Widespread of the *piggyBac* transposon in various *Bactrocera* species

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The *piggyBac* transposable element from the Lepidopteran species *Trichoplusia ni* is currently the most widely used vector for insect transgenesis. Consequently, the presence of *piggyBac*-like sequences has been investigated, by PCR and Southern analysis, in different species of target genera such as *Ceratitis*, *Bactrocera* and *Anastrepha,* along with *Tirhitromina* and *Rhagoletis*. *PiggyBac*-like sequences were detected on genomic DNA from several *Bactrocera* species. The evolution of the *piggyBac*-like sequences is discussed with respect to the phylogeny of the host.

Amplification of *piggyBac*-like sequences

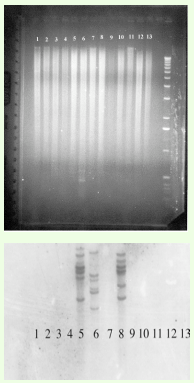
PCR primers pairs designed on the *B. dorsalis piggyBac*-like sequence AF289123:

For 5' GCGTAGCCGAGTCTCTGC

Rev 5' GTAAGGGGTCCGTCAAAACA

This set of primers was used on the genomic DNA of different Tephritidae species (*Ceratitis capitata*, *C. rosa*, *C. fasciventris*, *C. cosyra*, *Bactrocera dorsalis*, *B. zonata*, *B. philippinensis*, *B. cucurbitae*, *B. oleae*, *B. neohumeralis*, *B. tryoni*, *B. jarvisi*, *Anastrepha suspensa*, *A. ludens*, *Dacus dammerezi*, *Tirhitromina cyanescens* and *Rhagoletis cerasi*). PCR positive signals were detected only in *B. dorsalis*, *B. zonata*, *B. philippinensis*, *B. jarvisi*, *B. neohumeralis* and *B. tryoni*. For each of these species, the PCR product of two individuals was cloned and sequenced. The longest fragment was cloned from *B. zonata*: BZC of 890 bp.

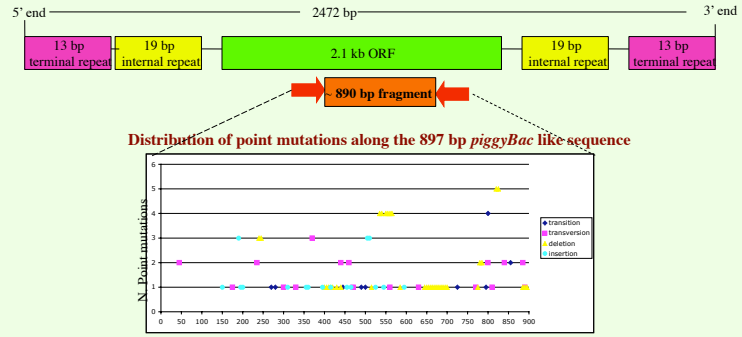
Southern DNA hybridization analysis



1. *C. capitata*
2. *C. rosa*
3. *C. fasciventris*
4. *C. cosyra*
5. *B. dorsalis*
6. *B. zonata*
7. *B. cucurbitae*
8. *B. philippinensis*
9. *B. oleae*
10. *T. cyanescens*
11. *D. dammerezi*
12. *A. ludens*
13. *A. suspensa*

genomic DNA. Each genomic DNA was EcoRI cut and hybridized with the BZC-890bp-*piggyBac* like sequence cloned from *B. zonata*.

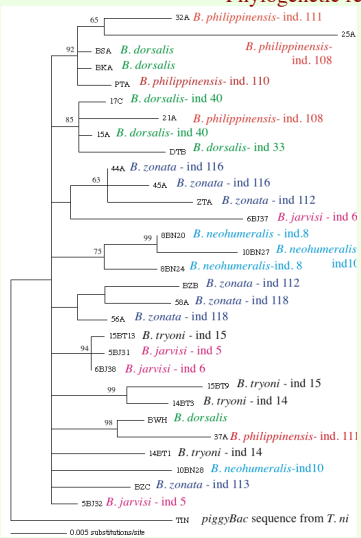
Schematic representation of the *piggyBac* element from *Trichoplusia ni* (Cary *et al*., 1989)



Matrix of the mean P distance±S.D. for all the *piggyBac* related sequences derived from six *Bactrocera* species

	B. dorsalis	*B. philippinensis*	*B. zonata*	*B. tryoni*	*B. nehoumeralis*	*B. jarvisi*
B. dorsalis	0.0099±0.0043					
B. philippinensis	0.0185±0.0090	0.024±0.0082				
B. zonata	0.0130±0.0038	0.0206±0.0072	0.0130±0.0035			
B. tryoni	0.0180±0.0090	0.0218±0.0067	0.0166±0.0033	0.0148±0.0042		
B. neohumeralis	0.0184±0.0054	0.0249±0.0070	0.0195±0.0041	0.0183±0.0039	0.0168±0.0075	
B. jarvisi	0.0133±0.0070	0.0203±0.0087	0.0144±0.0056	0.0137±0.0079	0.0179±0.0062	0.0122±0.0085

Phylogenetic relationships



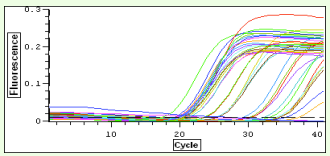
Phylogenetic relationships among the *Bactrocera piggyBac* like sequences. The maximum likelihood tree is based on the HKY+G evolution model (Hasegawa *et al*., 1985) and was derived with PAUP 4.0b10, after 100 bootstrap of the original data set (Swofford, 1998). Only bootstrap values > 50 are shown. The original sequence from *T. ni* (TIN) was used as outgroup.

- The *B. dorsalis* sequences group together with the sequences derived from *B. philippinensis*
- The *B. zonata* sequences tend to form separated clusters
- The separation among the Australian *Bactrocera* (*B. jarvisi*, *B. neohumeralis* and *B. tryoni*) is less definite

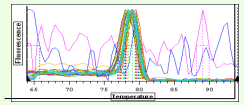
quantitative PCR

The qPCR primers are based on a highly conserved region on the alignment of all our sequences

Results of the *piggyBac* fragment amplification from different *Bactrocera* species. The SYBRGreen technology was used.



Primers melting plot



Relative quantity of the *piggyBac*-like fragment amplified by quantitative PCR in the different *Bactrocera* species. Two flies were analyzed for each of the six species.

		B. dorsalis		*B. philippinensis*		*B. zonata*		*B. tryoni*		*B. jarvisi*		*B. neohumeralis*	
		Ind.1	Ind.2	Ind.1	Ind.2	Ind.1	Ind.2	Ind.1	Ind.2	Ind.1	Ind.2	Ind.1	Ind.2
B. dorsalis	ind.1	1											
B. dorsalis	ind.2	0.93	1										
B. philippinensis	ind.1	0.76	0.82	1									
B. philippinensis	ind.2	0.88	0.95	1.16	1								
B. zonata	ind.1	0.96	1.04	1.27	1.09	1							
B. zonata	ind.2	0.47	0.51	0.62	0.53	0.49	1						
B tryoni	ind.1	0.35	0.38	0.46	0.39	0.36	0.74	1					
B. tryoni	ind.2	0.84	0.90	1.11	0.95	0.87	1.79	2.4	1				
B. jarvisi	ind.1	0.57	0.61	0.74	0.64	0.59	1.21	1.63	0.68	1			
B. jarvisi	ind.2	0.47	0.51	0.62	0.54	0.49	1	1.35	0.56	0.83	1		
B. neohumeralis	ind.1	0.73	0.79	0.97	0.83	0.76	1.56	2.10	0.88	1.30	1.56	1	
B. neohumeralis	ind.2	0.53	0.70	0.69	0.60	0.55	1.12	1.52	0.63	0.93	1.12	0.72	1

Conclusions:

- Among the sixteen Tephritidae species analyzed, *piggyBac*-like sequences were detected only in six *Bactrocera* species: *B. dorsalis* and *B. philippinensis*, which are found in the South-East Asia and in parts of the Pacific, *B. zonata*, which extends its range to the east-southern Mediterranean coast and the three Australian species *B. tryoni*, *B. jarvisi* and *B. neohumeralis*,
- Most of the point mutations are deletions and insertions. Only the BZC sequence from *B. zonata* contains a putative intact ORF, but shows several point mutations leading to synonymous and non-synonymous mutations..
- The *B. philippinensis* sequences seem to harbor most of the both within species and among species sequence divergence
- In the tree, few bootstrap values are higher than 50%, suggesting a low sequence divergence. In any case, the *B. zonata* and the *B. neohumeralis* sequences tend to form separate clusters with respect to the sequences from the other species. On the contrary, sequences from *B. dorsalis* and *B. philippinensis* on one hand and those from *B. tryoni* and *B. jarvisi* on the other tend to cluster together.
- The relative quantity of the *piggyBac*-like fragment is very similar in the different species. The highest difference is between *B. dorsalis* and *B. tryoni*, with the first having between 1.98 and 2.87 more *piggy-Bac* fragments than the second. In general, the Australian *Bactrocera* show the lowest quantity of *piggyBac*-like fragment.
- All of these results suggest a recent horizontal trasmission of the *piggyBac* element from *T. ni* and its recent inactivation.

Improved Attractants for Fruit Fly Management Programmes

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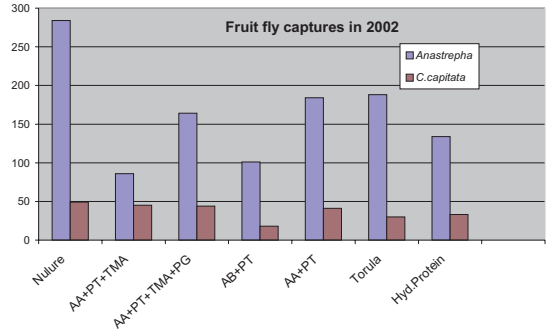
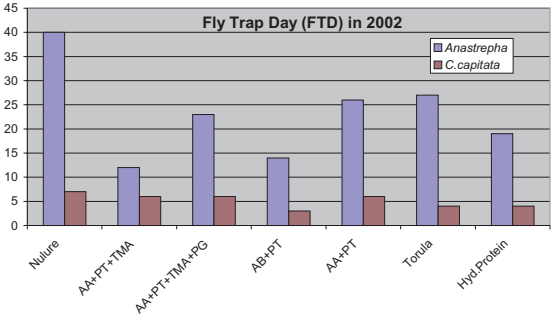
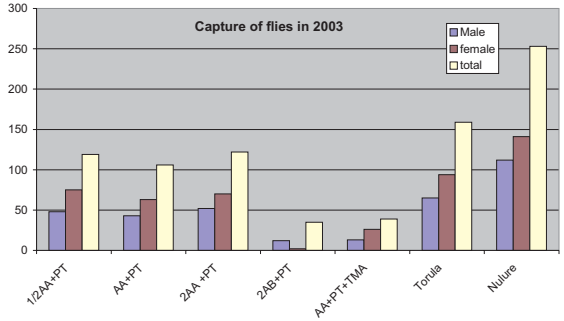
RESULTS

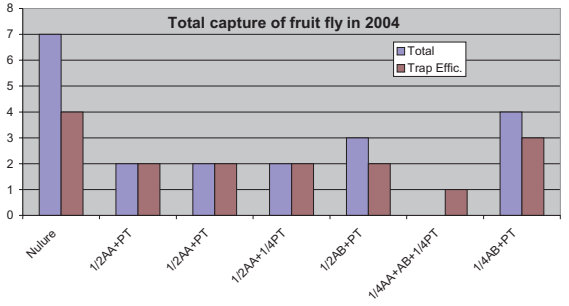
The food attractant Nulure captured the highest numbers of *Anastrepha* during three-year study. Fly Trap Day (FTD) indices, Relative Trap Efficiency (RTE %) and percentage of female per trap were also the highest figures with the product Nulure. The second best products to catch *Anastrepha* complex were the combination of the synthetic lures AA + PT (Ammonium Acetate + Putrescine) during 2001/2001 and 2002/2003 experiments. However during 2003/2004 experiments the combination 1/2AB + PT (Ammonium Bicarbonate + Putrescine) captured more flies than 1/2AA + PT but there was not statistical difference. The combination AA+PT+TMA (Trimethylamine) in two years had the worst performance in the capture of *Anastrepha* species but high capture of *C. capitata*. It seems that fractions of the synthetic lure are more efficient than the whole lure to attract flies. The posibly explanation for this fact is that fractions of lures liberate more slowly the active ingredient making it more efficient than the whole bait. Other parameters analyzed such FTD and RTE had similar response for the same combinations commented above. All treatments captured more fruit fly females. The predominant species, over 80%, was *Anastrepha zenildae*.

The numbers of males and females of *Anastrepha* captured with Nulure were significantly superior compared to the others food attractant and lures. Traps with the treatment AB + PT captured significantly lower numbers of adult males and females of *C. capitata* during the experiment in 2002 compared to other food attractants and lures. The population of *C. capitata* in the years 2002/2003 and 2003/2004 was very low. This population was due to low occurrence of rain during those years. The peak of captures was concentrated during of the rain season from January to June and the lowest captures was during the second semester where the rain fall was very low suggesting that population density is highly influenced by relative humidity and period of rain.









CONCLUSIONS

- The best food attractant in all years was Nulure.
- The second best products to catch *Anastrepha* complex were the combination of the synthetic lures AA + PT (Ammonium Acetate + Putrescine) during 2001/2001 and 2002/2003 experiments.
- During 2003/2004 experiments the combination 1/2AB + PT (Ammonium Bicarbonate + Putrescine) captured more flies than 1/2AA + PT but there was not statistical difference.
- The combination AA+PT+TMA (Trimethylamine) in two years had the worst performance in the capture of *Anastrepha* species but high capture of *C. capitata*.
- All treatments captured more fruit fly females.
- The predominant species, over 80%, was *Anastrepha zenildae.*

TEPHRITID DIVERSITY IN FRUIT PLANTATIONS IN COSTA RICA.

The fruit production is one of the more important agricultural activities in Costa Rica. Banana, pineapple, melon, orange, mango and guava are the more important. They contribute to the population's diet, to produce materials necessary for the alimentary industry, to generate work sources, foreign currencies and other related activities.

Part of this production is threatened by the tephritids that use them to spawn and cause severe losses, damages and limitation for the fruit exportation.

For this reason, the study of the tephrtitid diversity present in the fruit plantations is a necessary and important contribution to the definition of political and for the definition of the best strategies to reduce the populations of these plagues.

This poster presents the results of nine evaluations of the tephritid diversity present in fruit plantations in Costa Rica.

RESULTS.

Tables and graphics show the diversity, quantity and percentage of tephritids species collected in each fruit plantation and evaluation.

MATERIAL AND METHODS.

Tephritids were captured in fruit orchards in Costa Rica during four years [2001 - 2004] with MultilureRM Traps with different attractants: A) *Nu - Lure (Nu), B) Ammonium Acetate (AA)+ Putrescine (PT), C) Ammonium Bicarbonate (AB) + PT, D) AA + PT + Trimethylamine (TMA) and E) Torula yeast.*

These attractants were used to attract them to the traps, in a mixed plantation of coffee and citrus in Grecia Canton [year 2001] and in Corralar District [2002 and 2004], in a mango plantation in Esparza Canton [2001 and 2003], in a guava orchard in Pocora District [2002 and 2004] and in a citrus plantation in San Carlos Canton, [2003].

Table 1.
Tephritids captured in a coffee and citrus plantation in Grecia Canton, Costa Rica. June- July, 2001.

Species	Quantity	Percentage
C.capitata	3837	84.34%
A. ludens	634	13.93%
A striata	49	1.07%
A. fraterculus	29	0.63%
TOTAL	4549	99.97%

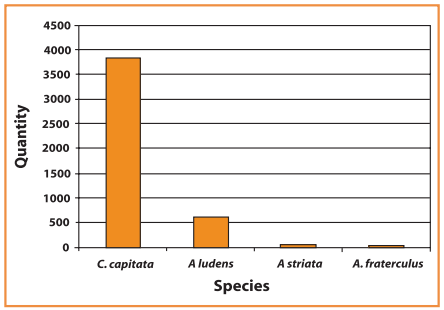


Fig. 1. Diversity of Tephritids captured in a coffee and citrus plantation in Grecia, Costa Rica. 2001

Table 2.
Diversity and quantity of Tephritids collected in two evaluations in a mango orchard in Esparza Canton, Costa Rica. Mars - April, 2001 and April - June, 2003.

Species	2001		2003	
	N	%	N	%
C. capitata	1107	49.50	518	65.40
A. obliqua	875	39.13	216	27.27
A. striata	156	6.97	15	1.89
A.serpentina	73	3.26	18	2.27
A. ludens	1	0.04	1	0.12
H. obscura	24	1.07	24	3.03
TOTAL	2236	99.97	791	99.98

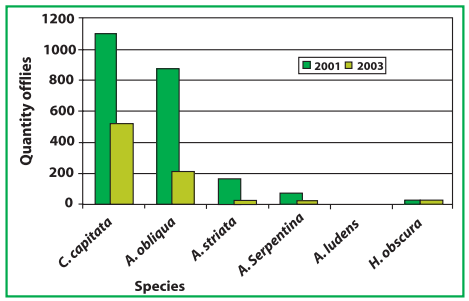


Fig. 2. Tephritids captured in a mango orchard in Esparza, Costa Rica. 2001 and 2003.

Table 3.
Tephritids collected in three evaluations in a guava orchard in Pocora, Costa Rica. .

Species	2002		I-2004		II-2004	
	N	5	N	%	N	%
A. striata	1526	98.96	8071	87.26	1786	87.67
A.obliqua	3	0.001	933	10.08	251	12.32
A. serpentina	0	0	6	0.06	0	0
A.ciclayae	0	0	2	0.002	0	0
A. fraterculus	0	0	0	0	0	0
A. zulianae	3	0.001	0	0	0	0
C. capitata	0	0	235	2.54	0	0
T. curvicauda	1	0.0006	1	0.01	0	0
H. obscura	0	0	1	0.01	0	0
Pseudocrataenea spp.	2	0.001	0	0	0	0
Pyrgotoides spp.	1	0.0006	0	0	0	0
TOTAL	1542	98.96	9249	99.96	2037	99.99

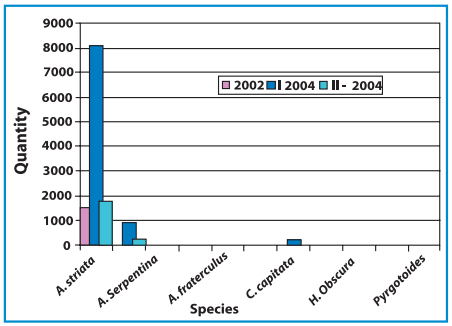


Fig. 3. Tephritids collected in three evaluations in a guava orchard in Pocora, Costa Rica.

Table 4 .
Tephritids captured in a coffee and citrus plantation in Corralar, Costa Rica. November - December, 2001 and June - August, 2004.

Species	*C.capitata*		*A.ludens*		*A.striata*		*A..obliqua*		*Tetreuaresta*		*Molynocoelya*		*Paroxyna sp*		TOTAL
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
2001	2323	60.2	1414	36.6	96	2.49	20	0.5	0	0	0	0	0	0	3853
2004	270	45.2	181	30.3	6	1.00	19	3.18	2	0.33	105	17.5	14	2.34	597

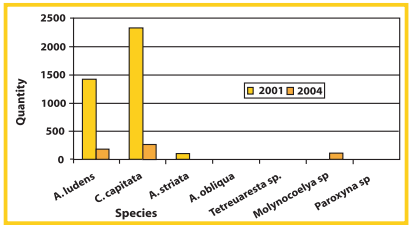


Fig. 4. Tephritid flies collected in a mixed coffee and citrus plantation in Corralar, Costa Rica. November - December, 2001 and June - August, 2004.

CONCLUSIONS.

The study of the diversity of fruit flies in Costa Rica shows the variety of this family present in the most important fruit plantation and it is an important contribution to know which are the tephritids pests present in them.

The *Medfly, Anastrepha striata, A. obliqua and A. ludens*, were the more frequent species captured in the evaluations.

Most of these species are polyphagous pests and able to share the hosts. It is possible that the reduction or eradication of one of them will leave its ecological niche free, which could be occupied by another and this is an important aspect to considerer in the Area Wide Programs.

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Responses of *Anastrepha striata* to various attractants in Costa Rica.

Anastrepha striata (Diptera: Tephritidae) is known how the "guava fruit fly " in Costa Rica because it spawns fundamentally in fruits of the Myrtaceae Family and the guava *(Psidium guajaba)* is the most common, frequent and with more dispersion species in the country. This is a nutritious fruit and its pulp is used to produce marmalades, juices yogurs and other foods.

It also spawns in the "cas" *(P. friedrichsthalianum)*, a wild Myrtaceae called "guísaro" *(P. savanarum)* and in an exotic species called popularly "Spanish guava" or "Peru guava" *(P. catlleianum)*. These hosts grow in cultivated and wild form in almost the national territory and they have various fructification periods during the year for what this pest has substratum to spawn during almost the time. *Anastrepha striata* has also been found in other fruits of more economic importance as orange (Citrus cinensis: Rutaceae), mango *(Mangifera indica: Anacardiaceae)*, avocado *(Persea americana:* Lauraceae), "jocote" or "jobo" (*Spondias mombim*: Anacardiaceae), and nance (*Birsonima crassifolia*; Malphigiaceae).

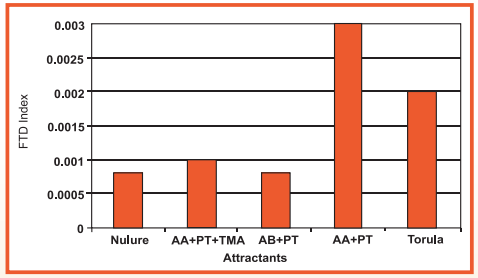


Fig. 1. Fly per Trap per Day Index for *Anastrepha striata* captured in a coffee and citrus plantation in Grecia Canton, Costa Rica, during the rainy season. June - July, 2001

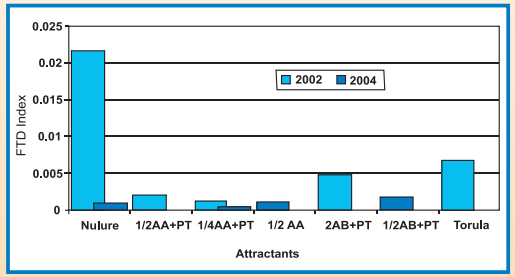


Fig. 2. Fly per Trap per Day Index for *Anastrepha striata* captured in a coffee and citrus plantation in Corralar, Costa Rica, during the transition between the rainy to the dry season (November - December, 2002) and in the rainy season June . August, 2004.

MATERIAL AND METHODS.

The purpose of this research was to determine the responses of *A. striata* to the attractants: A) Nu - Lure® (Nu), B) Ammonium Acetate (AA) at 150 µg NH4/hour + Putrescine (PT), C) AA at 300 µg NH4/hour + PT, D) AA at 600 µg NH4/hour +PT, E) Ammonium Bicarbonate (AB) at 300 µg NH4/hour + PT, F) AA at 300 µg NH4/hour + PT + Trimethylamine (TMA) and G) Torula yeast. Each evaluation lasted eight weeks, with a change of the attractants at four weeks and biweekly gatherings of the captured insects. The data are expressed by means of the Flies per Trap per Day Index (FTD Index).

This poster shows the results of attractant evaluations carried out during the last four years in several fruit plantations that are hosts of Anastrepha striata in Costa Rica.

Table 1.
Quantity of flies and *Anastrepha striata* (N and %) captured in each evaluation.

LOCALITY	Grecia	Esparza		Pocora			Corralar	
HOST	(Coffee & citrus)	(Mango)		(Guava)			(Coffee & citrus)	
YEAR	2001	2001	2003	2002	I-2004	II-2004	2002	2004
FLIES CAPTURED	4549	2239	792	1491	9251	2037	3853	447
A. striata	49 (1.07%)	50 (4.03%)	15 (1.89%)	1477 (99.06%)	8071 (87.49%)	1786 (87.67%)	114 (2.95%)	5 (1.11%)

Table 2.
Fly per Trap per Day Index to *Anastrepha striata* captured with various attractants, with different hosts and population density in Costa Rica. Years 2001 - 2004.

LOCALITY	Grecia	Esparza		Pocora			Corralar	
YEAR	2001	2001	2003	2002	I-2004	II-2004	2002	2004
Nulure	0.0008	0.005	0.0015	0.15	0.92	0.29	0.0215	0.01
AA+PT+TMA	0.001	0.004	0.0003	0.01	NE	NE	0.002	0
AB+PT	0.0008	0.008	0.001	0.02	0.08	NE	0.0012	0.00036
AA+PT	0.003	0.003	0.002	0.03	NE	NE	0.0011	0
½ AA+PT	NE*	NE	0	0.06	NE	NE	0.0048	0
2AA+PT	NE	NE	0.0003	0.05	NE	NE	0.0018	0
Torula	0.002	0.20	0.0015	0.17	0.65	NE	0.0068	NE

*NE = No evaluated

CONCLUSIONS.

The results of the evaluations carried out in Pocora [I and II 2004] in a guava orchard and in Corrralar [2002 and 2004] in a coffee and citrus plantation the largest FTD Index was obtained with NuLure (0.92, 0.29, 0.0215 and 0.01 respectively).

In Esparza [2002] in a mango orchard and Pocora [2002] in a guava plantation the largest FTD Index was obtained with Torula (0.02 and 0.17, respectively). The second best attractant was NuLure. In Pocora 2002 and in Grecia [2001], Pocora [2004] and Corralar [2002] it was Torula.

In Grecia [2001] and in Esparza [2003] the best attractant was the mixture of Ammonium Acetate with Putrescina.

The responses of *A. striata* to different attractants is variable. Nu - Lure and Torula were the best attractants in most of the evaluations.

Possibly there are specific *microclimatic and phenologycal conditions* that affect the responses of the flies to the attractants and produce variations in their behavior.

There was obtained basic and necessary information to evaluate the presence and density of the population of *A. striata* under diverse climatic conditions, *phenologycal* conditions and population densities. These evaluations are an important base to the area wide programs and eventually study its use in bait stations.

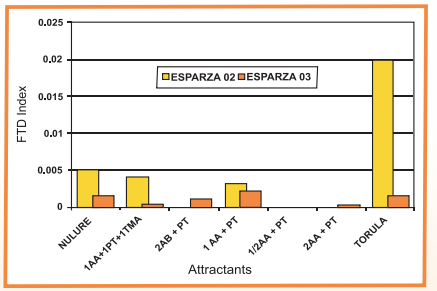


Fig. 3. Fly per Trap per Day Index for *Anastrepha striata* captured in a mango orchard in Esparza Canton, Costa Rica, during the dry season (2002) and the transition period between the dry to wet season (2003).

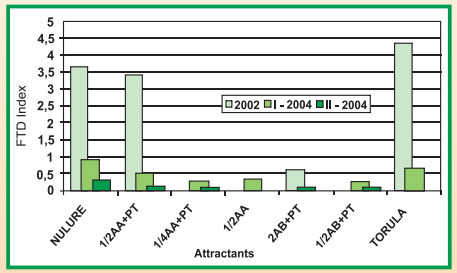
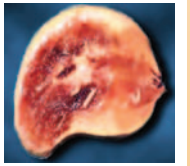


Fig. 4. Fly per Trap per Day Index for *Anastrepha striata* captured in three evaluations (2002, I - 2004 and II - 2004) in a guava plantation in Pocora, Costa Rica, during the rainy season, with mature fruits and high density of the population.



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IAEA-CN-131/7P

Evaluation of traps and killing agents in Mediterranen fruit fly captures

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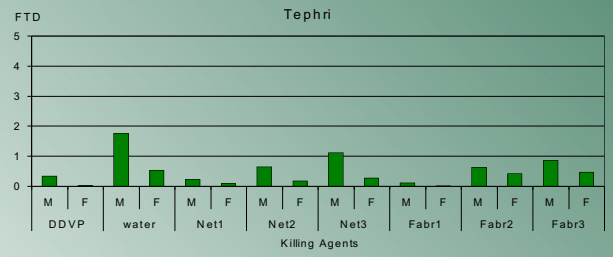
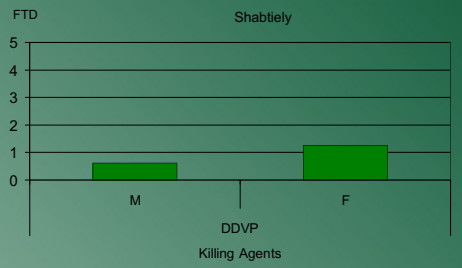
Introduction

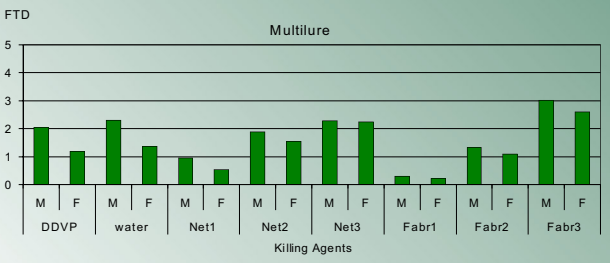
An effectiveness use of mass trapping as tool of fruit fly control needs to be improved. Simultaneously, this can be an enormous contribution for population suppression in SIT programs. The combination of traps design, attractants and killing agents, their effectiveness in males or females captures, trap cost, time that can remain in the field and labour, are very important aspects for the growers and action programs who are looking for mass trapping as population suppression.

Material

Traps: Tephri, Multilure (MLT), Shabtiely and Easy.
Attractants: Putrescine (PT), Ammonium acetate (AA) and Trimethylamine (TMA).
Killing agents: starvation, DDVP, water and triton, three different sizes of netting (UV, permaNet(R)): Net and Fabrics: 1 (16 cm^2), 2 (64cm^2) and 3 (256 cm^2).







Conclusion

To be used under male only SIT programme the Multilure trap is the best option, when baited whit the TMA+AA+PT and Fabrics (UV, permaNet(R)) 256 cm^2 as killing agent, capturing more females than the other traps.
In total capture numbers easy trap show to be the best trap as tool to be used in Mediterranean fruit fly suppression.

Acknowledgements
The join FAO/IAEA Division supported this research under the research agreement POR 29114.

Measuring Insect Characteristics by Near-infrared Spectroscopy and Applications to the SIT

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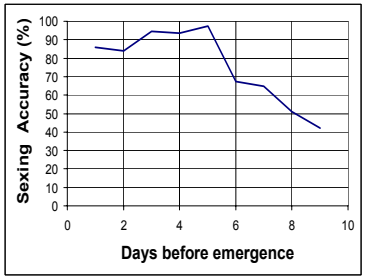
Introduction. Near-infrared spectroscopy (NIRS) is commonly used to measure characteristics of biological materials such as grain protein, milk fat content, and pharmaceutical quality. This poster reports the application of NIRS to measuring sex, species, and chronological age of insects.

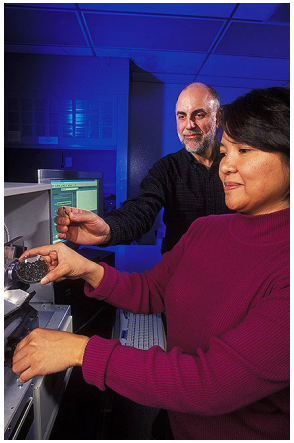
Sexing. We have used NIRS to determine the sex of tsetse (*Glossina* spp) pupae where significant differences have been found between the spectra of male and female. The differences appear to be maximized four to five days before emergence of the adults. Fly pupae can be sexed and automatically sorted up to five days before emergence with accuracies that ranged from 80 to 100%. Thus, males can be separated and irradiated for SIT applications, and females returned for colony production.

NIRS was also used in sexing pupae of the house fly, *Musca domestica* Linnaeus, face fly, *M. autumnalis* DeGeer, secondary screwworm, *Cochliomyia macellaria* (Fabricius), primary screwworm fly, *C. hominivorax* (Coquerel), and stable flies, *Stomoxys calcitrans* (L.) but had lower classification accuracies of 50-74% when compared with those of *Glossina* spp. pupae.

We have also conducted preliminary studies to sex mosquito pupae and adults, but additional testing is needed.



Accuracy of sexing tsetse flies



Placing pupae in automated sorting instrumentation

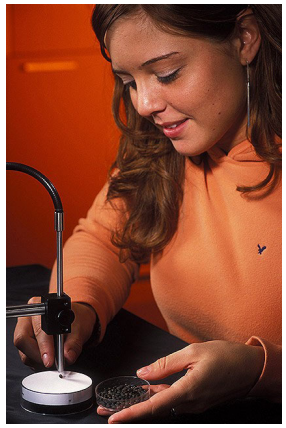
Instrumentation for manually scanning mosquito pupae and adults

Species ID. We also used NIRS to identify species of 11 adult stored-grain beetle pests with up to 99% accuracy. Species studied included the flat grain beetle, *Cryptolestes pusillus* (Schönherr); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); merchant grain beetle, *Oryzaephilus mercator* (Fauvel); confused flour beetle, *Tribolium confusum* Jacquelin du Val; red flour beetle, *Tribolium castaneum* (Herbst); lesser grain borer, *Rhyzopertha dominica* (F.); larger grain borer, *Prostephanus truncatus* (Horn); granary weevil, *Sitophilus granarius* (L.); rice weevil, *Sitophilus oryzae* (L.); and maize weevil, *Sitophilus zeamais* Motschulsky. Differences in cuticular lipids between the species likely affected the NIR spectra and contributed to classifications.

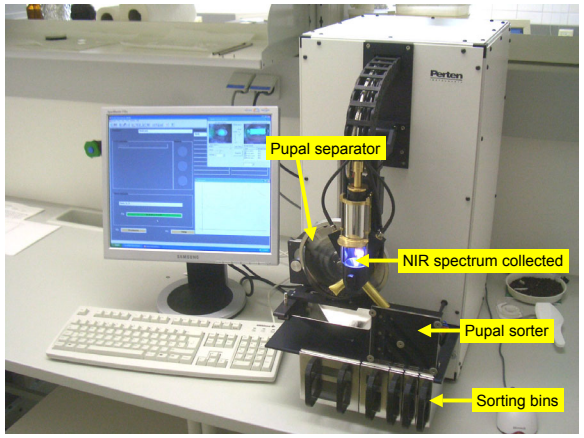
In preliminary studies, we differentiated 5 species of mosquitoes (*Aedes aegypti, Anopheles freeborni*, *An. gambiae*, *An. stephensi* and *An. dirus)* with 75-100% accuracy. Thus, NIRS shows potential to rapidly determine insect species.

Age Grading. We were able to age-grade house flies, *M. domestica* (L.), face flies, *M. autumnalis* DeGeer, and stable flies, *Stomoxys. calcitrans* (L.), with accuracies that exceeded the current pteridine fluorescence age-grading technique. NIR spectra may have been influenced by pteridine levels, lipid composition, or by an increase in cuticular lamellae. We were also able to age-grade stored-product beetles. The NIR spectra appeared to be sensitive to decreasing water content in older insects. Thus, NIRS may be a useful tool to study insect population dynamics.

Instrumentation. All insects were either hand-placed into the viewing area of a NIR spectrometer (DA7000, 400-1700 nm, Perten Instruments, Stockholm, Sweden; or ASD, 400-2500 nm, Boulder Colorado), or automatically scanned at a rate of about 1 pupae/2s using an automated scanning and sorting NIR spectrometer (950-1700 nm, SKNIR model, Perten Instruments, Stockholm, Sweden). This automated system can sort pupae into 4 categories based on user-defined sorting criteria. Instruments for determining characteristics of individual hand-placed insects can be field portable. Instruments for automatically scanning and sorting pupae are commercially available.



Manually scanning tsetse pupae for feasibility studies



Automated scanning and sorting instrumentation

ISOLATION AND CHARACTERIZATION OF *HSP 70* GENES IN *BACTROCERA OLEAE*

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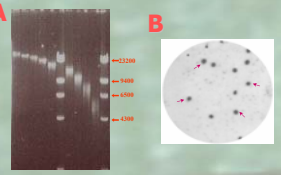
Bactrocera oleae **is an insect pest of great economical importance to the Mediterranean countries, causing annually great losses to the olive fruit crops. Molecular studies of the insect can significantly contribute to the efforts of its biological control. The *hsp*70 gene, a member of the Hsp70 family, presents high levels of conservation and plays a crucial role during heat-shock response. In *Drosophila* and other Diptera species multiple copies of the *hsp*70 gene have been identified, while its promoter has been widely used for the expression of several genes in insect transformation systems.**

Library construction and screening

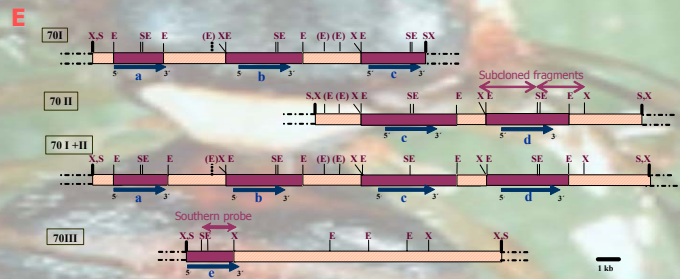
- Isolation of **total genomic DNA from adult flies**
- Partial digestion with ***Sau*3AI**
- Selection of DNA fragments after **sucrose gradient ultra-centrifugation (A)**
- **Cloning in λFIX II** (Lambda FIX II/*Xho*I Partial Fill-In Vector kit, Stratagene, Calif.)
- Screening of **4x10⁵** plaques **(B)** with a ***Ceratitis capitata hsp* 70 gene copy (C)** **(2 X Denhardt's** solution, **55°C)**



Three λ clones bearing hsp70 sequences have been isolated from the constructed B. oleae genomic library (8x10⁶ pfu/ml , 16 kb average insert size)

Restriction mapping of the isolated *hsp 70* clones

- Isolation of **λ DNA**
- Digestions with ***Eco*RI, *Sal*I and *Xba*I**
- Analysis on **0,7% agarose gel**
- **Southern transfer** and **hybridization** with **5' and 3'** fragments of a ***Ceratitis capitata hsp* 70 gene copy (C)** **(Church** solution, **55°C)**



Sequence analysis of an *hsp70* copy

- Isolation of ***Sal*I /*Xba*I fragments** of **d** *hsp*70 copy **(E)**
- **Subcloning in pGEM-3Zf** plasmid vector
- **Automated sequencing** by Lark Technologies
- **Bioinformatic** analysis

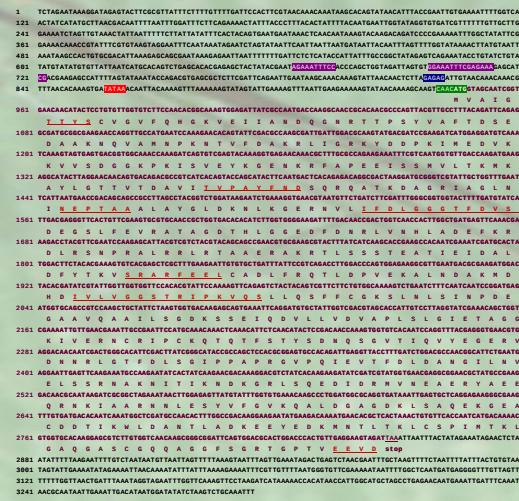
G

	Bohsp70	Cchsp70	Dmhsp70	Aahsp70	Cthsp70	Cchsc70	Dmhsc4
Bohsp70		92	85	82	77	75	74
Cchsp70	84		84	78	75	73	73
Dmhsp70	72	75		81	79	73	73
Aahsp70	71	72	75		79	76	75
Cthsp70	71	69	65	67		72	72
Cchsc70	67	65	67	65	64		96
Dmhsc4	64	65	72	69	54	82	

% identity of nucleotide sequence

% identity of aminoacid sequence

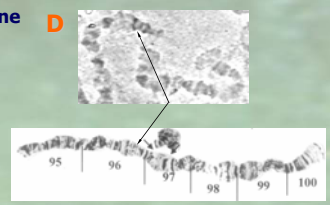
H



Chromosomal localization of the isolated *hsp*70 clones

- Preparation of **salivary gland polytene chromosomes**
- **Labelling** of λ clones by random priming with **dig-11dUTP**
- **Hybridization** at **65°C**
- **Detection: Anti-dig-AP** fragment – **Colour reaction** with BCIP and NBT

D

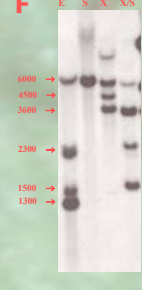


All isolated clones map on the locus of the major heat-inducible puff of B. oleae at region 96 on the VR polytene chromosome arm

Genomic Southern blotting

- Isolation of **genomic DNA from adult flies**
- Digestions with ***Eco*RI, *Sal*I and *Xba*I**
- Analysis on **0,7% agarose gel**
- **Southern transfer** and **hybridization** with **a fragment of the e *hsp*70 sequence** included in λ clone 70III **(E)** **(5 X Denhardt's, 50% formamide, 42°C)**

F



Five copies of the hsp70 gene have been identified in the genome of B. oleae. Four of these copies are arranged in parallel orientation

Analysis of the B. oleae hsp 70 copy suggests for a cytoplasmic heat-inducible member of the Hsp70 family, bearing high homology with other Dipterans hsp 70 genes

Postharvest Irradiation Treatments: Generic Dose, High Dose, and Less-Than-Probit 9 Applications

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GENERIC TREATMENTS

Quarantine or phytosanitary treatments eliminate, sterilize, or kill regulatory pests in exported commodities to prevent their introduction and establishment into new areas. Development of generic dose, high dose and less than probit 9 concepts with irradiation have the potential to accelerate approval of quarantine treatments and rapidly expand exports, thereby promoting irradiation as a postharvest treatment technology.

A generic quarantine treatment is one that provides quarantine security for a broad group of pests. For example, a generic treatment could be applied to all Diptera (flies), or to flies in the family Tephritidae (fruit flies), or to tephritid fruit flies in the genus *Anastrepha*. Irradiation is the ideal technology for developing generic treatments because it is effective against most insects and mites at dose levels that do not affect the quality of most commodities.

USDA APHIS approved a generic irradiation treatment for tephritid fruit flies (150 Gy) based on data for *Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis* fruit flies. Publication of this treatment in a rule is forthcoming. Before generic treatments can be recommended for more insect taxa, information from coordinated research projects and large-scale testing is needed on effective irradiation doses for key pests and under-represented groups.

HIGH DOSE APPROACH

Use of an irradiation dose at the upper limit of what is believed to control the insect groups that infest a commodity without specific data on the quarantine species of concern is termed the high dose or default dose approach. Sweetpotato growers in Hawaii are unable to ship sweetpotatoes to California and the U.S. mainland without a quarantine treatment because of the presence of three regulatory pests: West Indian sweetpotato weevil, *Euscepes postfasciatus* (Coleoptera: Curculionidae), and sweetpotato vine borer, *Omphisa anastomosalis* (Lepidoptera: Pyralidae), and sweetpotato weevil, *Cylas formicarius elegantul*us (Coleoptera: Curculionidae) (see photos above). An irradiation treatment of 400 Gy for sweetpotatoes was published as a Final Rule in the Federal Register on February 2004 based on preliminary data on radiotolerance of the insect pests and a recommendation for a high-dose. The 400 Gy dose is believed to control most species of Coleoptera and Lepidoptera. This was the first time APHIS considered the high-dose approach for controlling a pest complex until research is completed to confirm a lower dose. Recent research indicates the dose to control the sweetpotato pests can be reduced to 150 Gy.

LESS-THAN-PROBIT 9

In the U.S., probit 9 (99.9968% mortality) has been the standard for quarantine treatment efficacy for highly infested commodities. Use of an irradiation treatment with less-than-probit 9 efficacy may be practical if the commodity is sensitive to radiation treatment and a system of other measures are in place to cumulatively provide quarantine security. Components of the systems approach may include poor host status, protein bait sprays and/or traps, fruit cutting and inspection, a postharvest treatment, limited distribution, and limited geographic area. A systems approach including irradiation is being considered against oriental fruit fly (*Bactrocera dorsalis*) in Sharwil avocados for export from Hawaii to the U.S. mainland. Use of irradiation alone to provide quarantine security for oriental fruit fly requires dose levels (150-250 Gy) that adversely affect the quality of fruit. Less than probit 9 irradiation doses (50-75 Gy) will kill >90% of flies and could be integrated with other components for sequential mortality providing quarantine security if avocado quality is preserved.

Table 1. Irradiation doses shown to control various pest groups. Generic doses for each group could be set at the upper values of the range

Pest group	Target life stage	Dose range (Gy)
Bruchid seed weevils	Adult	70-300
Curculionid weevils	Adult	80-150
Hemiptera	Adult	50-250
Lepidopera	Larva	100-250
	Pupa	200-400
Mites	Adult	200-400
Scarab beetles	Adult	50-150
Stored products beetles	Adult	50-250
Stored products moths	Adult	100-600
Tephritid fruit flies	Larva	50-150
Thrips	Adult	150-350

Source: Follett and Griffin. In press. Irradiation as a phytosanitary treatment for fresh horticultural commodities: research and regulations. In Food Irradiation Research and Technology, C. H. Sommers and X. Fan eds., Blackwell Publishing, Ames, Iowa. Modified from IPPC (2003) "Guidelines for the use of irradiation as a phytosanitary treatment".

ARTHROPOD RADIOTOLERANCE

Animal groups vary in their tolerance to irradiation (Table 1). Among insects, Diptera (such as fruit flies), Coleoptera (beetles), Hemiptera (true bugs) tend to be less radiotolerant than Lepidoptera (moths and butterflies), although there is considerable variation among the species that have been tested within these groups. Estimates for Hemiptera (scales, mealybugs, aphids and whiteflies) and Thysanoptera (thrips) are based on a small number of studies. Two of the most radiotolerant insects are the Indianmeal moth, *Plodia interpunctella*, and the Angoumois grain moth, *Sitrotroga cerealella*, both stored products pests. Several species of mites have been tested and they appear to be relatively tolerant of ionizing radiation. Few studies have conducted the large-scale tests needed to confirm the efficacy of an irradiation dose predicted to give 100% mortality. Most insects are sterilized at doses below 300 Gy.

Capture of *Anastrepha* Spp. Fruit Flies In North America and the Caribbean Basin

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Introduction

Research is being conducted to develop improved attractants for *Anastrepha* species fruit flies. This study compared different doses and formulations of ammonia with liquid protein baits. Comparative data on effectiveness of synthetic lures among different species and hosts will advance detection, monitoring and control of these pests.

Materials & Methods

Traps - All baits and lures were used in Multilure Plastic McPhail Traps (Better World, Miami, FL)

Lures -

I. Liquid protein baits
- Nulure - 300 ml of 9% Nulure, 3% borax, 88% water
- Torula - 3 torula yeast/borax pellets in 300 ml water

2. Ammonium acetate (AA) with Putrescine (Pt) (BioLure, Suterra LLC., Bend, OR)
- ¼x AA + Pt
- ½x AA + Pt
- 1x AA + Pt
- 2x AA + Pt

3. Ammonium bicarbonate (AB) (AgriSense BCS Ltd, UK) with Pt
- 1x AB + Pt
- 2x AB + Pt

Retention solutions -
- PPG - 25 ml polypropylene glycol/275 ml water
- Triton – 3 drops in 300 ml water

Field protocol -
- Traps were checked twice a week for 8-17 wk
- Liquid protein baits were replaced weekly
- Synthetic lures were used for 4 wk
- Population level is flies per trap per day for Nulure (*A. obliqua*) or AA + PT (*A. ludens* and *A. suspensa*)

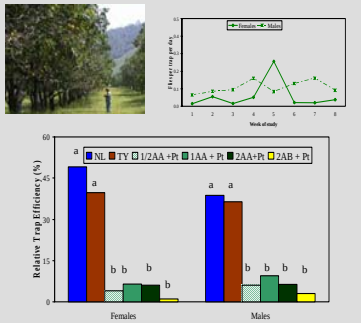
Results

Anastrepha obliqua West Indian fruit fly

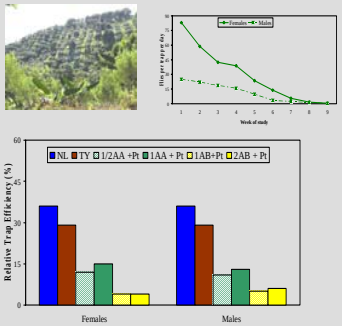
weslaco.ars.usda.gov/photos/dmfly.html

Numbers captured were very low in Honduras, but very high in the tests in the Dominican Republic. Liquid protein baits captured the most of flies in both tests. Few flies were captured by synthetic baits in citrus, but AA + Pt captured more than AB + Pt in tests in mango.

- Test conducted for 8 wk, Summer 2002 in grapefruit in Atlántida, Honduras



- Test conducted for 9 wk, Summer 2004 in mango orchard, Dominican Republic

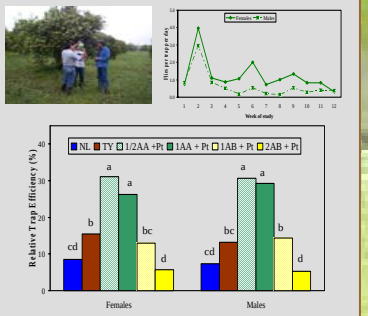


Anastrepha ludens Mexican fruit fly

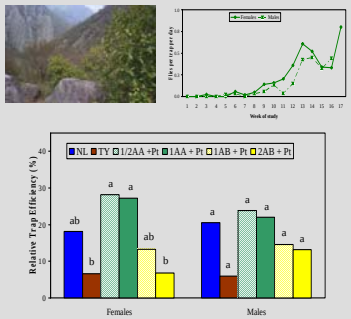
ars.usda.gov/is

Numbers of flies captured were high in the citrus orchard and low in the native host. At both sites, AA + Pt at either AA dose captured the highest percentage of flies. TY captured more flies in the test in a citrus orchard while Nulure captured more flies in the test in the native host.

- Test conducted for 12 wk, starting 3/3/04 in citrus orchard in Monterey, Mexico



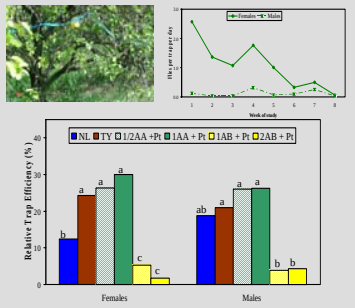
- Test conducted for 17 wk, starting 3/8/04 in yellow chapote Nuevo Leon, Mexico



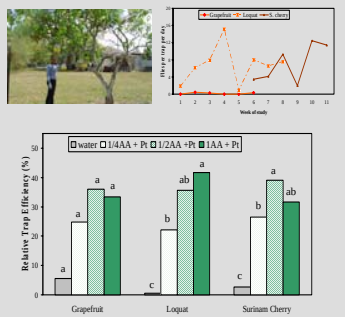
Anastrepha suspensa Caribbean fruit fly

Numbers captured were low in the grapefruit orchard and high in preferred hosts. AA + Pt at either full or half dose and TY tended to capture the highest percentage of flies. Decreasing the AA dose further decreased capture and few flies were captured in AB + Pt traps.

- Test conducted for 8 wk, starting 6/7/04 in grapefruit orchard in Ft. Pierce, FL



- Test conducted for 6-8 wk, starting 3/8/04 in host fruit trees in Miami, FL



Multilure trap baited with ½x AA + Pt: Release rate of the ammonium acetate lure was reduced by taping off half of the release membrane

Multilure trap baited with 2x AB + Pt: Release rate of the ammonium bicarbonate lure was increased by baiting with two lures

Discussion

Studies were conducted in commercial citrus orchards, and in commercial and non-commercial host trees. The primary host of *A. obliqua* is mango, but it will attack citrus. *A. ludens* is a citrus specialist. *A. suspensa* prefers guava, loquat and Surinam cherry and only infests citrus late in the season when the fruit skin is soft.

Relative trapping efficiency was variable among species and hosts. Nulure and TY traps captured the highest percentage of *A. obliqua* in all tests. However, synthetic attractants were the best for the other two species.

Among synthetic lures, AA+Pt was the most and AB+Pt the least effective. Increasing AB dose did not improve efficacy, decreasing AA dose had variable effects. Determination of mating status of females is ongoing and may indicate physiological differences among flies captured by the different attractants. In comparison with results from Central and South America, synthetic lures tend to capture more flies relative to liquid protein baits in North America and the Caribbean basin.

IAEA-CN-131/13P

Mass Rearing and Quality Control of *Aedes aegypti* for use in an SIT Control Programme

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Why?

- No vector control strategies used to decrease disease transmission by mosquitoes have proven to be totally successful or sustainable in the long term!
- Growing demand for solution to mosquito borne disease, which will probably incorporate new transgenic approaches.
- SIT set to be a key tool in control strategy tool box.
- Key requirement for SIT success: ability to economically mass-rear and high quality insects.

Aim

- Re-assess feasibility of reducing disease transmission by mosquitoes using area-wide pest management methods through the SIT.

Objectives

- Develop efficient method for mass rearing a stock, ***Ae. aegypti*** **(Bangkok)**, and transformed strain, ***Ae. aegypti*** **(kh^{w})**.
- Examine the stability and expression of the transgene under mass rearing conditions.
- Produce a “Product QC Standards Manual” with key standards for evaluating a mass reared strain.

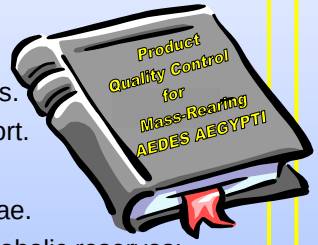
Progress & Plans

- **Egg Storage Capacity:** Establishing optimum storage time (potentially 10wks).
- **Larvae Rearing Depth & Density:** Mass rearing demands minimum handling and maximum space efficiency, therefore establishing rearing efficiencies in large-scale containers at increased larvae densities + depths.

- **Blood Feeding:** Comparing alternatives to membrane feeding (chick skin, latex, parafilm), and adapt for mass rearing.
- **Pupae / Larvae Separation:** Not an issue at a mass rearing level, if food is always available and only the high quality first 2 pupae collections used.

Product QC Manual

- **Draft:** QC Manual developing describing current standards. Proposes tests and specifications. Data needed to justify and support.



Key Indicators:

- Egg: Hatch & Develop to pupae.
- Larvae: 1st Instar survival + metabolic reserves;
- Adult: Wing Length = key quantifiable indicator of quality on many levels.
- Others: Adult competitiveness, sexing, size, sterility

Why *Aedes*?

- Most potential for inclusion as a genetically transformed insect for use in a SIT programme.
- Widespread urban vector of 3 major diseases (Dengue, yellow fever, filariasis).
- Widely studied, easy to rear.
- Already stably transformed (EGFP expressed); good data on linkage groups; evidence of meitotic drive.

THE UNIVERSITY of LIVERPOOL

PRELIMINARY EXPERIMENTS ON THE MASS TRAPPING TECHNIQUE AND RELEASING OF THE PARASITOID (*PSYTTALIA CONCOLOR* SZEPL.) TO CONTROL THE OLIVE FRUIT FLY (*BACTROCERA OLEAE* GMEL.) IN TURKEY

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INTRODUCTION

Olive fruit fly, *Bactrocera oleae* Gmel.(Diptera:Tephritidae) is the major pest in olive growing areas in Turkey like other Mediterranean countries. To date, a great deal of investigations and methodologies have been carried out to suppress the pest in order to lessen the crop losses from its damage and increase the quality of olive and olive-oil all the countries in Mediterranean basin. In this study we have aimed to investigate the possibility of control the olive fly by combining of two techniques as an environmental friendly concept.

MATERIAL AND METHOD

This study was carried out in Gökçeada Island (Imbroz) in Turkey in 2002.

Mass-trapping applications against *Bactrocera oleae* in the experimental area.

For this purpose, Eco-traps containing a food attractant (ammonium bicarbonate) and a sex attractant (1,7-dioxaspiro [5,5] undecane) and attracts the adults of the insect in an entomotoxic surface (paper covered by deltamethrine, on powerful contact insecticide) and kills them were used.

The traps were applied in the experimental area on 26 June 2002 at the density of one trap / one or two tree. Accordingly 2000 traps were distributed to 2500 olive trees. The traps were hung up the trees two meters high from ground and in the middle of the canopy, in the shade, before observing the first punctures. This process was aimed to decrease of ***B. oleae*** population before releasing of ***P. concolor***.

Releasing of *Psyttalia concolor* Szepl. (Hymenoptera:Brachonidae) in the experimental area

Releasing of ***P. concolor*** which mass-reared in our laboratory was initiated beginning of the September and was repeated four times with 12-22 days intervals (table 1). Parasitoids releasing were initiated after the number of trap catches tended to increase parallel to noticing of first punctures. ***P. concolor*** individuals were released through the nature after they had mated and gained parasitism ability. The number of males and females in the cages were calculated by means of eclosion grids before each releasing.

Population trend of *B. oleae*

Population trend of *B. oleae* was observed by means of McPhail traps containing DAP 2% and yellow sticky traps with pheromone.

Assessment:

Mass-trapping applications and parasitoid releases were evaluated together. Before every releasing and at the harvest,total 1000 fruits samples randomly taken from the trees were checked and the number of infested fruits were collected in each area (core, buffer, neighboring and control). Additional 500 fruits samples were collected from the ground. Associated efficiency of the method was determined by using Abbott formula.

Table 1. Number of *psyttalia concolor* released

Releasing date	No. of ♀	No. of ♂	Total	Av.parasitoids/tree
05.09.2002	37.884	28.571	66.455	26,58
17.09.2002	52.940	50.933	103.873	41,54
02.10.2002	45.308	20.385	65.693	26,27
24.10.2002	37.961	28.896	66.857	26,74
TOTAL	**174.093**	**128.785**	**302.878**	**121,13**

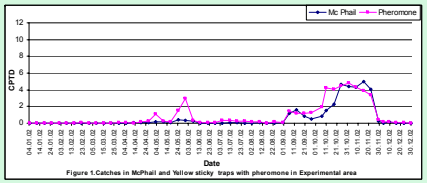

Figure 1.Catches in McPhail and Yellow sticky traps with pheromone in Experimental area

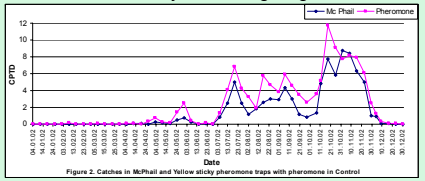

Figure 2. Catches in McPhail and Yellow sticky pheromone traps with pheromone in Control

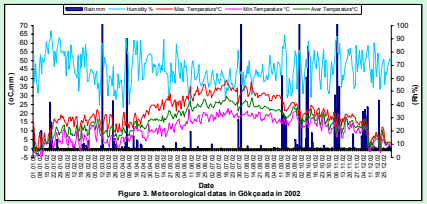

Figure 3. Meteorological datas in Gökçeada in 2002

Table 2. Effectiveness of combination mass-trapping technique and releasing of *P.concolor*

Parcel / Date	Damage %											
	Experimental area						Neighboring			Control		
	Core			Buffer								
	tree	ground	mean	tree	ground	mean	tree	ground	mean	tree	ground	mean
05.09.02	0,5	-	-	1,3	-	-	2,0	-	-	3,36	-	-
18.09.02	1,06	1,15	1,10	2,13	2,01	2,07	5,0	4,93	4,96	8,0	10,95	9,47
02.10.02	1,2	1,53	1,36	2,9	3,33	3,11	6,1	5,1	5,6	12,3	18,7	15,5
24.10.02	11,3	14,4	12,85	14,8	25,26	20,03	20,13	26,47	23,3	36,2	44,5	40,35
20.11.02 Harvest	12,35	15,8	**14,07**	16,12	28,16	**22,14**	39,9	48,71	**44,30**	81,0	94,2	**87,6**
Efficiency %			**83,93**			**74,72**			**49,42**			

RESULTS AND DISCUSSION

When the olive fly trap catches is reviewed (Figs. 1 and 2) it is seen that the population shows the highest tendency continuously two months from the early October to early December. Within this period, CPTD were 31,46 and 27,36 in yellow sticky traps with pheromone and McPhail traps in the experimental area. The catches were 62,12 and 49,34 in control area indicating two times more catch. Meteorological data shows that the relative humidity was between 60-80% during the year of 2002,by indicating regular rain from early September till the end of December and average temperature was around 15ºC from beginning September to end of the November (Fig.3). These conditions probably caused a delay in the turn of the color of fruits to dark and available of green fruits increased the damage rate especially in the second half of October. As pointed out by Cirio (1971) ***B. oleae*** females tends to lay their eggs in green olives where no egg have previously been laid.

Damage rates in core and buffer zones in experimental area average was 18,10%. Combined efficiency of parasitoid releases and mass-trapping technique was estimated to be average 79,32% (table 2).

In our situation, Gökçeada Island where we have conducted our experiments ecological farm techniques have been undertaken for two years. Consequently although 2002 was a full production year from the olive growing standpoint, since the growers applied no chemical treatment, favorable meteorological conditions caused important damages in October and November. Under this circumstances quite satisfactory effectiveness was obtained (79,32%). If control measures had been applied in neighboring areas to prevent the pest from possible moving to our experimental area better results might have been a probability.

Acknowledgement

The author is much thankful to Jorge Hendrich, project coordinator, Head of Insect Pest control Section, Joint FAO/IAEA, for this valuable support and interest during the project that is number 10783/TUR.

IIAEA-CN-131/16P

Inherited Sterility for Area-Wide Control of Corn Stem Borer, *Sesamia cretica* (Led.)

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Results

Table 1. Inherited Sterility of *Sesamia cretica*, F1 and F2 Adults Emerged from Irradiated Parental Male Pupae with 200 Gray and Mated with Unirradiated Female.

Generation	Mating type (♀×♂)	Mating Percent	♂ Longevity (days) (Mean ± SD)	♀ Longevity(days) (Mean ± SD)	Eggs No./♀ (Mean ± SD)	Hatching Percent
Parent	N × N	92.6 a	6.7 ± 2.3 a	9.2 ± 2.3 a	128.0 ± 37.7 a	92.6 a
	N × T	88.9 a	5.6 ± 1.5 a	7.0 ± 2.2 a	104.1 ± 32.8 b	52.2 b
F1	1 × 1	90.9 a	6.1 ± 2.1 a	7.5 ± 2.1 a	112.6 ± 32.3 a	89.9 a
	1 × 2	83.3 a	5.9 ± 1.8 a	8.3 ± 1.1 a	112.0 ± 38.5 a	22.8 b
	2 × 1	83.3 a	6.4 ± 1.1 a	7.6 ± 2.0 a	96.8 ± 42.3 b	12.7 c
	2 × 2	76.7 a	5.7 ± 2.0 a	6.3 ± 2.3 a	80.0 ± 31.1 b	00.0 d
F_2	a × a	90.5 a	5.1 ± 2.0 a	6.7 ± 2.3 a	122.3 ± 30.6 a	91.8 a
	a × b	90.5 a	6.0 ± 1.5 a	7.7 ± 1.5 a	117.4 ± 25.7 a	41.4 c
	b × a	85.7 a	5.9 ± 2.1 a	6.8 ± 2.1 a	102.2 ± 38.7 b	50.2 b
	b × b	80.0 a	3.9 ± 1.1 a	6.2 ± 1.5 a	54.4 ± 35.1 c	35.0 b
	a × c	83.3 a	5.4 ± 1.6 a	8.0 ± 1.1 a	81.7 ± 28.2 b	44.7 c
	c × a	83.3 a	6.0 ± 1.9 a	6.3 ± 1.8 a	48.3 ± 37.1 c	59.2 d

Numbers within a column followed by the same letter are not significantly different according to DMRT (P?0.05).
N= Unirradiated parental adults, T = Irradiated parental adults.
1 = F1 adults emerged from N × N, 2 = F1 adults emerged from T × N.
A = F2 adults emerged from 1 × 1, B = F2 adults emerged from 2 × 1, C = F2 adults emerged from 1 × 2.

Table 2. Inherited Sterility of *Sesamia cretica*, F1 and F2 Adults Emerged from Irradiated Parental Male Pupae with 250 Gray and Mated with Unirradiated Female.

Generation	Mating type (♀×♂)	Mating Percent	♂ Longevity (days) (Mean ± SD)	♀ Longevity(days) (Mean ± SD)	Eggs No./♀ (Mean ± SD)	Hatching Percent
Parent	N × N	92.0 a	6.7 ± 2.3 a	9.3 ± 2.3 a	128.0 ± 37.7 a	92.6 a
	N × T	93.1 a	5.8 ± 1.5 a	7.6 ± 2.0 a	93.5± 31.8 b	34.3 b
F_1	1 × 1	90.0 a	6.1 ± 2.1 a	7.5 ± 2.1 a	112.6 ± 32.3 a	89.9 a
	1 × 2	85.2 a	6.6 ± 1.6 a	7.7 ± 1.6 a	116.9 ± 35.4 a	15.8 c
	2 × 1	89.5 a	6.4 ± 1.4 a	7.6 ± 1.9 a	102.6 ± 48.8 a	00.0 c
	2 × 2	90.0 a	5.3 ± 1.9 a	6.9 ± 1.8 a	51.1 ± 25.9 b	00.0 c
F_2	a × a	90.5 a	5.1 ± 2.0 a	6.7 ± 2.3 a	122.3 ± 30.6 a	91.8 a
	a × b	84.2 a	4.9 ± 1.2 a	7.1 ± 1.1 a	102.8 ± 47.1 a	29.1 c
	b × a	100.0 a	6.4 ± 1.2 a	6.7 ± 0.9 a	112.3 ± 30.4 a	39.8 b
	b × b	86.7 a	5.1 ± 1.2 a	6.9 ± 1.1 a	66.1± 29.7 b	27.0 c

Numbers within a column followed by the same letter are not significantly different according to DMRT (P?0.05).
N= Unirradiated parental adults, T = Irradiated parental adults
1 = F1 adults emerged from N × N, 2 = F1 adults emerged from T × N.
A = F2 adults emerged from 1 × 1, B = F2 adults emerged from 2 × 1.

Table 3. Inherited Sterility of *Sesamia cretica*, F1 and F2 Adults Emerged from Irradiated Parental Male Pupae with 300 Gray

Generation	Mating type (♀×♂)	Mating Percent	♂ Longevity (days) (Mean ± SD)	♀ Longevity(days) (Mean ± SD)	Eggs No./♀ (Mean ± SD)	Hatching Percent
Parent	N × N	92.0 a	6.7± 2.3 a	9.2± 2.3 a	128.0 ± 37.7 a	92.6 a
	N × T	100.0 a	6.1 ± 0.9 a	7.1 ± 1.4 a	127.6 ± 11.4 a	29.7 b
F_1	1 × 1	90.0 a	6.1 ± 2.1 a	7.5 ± 2.1 a	112.6 ± 32.3 a	89.9 a
	1 × 2	76.7 a	5.2 ± 1.8 a	7.5± 2.0 a	105.6 ± 49.2 a	00.0 b
	2 × 1	83.3 ab	6.2 ± 1.6 a	7.2 ± 1.9 a	120.1 ± 44.4 a	00.0 b
	2 × 2	51.9 b	5.2 ± 1.8 a	7.0 ± 1.2 a	67.6 ± 45.2 b	00.0 b

Numbers within a column followed by the same letter are not significantly different according to DMRT (P?0.05).
N= Unirradiated parental adults, T = Irradiated parental adults.
1 = F1 adults emerged from N × N, 2 = F1 adults emerged from T × N.

Table 4. Mating Competitiveness Values of Partially Sterile Adult Males of Corn Stem Borer *Sesamia cretica*, Irradiated as 8-10 Days Old Pupae with 250 Gray.

I♂	N♂	N♀	X No. of Eggs/♀	% Hatching	% Unhatched Egg	%expected	CV
0	1	1	148.2 ± 54.4 a	91.6 a	8.4	-	-
1	1	1	127.1±45.7 a	78.3 ab	21.7	38.4	0.56
2	1	1	125.4 ± 46.0 a	64.1 bc	35.9	48.4	0.74
3	1	1	125.9 ± 46.6 a	56.7 bcd	43.3	53.4	0.81
4	1	1	141.3 ± 59.3 a	48.4 cd	51.6	56.4	0.91
5	1	1	141.7 ± 49.7 a	45.7 cd	54.3	58.4	0.93
6	1	1	145.4 ± 46.8 a	42.4 cd	57.6	59.8	0.96
7	1	1	143.3 ± 45.9 a	38.7 d	61.3	60.9	1.01
8	1	1	156.1 ± 48.4 a	34.8 d	65.2	61.7	1.06
9	1	1	131.0 ± 55.7 a	40.0 cd	60.0	62.4	0.96
10	1	1	157.0 ± 39.1 a	35.4 d	64.6	63.0	1.03
1	0	1	128.2 ± 42.8 a	31.6 d	68.4	-	-

Numbers within a column followed by the same letter are not significantly different according to DMRT (P?0.05).
I= Irradiated, N=Normal, CV=Competitiveness value .

Table 5: Mating Competitiveness Values of Partially Sterile Adult Males of Corn Stem Borer *Sesamia cretica*, Irradiated as 8-10 Days Old Pupae with 300 Gray.

I♂	N♂	N♀	X No. of Eggs / ♀	% Hatching	%Unhatched Eggs	% Expected	CV
0	1	1	148.7±36.5 a	91.8 a	8.2	-	-
1	1	1	142.4±55.9 a	71.7 abc	28.3	41.95	0.67
2	1	1	150.1±51.6 a	76.4 ab	23.6	53.2	0.44
3	1	1	140.9±42.9 a	63.4 bcd	36.6	58.8	0.62
4	1	1	147.1±48.2 a	67.3 bcd	32.7	62.2	0.53
5	1	1	140.0±57.9 a	48.2 bcd	51.8	64.45	0.80
6	1	1	119.7±37.7 a	44.5 de	55.5	66.06	0.84
7	1	1	140.4±46.6 a	38.5 de	61.5	67.26	0.91
8	1	1	138.8±56.3 a	36.7 de	73.4	68.2	1.08
9	1	1	149.8±55.9 a	28.1 e	71.9	68.95	1.04
10	1	1	127.7±42.5 a	28.2 e	71.8	69.56	1.03
1	0	1	123.9±49.2 a	24.3 e	75.7	-	-

Numbers within a column followed by the same letter are not significantly different according to DMRT (P?0.05).
I= Irradiated, N=Normal, CV=Comparativeness value .

Table 6 : Average Infestation Percent of Field Caged Corn Plants After Releasing of Partially Sterile Adult Males of Corn Stem Borer, *Sesamia cretica*, Irradiated as 8-10 Days Old Pupae with 250 or 300 Gray.

Dose (Gray)	Ratio of Released Adults/Cage			Average Plants/Cage	Average Infested Plants	Average Infestation Percent
	I♂	N♂	N♀			
0	0	3	3	46.7	18.3	39.2 a
25	12	3	3	43.0	3.3	13.0 b
30	15	3	3	40.3	2	5.0 b

Numbers within a column followed by the same letter are not significantly different according to DMRT (P?0.05).
I= Irradiated, N=Normal.

Conclusion

A. Inherited Sterility

1) Irradiation of male pupae did not affect significantly spermatophores transfer to the females, and this fact was noticed also in F1 and F2 adults.
2) There were no significant differences in average male longevity at parental, 1st and 2nd generations.
3) At all doses used, there was a noticeable reduction in fecundates at all mating types of 1st and 2nd generations.
4) There was significant reduction in eggs hatching at all mating types of 1st and 2nd generations.
5) It was clear that 1st generation adults were more sterile than their parents at all irradiation levels.
6) The level of induced sterility in 1st generation females was higher than that of the males.
7) The males and females of the 2nd generation were less sterile than that of the 1st generation, but still more than that of the parent.
8) The females of the 2nd generation were less sterile than males of the same generation.

B. Mating Competitiveness Studies:

1) Females' fecundities were not significantly affected at all combination of mating types ratios tested for F1 and F2 adults.
2) Hatching percentages were decreased as the ratio of irradiated males to unirradiated ones was increased.
3) The calculated competitiveness values were good at ratio of 4-5 I♂: 1N♂: 1N♀

C. Field Trails:

*** Releasing of partially sterile adult males 4-5 I♂: 1N♂: 1N♀ significantly reduced infestation percentages of corn plants under field conditions.**

Acceptability of irradiated larvae of the Mediterranean flour moth, *Ephestia kuehniella*, by a parasitoid *Venturia canescens*

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INTRODUCTION

Irradiation of lepidopteran larvae with low doses prevents their further development.

Low doses of gamma radiation in the range from 0.1 kGy to 0.5 kGy cause that irradiated moth larvae remain alive for several weeks.

Irradiation with doses up to 0.5 kGy did not cause 100% mortality within 60 days after the treatment.

This can be advantageous if the irradiated larvae are stored for several weeks without decline in their suitability as hosts. Irradiated and parasitized by *V. canescens* of stored-product moths could be a final product for sale.

AIM OF STUDY

The main aim of the study project is to elaborate the utility of irradiation techniques in improving the production and realizing of parasitoids of high quality.

The suitability of irradiated larvae of the Mediterranean flour moth, *Ephestia kuehniella* (Hübner), as host for *V. canescens* parasitoids is the most important study aspect.

METHODS

Groups of old larvae (the "wandering" phase of the fifth instar, L_5) were selected from the laboratory colony, placed into jars (100 larvae per jar) with food, and irradiated with a dose of 0.0 (control), 0.2 or 0.4 kGy of gamma radiation (dose rate ca. 30 Gy/min).

After the irradiation treatment, moth larvae (100 larvae per treatment) were placed into little sacks made from gauze (25 larvae per sack) and provided a little portion of food (wheat germs). These sacks were introduced into a glass cabin with one–day old parasitoids (about 50 wasps), and were exposed to *V. canescens* parasitoids. After 24 hours the larvae were removed from the parasitoids' cabin, released from the sacks, transferred into 130 ml dishes with food (dried wheat germs). Parasitoid emergence was determined.

In a separate experiment, parasitized larvae were stored at the low temperature of 4±1°C for 2, 4 or 6 weeks. After these periods, moth larvae were transferred into a temperature 25±1°C, and the number of adult parasitoids emerged was recorded. Samples of 10 wasp adults emerged from irradiated host larvae were placed into glass cabins provided with gauze sacks containing ca. 100 larvae of *E. kuehniella* each. The oviposition time and number of ovipositing wasps were observed, recorded and compared to the control group.

After 24 hours larvae were removed from the cabin, released from the sacks and transferred into 130 ml dishes with food (dried wheat germs), and kept at a temperature of 25±1°C. Fecundity and longevity of adult parasitoids emerged from larvae that were irradiated and stored in cool was compared to the control.

RESULTS

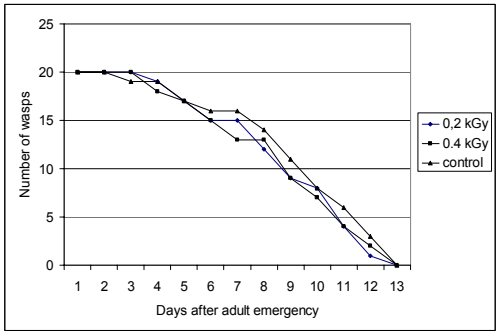
Number of adults of *V. canescens* emerged from moth larvae irradiated with a dose of 0.4 kGy were similar as the adults that emerged from untreated hosts (control). Moth larvae irradiated with a dose of 0.2 kGy yielded more parasitoids that the non-irradiated larvae.

TABLE 1.
Number of adults of *V. canescens* emerged from moth larvae irradiated with a dose of 0.2 or 0.4 kGy compared with non-irradiated (control)

Dose (kGy)	Mean number of parasitoid adults emerged from host larvae*
0.2	73.8b
0.4	58.1a
0.0 (control)	58.8a

Longevity of *V. canescens* adults emerged from host larvae irradiated with a dose of 0.2 or 0.4 kGy was similar to that of the adults that emerged from untreated hosts (control) (Fig. 1).

Figure 1. Longevity of *V. canescens* adults that emerged from irradiated and non-irradiated (control) larvae of the Mediterranean flour moth, *E. kuehniella*.



Body mass of *V. canescens* adults emerged from moth larvae irradiated with a dose of 0.2 or 0.4 kGy of gamma radiation was very similar to that of the adult wasps emerged from non-irradiated larvae (Table 2).

TABLE 2.
Body mass (mg) of *V. canescens* adults emerged from *E. kuehniella* larvae irradiated with a dose of 0.4 or 0.6 kGy of gamma radiation as compared to the control (wasps emerged from non-irradiated hosts)

Data	Dose (kGy) of gamma radiation		
	0 (control)	0.2	0.4
Number of insects weighted	100	100	100
Arithmetic mean	3,4358	3,3854	3,3862
Standard deviation	3,4723	3,4214	3,4235
Standard error	0.3472	0.3421	0.3423

TABLE 3.
Number *V. canescens* adults emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and cool stored for 2, 4, and 6 weeks as compared to the control (wasps emerged from non-irradiated hosts)

Dose of gamma radiation	Storage period (weeks) at low temperature*		
	2	4	6
0.0	38.7a	29.1a	7.5a
0.2	41.6a	26.9a	8.3a
0.4	25.0b	15.0b	5.1b

Storage period of irradiated and parasitized larvae at low temperature of 4°C reduced significantly the number of parasitoids emerged. Only a few parasitoids completed their development after 6 weeks' cool storage. Number of *V. canescens* adults emerged from moth larvae irradiated with a dose of 0.2 kGy and stored at a low temperature was similar to the control. Irradiation treatment of host larvae with a dose of 0.4 kGy reduced emergence of parasitoids (Table 3).

TABLE 4.
Fertility of *V. canescens* adults emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and cool stored for 2, 4, and 6 weeks as compared to the control (wasps emerged from non-irradiated hosts)

Dose (kGy)	Mean number of parasitoid adults emerged from host larvae after		
	2 weeks	4 weeks	6 weeks
0.0	55.1a	57.2a	41.6a
0.2	62.1b	56.4a	37.5a
0.4	54.3a	42.3b	25.2b

Number of progeny of wasp that emerged from larvae irradiated with 0.2 kGy and stored at a low temperature was similar to the control with except of a 2-week storage. In this combination treatment, more progeny was noted than in the control.

Less parasitoid progeny was obtained from adults emerged from irradiated larvae and cool stored for 6 weeks (Table 4).

V. canescens females that emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and then cool stored for 2 and 4 weeks were attracted by host larvae, and parasitoids of all combination treatments oviposited readily.

TABLE 5
Oviposition activity of *V. canescens* parasitoids that emerged from moth larvae irradiated with a dose of 0.2 kGy or 0.4 kGy of gamma radiation and cool stored for 2 and 4 weeks as compared to the control

Storage time at low temperature	Dose (kGy)	Number of ovipositing parasitoids		
		after 2 minutes	after 5 minutes	after 10 minutes
2 weeks	0.0	4	5	9
	0.2	2	4	8
	0.4	3	3	9
4 weeks	0.0	2	6	9
	0.2	1	4	10
	0.4	3	4	8

CONCLUSIONS

1. Number of wasp adults emerged from moth larvae irradiated with a dose of 0.2 or 0.4 kGy were similar to the adults that emerged from untreated hosts.
2. Longevity of *V. canescens* adults emerged from irradiated was not affected.
3. Body mass of parasitoid adults that emerged from moth larvae irradiated with a dose of 0.2 or 0.4 kGy was similar to that of the adults that emerged from untreated hosts.
4. Irradiated larvae of the Mediterranean flour moth, *E. kuehniella*, are accepted by and are suitable to *V. canescens* wasps.
5. Irradiated larvae may be used for mass-culture of *V. canescens*.

Maggot Infestation (Myiasis) in Humans from Western Jamaica (1999-2003):

has the National Screwworm Eradication Programme (NSEP) been a success?

IAEA-CN-131/18 P

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Abstract

Objective: To determine the distribution of cases of human myiasis admitted to the Cornwall Regional Hospital (CRH), Jamaica between 1999 and 2003, following the inception of the National Screwworm Eradication Programme (NSEP) in 1998, and the risk factors associated with the condition.

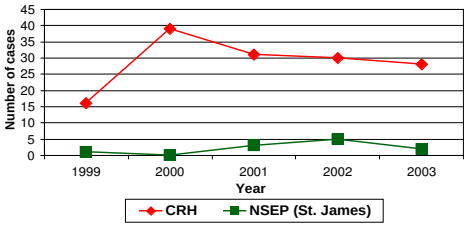
Method: A total of 144 cases of myiasis were retrieved from the database of the department of Medical Records at the CRH for the years 1999 to 2003. A data extraction form was devised to review the circumstances of each case. The data were analyzed using EpiInfo version 6.

Results: Of 144 cases, 54.9% female and 45.1% male. The largest groups were <10yrs 52.8% [76/144 (M-21, F-55)] and 60yrs and over 18.8% [27/144 (M-16, F-11)] (p<0.0001). Case distribution for the years 1999-2003 showed 16, 39, 31, 30, and 28 cases respectively. Three-quarters (74.6%) of all cases affected the scalp/head, one-fifth (20.3%) affected the lower limbs. Ninety-six percent of <10yrs had scalp/head myiasis (p<0.0001; OR= 23.29; CI: 6.14<OR <104.11). Two-thirds (66.6%) of 60yrs and over had lower limb myiasis (p<0.0001; OR= 19.09; CI: 6.20<OR<61.12). Mean duration of treatment was 3.5 days (SD = +/–1.4 days) and 69.7% required hospitalization for 7 or more days. There was no difference in duration of treatment for myiasis or in length of hospitalization in relation to method used to eliminate maggots. Risk factors identified included tinea capitis for myiasis of the scalp/head (p<0.0001) and diabetes mellitus for lower limb myiasis (p<0.0001; OR= 14.48; CI: 2.37<OR<133.25).

Conclusion: Human myiasis remains a public health issue in western Jamaica with no significant decrease in the number of cases admitted to the CRH since 1999. It is recommended that this zoonosis become a Class 1 Notifiable disease to the Ministries of Health and of Agriculture, because of the existing NSEP.

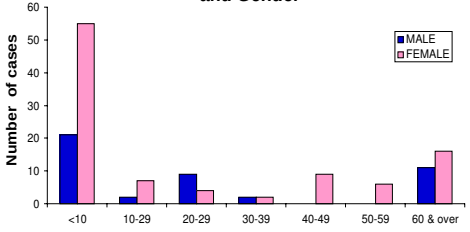
RESULTS

Fig. 1 Cases of Human Myiasis at CRH vs. NSEP (St. James)



- Human myiasis cases remained unchanged for 5 years following the NSEP. There was no annual variation (p = 0.22).
- Poor correlation between hospital data and NSEP figures, indicated significant underreporting.

Fig. 2 Distribution of Cases of Myiasis by Age and Gender

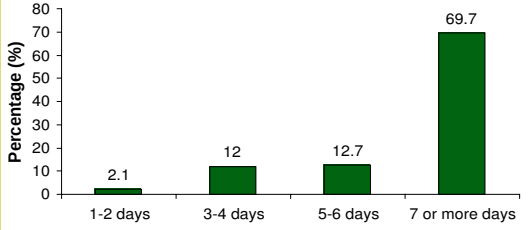


- The young and the elderly were most at risk for maggot infestation.
- Females were marginally more affected more than males.

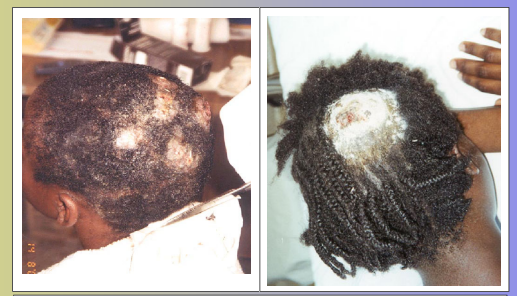
Fig 3. Sites on body affected by myiasis

- The scalp/head was the most affected site, followed by the lower limb.
- Children <10yrs and those with tinea capitis were mostly affected in the head/scalp. Patients >60 yrs and diabetics were predisposed to lower limb myiasis. These associations were significant (p<0.0001).

Fig 4. Duration of Hospitalization



- One-third (33.9%) of patients required 5 or more days to eliminate maggots with a mean duration of 3.5 days (SD = +/– 1.4 days). Hospital admission for the majority of patients beyond this time was most likely the result of wound care.



Pictures of Scalp Myiasis in Jamaican Children (Source: NSEP)

CONCLUSION

- The problem of human myiasis continues to be a public health concern in western Jamaica.
- There has been no decline in cases of human myiasis seen at the CRH since 1999, following the inception of the NSEP.
- Poor support of NSEP from medical services, as there was failure in reporting human myiasis cases. No accurate figure for human screw-worm myiasis, as specimens not submitted for identification.
- Persons at the extremes of age (young & old) were mostly affected.
- Patients often had comorbidity which predisposed them to maggot infestation.
- There was considerable time spent for in hospital treatment, following the diagnosis of myiasis.
- A protocol is needed for the management of active human myiasis. This must include the classification of “MYIASIS” as a Class 1 Notifiable Disease, to ensure the future success of the NSEP.

Towards the development of lure and kill systems for *Ceratitis capitata* and *Bactrocera oleae* combining food lures and coloured spheres

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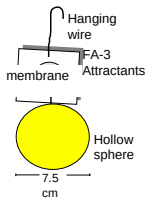
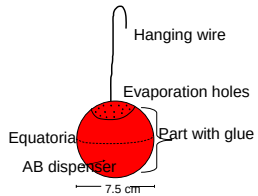
This study aimed at developing lure and kill systems for both the Mediterranean fruit fly and the olive fruit fly. The experiments were conducted in the Greek island of Chios in a citrus and an olive orchard. Sticky coloured spheres of 7.5 cm diameter were combined with food attractants and compared against unbaited spheres and other effective trapping systems for these flies. For the medfly was used a combination (referred to as FA-3) of the attractants ammonium acetate, putrescine and trimethylamine by BioLure. For the olive fruit fly ammonium bicarbonate dispensers (AgriSense) were tested against an aqueous solution of NuLure. Each experiment was replicated at least 10 times.

Baited Yellow sphere

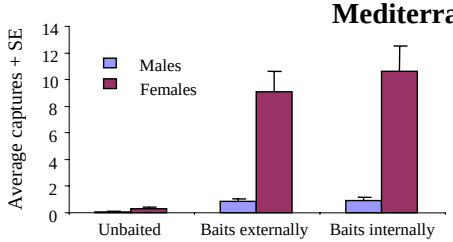


McPhail trap

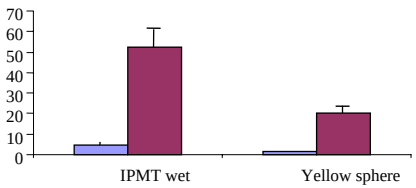
Internally baited red sphere



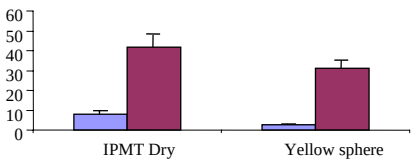
Mediterranean fruit fly



Unbaited spheres against spheres baited internally or externally: Baits increased 10 and 30 time the performance of the spheres for males and females respectively.

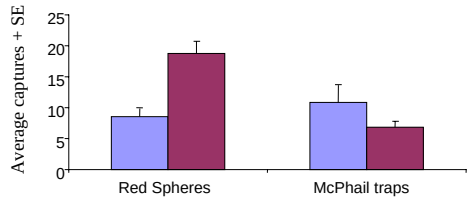


Wet McPhail traps against baited spheres: Wet IPMT traps baited with FA-3, captured 3 times more males and 2 times more females than likewise externally baited yellow spheres.

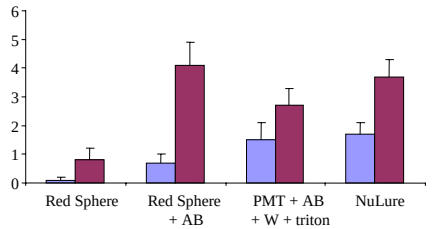


Dry McPhail type traps against baited spheres: Dry IPMT baited traps captured 2 times more males and only 1.5 more females than likewise baited yellow spheres.

Olive fruit fly



Unbaited spheres vs McPhail traps with aqueous solution of ammonium sulfate: Unbaited red spheres were 2 times more attractive for females compared with McPhail traps baited with the conventional lure ammonium sulfate.



Spheres with ammonium bicarbonate (AB) vs McPhail traps with NuLure: Red spheres baited with AB were slightly more attractive for females compared with McPhail traps baited with AB or NuLure.

Conclusions

- FA-3 dispensers increase dramatically the performance of yellow spheres and render them comparable with likewise-baited dry IPMT traps.
- Observations revealed that a high proportion of medflies left the spheres without being captured. This suggests that the performance of baited spheres can be further increased if a more appropriate trapping or killing system is developed.
- The higher performance of red spheres baited with ammonium bicarbonate, compared with traps baited with ammonium bicarbonate and NuLure or with McPhail traps, suggests that baited red spheres can be used as a powerful lure and kill system for the olive fruit fly.

Relevant References

Katsoyannos, B. I., and N. T. Papadopoulos. 2004. Evaluation of synthetic female attractants against *Ceratitis capitata* (Diptera: Tephritidae) in sticky coated spheres and McPhail type traps. Journal of Economic Entomology 97: 21 - 26.

Katsoyannos, B. I., and N. A. Kouloussis. 2001. Captures of the olive fruit fly *Bactrocera oleae* on spheres of different colours. Entomologia Experimentalis Et Applicata 100: 165-172.

Population models for optimising SIT eradication strategies

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Introduction

Successful eradication using the **sterile insect technique** (SIT) relies on (a) optimising the competitiveness of irradiated insects, and (b) implementing a strategy of releases in **space and time** which effectively infiltrates the wild population.
We explored simple general population models with respect to:

- complete vs. inherited sterility
- optimal release frequency in time
- optimal release locations in space.

Releasing irradiated painted apple moth males.

Models

Simple models were developed for the effects of releasing irradiated males on pest insect populations. The models allow for matings between irradiated males, wild moths, and irradiated lineage (IL) moths.
Special cases arise for **complete sterility** (only matings between wild ♂ and wild ♀ are successful), and for **IL-incompatibility** (matings between IL moths, or between irradiated ♂ and IL ♀ are unsuccessful).
Separate models were developed for populations with **discrete** vs **overlapping** generation structure, and the models were parameterised for important Lepidopteran pests from the literature (see table below).
Spatial versions of the models were also developed.
The results could be expressed in terms of the **critical overflooding ratio** Φ_c, the ratio of irradiated to wild males needed to prevent population increase. If Φ_c is exceeded then SIT leads to extinction.

Estimated critical overflooding ratios for some Lepidoptera parameterised from the literature.

Species	Generation structure	Dose (Gy)	Critical overflooding ratio, Φ_c
Painted apple moth (*Teia anartoides*)	overlapping	100	0.64
		160	1.89
Codling moth (*Cydia pomonella*)	discrete	100	1.04
		200	2.04
Diamondback moth (*Plutella xylostella*)	overlapping	200	5.92
Gypsy moth (*Lymantria dispar*)	discrete	100	1.18

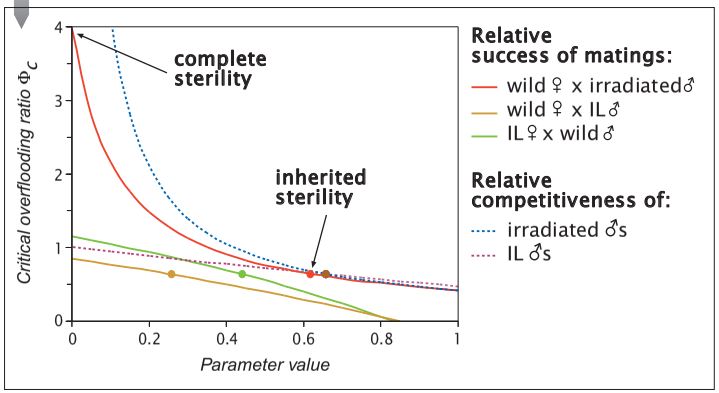
Photos : USDA Forest Service Archives. www.forestryimages.org

Complete vs inherited sterility

The model suggests that as long as there is IL-incompatibility, then inherited sterility is always better than complete sterility.

This is not necessarily so if there is not IL-incompatibility, or if there is fertility recovery in subsequent generations as is seen in some species.

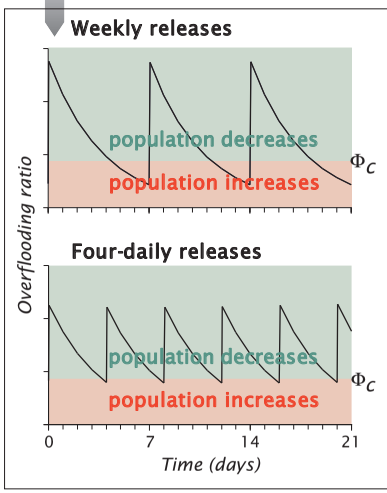
Effects of model parameters on the critical overflooding ratio for the painted apple moth at 100 Gy. Dots show default values.



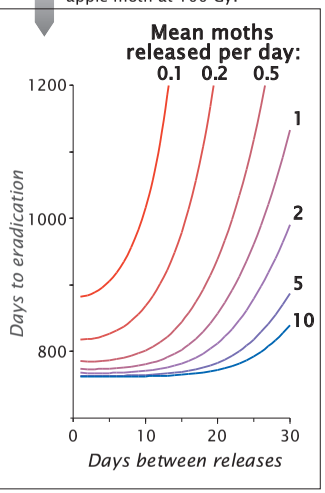
Frequency of releases

For species with overlapping generation structure, the realised overflooding ratio is kept above Φ_c for a greater proportion of the time when releases are small and frequent, rather than large and infrequent.

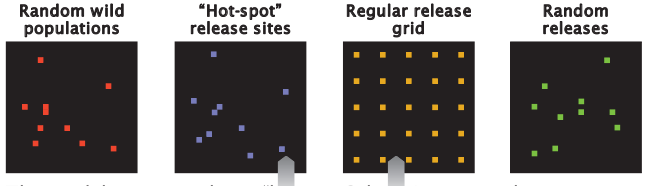
Effects of release frequency on the overflooding ratio.



Effects of release frequency on the time to extinction for painted apple moth at 100 Gy.



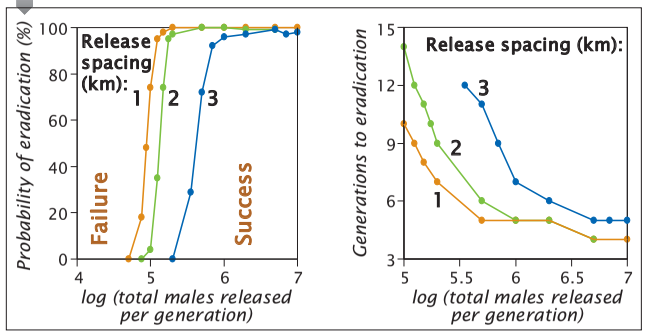
Spatial release strategies



The model suggests that a "hot-spot" release strategy is the most effective, but this relies on knowing the approximate locations of wild populations.

Otherwise, a regular pattern was optimised by releases of fewer males in more sites, rather than more males in fewer sites.

Effects of regular release spacing on the chance of success (left) and time to eradication (right). The model is parameterised for Asian gypsy moth.



Conclusions

- **Inherited sterility is always more effective** than complete sterility when there is irradiated-line incompatibility and no fertility recovery.
- For target species with overlapping generations, **small but frequent releases** often give better control than infrequent large releases.
- Given a certain number of sterile males available per release, the best strategy is to **release close to the wild populations**. If the location of wild populations is not known, then **many small releases, regularly spaced**, are more certain to achieve eradication sooner than few large release sites.
- The models discussed here are also applicable to other SIT target species, such as Diptera.

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agresearch Farming, Food and Health. First.

HortResearch

Dose distribution in pupae treated by gamma irradiation using GEANT4 toolkit

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Detailed dose mapping of the irradiation cell are performed to establish plant operational parameters, such as dose uniformity, source utilization efficiency, maximum and minimum dose positions in pupae and absorbed dose for inducing sterility. This mapping could be obtained by dosimetric experiments as well as by computer calculations.

Monte Carlo method is being widely used to achieve reliable radiation transport calculations for various investigations. The main aim of this work is to validate the use of Monte Carlo code GEANT4 of CERN to predict the dose distribution for measurements carried out with the Tunisian irradiator.

The strategy adopted in this work is to perform the dose mapping using simulation technique in some specific locations in the irradiation cell space. The dose rate was calculated and measured inside pupae, transversally and laterally. Simulation dose rate values are in agreement with measurements.

This agreement permits to use GEANT4 for dose mapping of the irradiation cell to establish plant operational parameters with a very little monitoring of dosimeters, man powers and products. However, some experimental measurements should be performed to check the simulation procedure.

Irradiation plant description

The Tunisian gamma irradiation facility is designed for sterilization of pupae. Gross weight of the irradiator is 8300 kg, main dimensions are L = 2380 mm, W = 960 mm and H = 2350 mm. The sample door is located at 1090 mm from the ground. Source is composed by twenty four cylindrical pencils of Co-60 (radius 7 mm, height 235 mm). The pencils are placed on a circle of diameter 217 mm. Source cage can hold maximum 36 pencils of C0-60. Loaded Co-60 activity must not exceed (18000 ±10%) Ci. The present total activity (2005) is (12300 ±10%) Ci. The source cage is moved down and up by a propel pneumatic drive mechanism. Pencils are stored in a cylindrical container of lead shielded cask protecting the personnel and the environment against gamma radiation (radius = 125-850 mm, height = 250 mm). A cylinder of aluminum (radius 130 mm, height 240 mm) is used to introduce product to be irradiated in source.

Experimental procedure

Dose measurements were carried out using Gammachrome dosimeters in order to validate Monte Carlo predictions. Gammachrome are polymethyl methacrylate (PMMA) routine dosimeters with an overall uncertainty of 6%, at a 95% confidence level, in the range of 5-50 kGy (ISO/ASTM 51276). Determination of absorbed dose was carried out indirectly through spectrophotometric evaluation (Spectronic Genesys 5 UV-VIS spectrophotometer + Kafer KMF30 thickness jauge + Aer'ODE software) of the specific absorbance at an auto-controlled wavelenght. Three measurements of the dose rates were realized in the irradiation cell. The first measurement of dose rates was performed at 7 points along the Z-axis (vertical direction). The second measurement was realized in 13 points along the Y-axis (horizontal direction). The third measurement is illustrated in figure 4 which indicates the location of 36 dosimeters around a circle of a diameter 130 mm at z = 0 mm, the angle between tow consecutive dosimeters is 10°.

Validation

GEANT4 is a toolkit that simulates accurately the passage of particles through matter. It contains a complete range of functionalities including tracking, geometry, physics models and hits. This toolkit based on object-oriented technology is implemented in the C++ programming language. The platform used for GEANT4 version 6.1 code was a Linux (Red Hat 9.0) Workstation running with 512 MB RAM and 2.30 GHz CPU.

Comparison between predicted and experimental dose rate along the vertical (horizontal) direction of the irradiation cell shows in figure 1 (2). In Both cases detailed behaviors are reproduced by simulation with a good accuracy.

As shown in Figure 3 experimental dose rate around a circle at z = 0 mm, is reproduced by the Monte Carlo simulation with a good agreement.

Such satisfactory agreement confirms that GEANT4 reproduces accurately the dose distribution in the irradiation cell.

Dose mapping

The dose rate was simulated at 140 points at the horizontal plans (z=-100 mm, z=0 cm, z=100 mm) in the source rack. Figures 5, 6 and 7 show dose rate mapping obtained in this plans using GEANT4. The maps are obtained by interpolation of dose rate values.

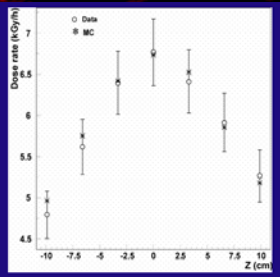

Figure 1 : Simulated (MC) and measured (Data) dose rate along the vertical axis .

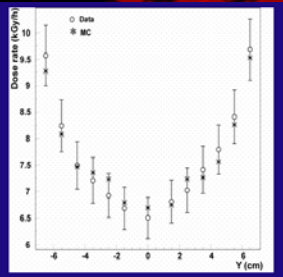

Figure 2 : Simulated (MC) and measured (Data) dose rate along horizental axis.

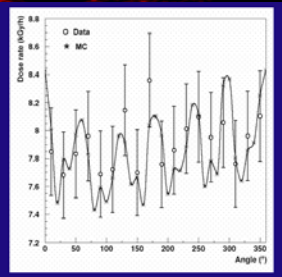

Figure 3 : Simulated (MC) and measured (Data) dose rate along perimetre of circle of radius 65 mm.

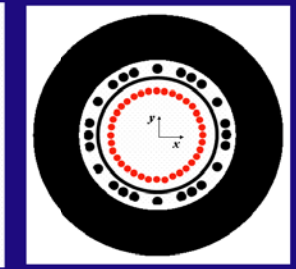

Figure 4 : 2D figure of the Tunisian irradiator for sterile insects. Pencils are represented in black, dosimeters are in red.

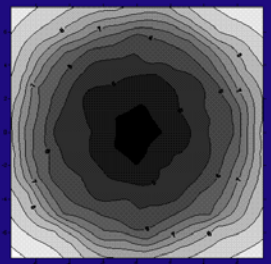

Figure 5 : Simulated dose rate maps for the horizontal plane at z = -100 mm.

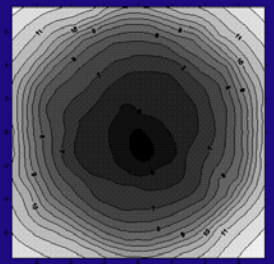

Figure 6 : Simulated dose rate maps for the horizontal plane at z = 0 mm.

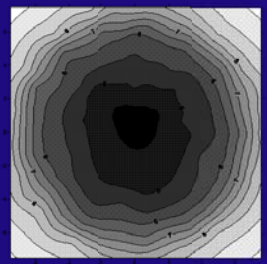

Figure 7 : Simulated dose rate maps for the horizontal plane at z = 100 mm.

Sex chromosome pairing and heterochromatin body appearance in *Cydia pomonella* females.

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Introduction

Codling moth, *Cydia pomonella* L. (CM) is the most economical insect pests of apple tree. The sterile insect technique (SIT), and its variant the inherited sterility technique (IST), are considered as important components of an approach to insect control and their use against *C. pomonella* populations has been suggested. The efficiency of SIT against CM could be significantly improved by releasing males only. To date, the only genetic sexing available in Lepidoptera is based on construction of balanced lethal (BL) strains. Such strains have been constructed in *Ephestia kuehniella* (Marec, 1991) and *Bombyx mori* (L.) (Strunnikov 1975).

When these males mate with wild females, the F1 females will die during their early developmental stage (egg stage), while F1 males will be able to survive. Therefore, the lack of females will directly lead to reduction in pest population. This could be illustrated as follows:

$$Z^{l1}Z^{l2}\,♂ \;X\; ZW\,♀$$

$$\downarrow$$

F1 $\quad Z^{l1}Z : Z^{l1}W : Z^{l2}Z : Z^{l2}W$

Sex ratio $\quad 1\,♂ : 0\,♀ : 1\,♂ : 0\,♀$

The most essential requirements for the construction of such stain is the induction and isolation of translocated females where a Z-chromosome is translocated onto W chromosome, which called T(W;Z) translocation. Following special breeding program between $Z^{l1}Z^{l2}$ males and T(W;Z) females a female with one recessive lethal mutation T(WZ/Z^{l1}) or T(WZ/Z^{l2}) could be obtained. To keep the balanced lethal strain (BLS) and establish the required colony, the $Z^{l1}Z^{l2}$ males have to be crossed with their sisters. The following illustration shows that:

$$Z^{l1}Z^{l2}\,♂ \;X\; TWZ/Z^{l1}\,♀$$

$$\downarrow$$

F1 $\quad Z^{l1}Z^{l1} : TWZ/Z^{l1} : Z^{l2}Z^{l1} : TWZ/Z^{l2}$

Sex ratio $\quad 0\,♂ : 1\,♀ : 1\,♂ : 1♀$

The main objective of our current study was to investigate the sex chromatin body and identification of sex chromosomes. Such study could help in detection and isolation of induced T(W;Z) translocations which are essential in developing BLS in CM.

Materials and methods:

Newly emerged adult females were irradiated at different doses: 20 and 30 Gy. Twenty five females at each experimental dose were used. Twenty five newly emerged adult females were used as the control. Irradiated and unirradiated females were singly paired with 1-d-old males. Females and males were kept together until death. Eggs were removed daily, counted, and left to determine the percentage of egg hatch (female fertility). All newly hatched larvae from irradiated and unirradiated females were fed individually on artificial diet. The number of emerged adults and the sex ratio of F_1 progeny were determined.

F_1 Generation

Newly emerged F_1 females were singly crossed with normal males. Immediately after death, females which laid a sufficient number of eggs were dissected to detect the appearance of the sex-heterochromatin body in the Malpighian tubule cells.

All newly hatched larvae (F_2) were fed on waxed potato slices to form specific family lines at each tested dose. Before pupation, a number of female last instar larvae (n=4-5) from each constructed line were dissected and their ovaries were taken. Chromosomal analysis during pachytene stage was carried out to identify the status of the W chromosome. After emergence, the number of emerged F_2 individuals and the sex ratio of F_2 progeny were determined for each constructed line.

W chromatin appearance

To study the appearance of W chromatin in progeny of normal and irradiated females, highly polyploid nuclei of Malpighian tubule cells were used. The Malpighian tubules were dissected from female last instar larvae and adults and fixed in Carnoy,s fixative (ethanol :chloroform : acetic acid 6:3:1) for 2 min (Truat et al. 1986; Marec and Traut 1994). The tubules were mounted in lactic acetic orcein for 5 min, and then inspected under a light microscopic (LM).

To detect the presence of sex-heterochromatin in *C. pomonella* male, highly polyploid nuclei of Malpighain tubule cells were taken from male last instar larvae and adults. Dissection, fixation and inspection were carried out as described with the female.

Chromosomal analysis under a LM.

For chromosomal analysis of the sex chromosome bivalent, spread preparations of pacytene oocytes were made from progeny of normal and irradiated as mentioned by Marec and Traut (1994). In brief, ovaries of female last instar larvae were dissected and fixed in freshly prepared Carnoy,s fixitive for 30 min .Then the ovaries were transferred on a slide, shortly before drying a drop of 60% acetic acid was added and the ovaries completely torn into pieces with fine tungsten needles. The slide was then placed on a heating plate at 45 $_{o}$C and the drop was moved for some millimeters on the slide by pushing it with a needle. Afterwards, it was allowed to settle for ½ min and then moved again and so on. This procedure was repeated for 5 min until the acetic acid evaporated.

Then the preparation was stained and mounted in lactic acetic orcein for 5 min. The cover glass was sealed with nail polish. Morphology of the sex chromosomes was examined in phase contrast micrographs.

Results

Appearance of W chromatin

Our observations showed that when highly polyploidy nuclei of F_1 normal (control) females' larvae were inspected each nucleus had a single spherical W chromatin body (Fig 1). Sex-heterochromatin bodies were not observed when highly polyploidy nuclei of normal male larvae were examined (Fig 1). There were no differences between last instar larvae and adults of both sexes in the presence and the appearance of W chromatin body.

Regardless of applied dose, polyploid nuclei of F_1 females manifested various shapes of W chromatin body. Single normal or abnormal W chromatin body could be seen in Malpighian tubule nuclei depending on the applied dose of gamma irradiation. Therefore, according to the appearance of W chromatin F_1 females were classified in four different lines: normal, elongated, fragmented and dispersed lines (Figs.3a, 4a and 5a)

Chromosomal analysis under a LM:

From F_1 and F_2 progenies 4 to 5 larval females of each line were inspected to analyze the pachytene chromosome sets. The results showed that in normal female (control) pachytene set there were 28 bivalents. There were 27 autosomal bivalents; each homolog bivalent exhibits a homologous chromomere and interchromomere pattern (Fig. 2). The sex chromosome bivalent ZW was easily distinguished in all pachytene chromosome sets; and it was very similar to that of *E. kuehniella* (Marec and Traut 1993). The W chromosome forms a deeply-stained heterochromatic thread while the Z chromosome displays a chromomere/interchromomere pattern. While Z chromosome was longer than W and in some cases it was twisted along W axis (Fig 2).

In sex chromosome bivalents of females of elongated lines a clear translocated segment of Z chromosome in the terminal part of W chromosome was observed (Fig 3b). The translocated Z segment was homologously paired with the corresponding region of Z chromosome.

When sex chromosome bivalents of the females of fragmented line were inspected, two clear visible fragments, unequal in length, of W chromosomes were observed (Fig 4b).

In females of dispersed line, the deeply stained heterochromatic thread (W chromosome) was divided into several parts (Fig 5).

The results showed that F_1 females with elongated W chromatin bodies were detected at all applied doses (Fig. 6). However, in F_1 progeny of 20 Gy-irradiated female parents the percentage females with elongated W bodies was significantly higher than that of 30, and 50 Gy-irradiated female parents. Nevertheless, there was no significant difference in percentage females with elongated bodies between F_1 females of 20 and 30-Gy-irradiated female parents. The results illustrated that the females with small W bodies were observed only in F_1 progeny of 30 and 100 Gy-irradiated female parents (Fig 4). The percentage females with small bodies at 100 Gy was significantly higher than those at 30 Gy. The results illustrated that the females with fragmented W bodies were observed when female parents were irradiated at high doses (50,100and 150 Gy). However, the percentage of females with fragmented bodies at 150 Gy was significantly higher than those at 50 and 100 Gy (Fig 4).

Conclusion

The forgoing study showed that the sex heterochromatin could be very easily observed in polyploid cells; therefore it could be effectively used in: 1) sex identification during early stage of larvae developmental time; 2) detecting sex chromosome aberrations after irradiation and mutagen treatments; and 3) cytogentic marker for isolation of translocated females T(W;Z). Moreover, it should be emphasized that our isolated T(W;Z) translocated lines could be used in the construction of a balanced lethal strain against *C. pomonella*.

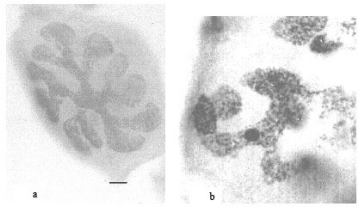

Fig 1: Highly polyploid of the Malpighian tubule cells of *C. pomonella*. a. From wild adult male without sex chromatin. b. From wild adult female showing sex-chromatin body (arrow). Bar = 10 μm.

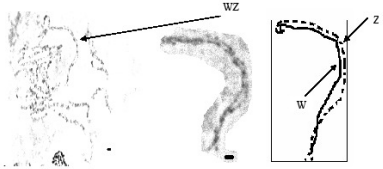

Fig 2. Spread pachytene oocyte complement of C. pomonella larval female, showing 28 and WZ bivalents stained with orcein. Bar= 1μm.

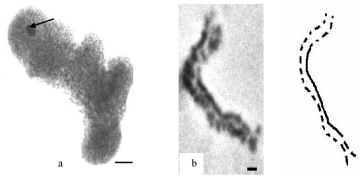

Fig 3. a, Highly polyploid of the Malpighian tubule cells of F1 larval female of irradiated *C. pomonella* females with elongated sex-chromatin body Bar= 10μm. b, WZ chromosome bivalent from oocyte of F_1 larval female of irradiated *C. pomonella* females with elongated sex-chromatin body Bar= 1μm.

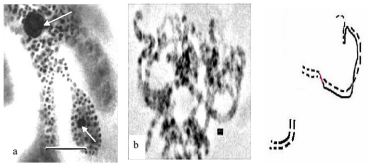

Fig 4. a, Highly polyploid of the Malpighian tubule cells of F_1 larval female of irradiated *C. pomonella* females with fragmented sex-chromatin body Bar= 10μm. b, WZ chromosome bivalent from oocyte of F_1 larval female of irradiated *C. pomonella* females with fragmented sex-chromatin body Bar= 1μm.

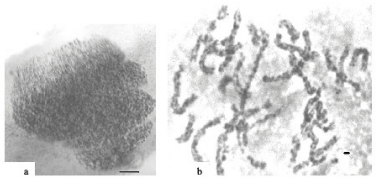

Fig 5. a, Highly polyploid of the Malpighian tubule cells of F_1 larval female of irradiated *C. pomonella* females with dispersed sex-chromatin body Bar= 10μm. b, WZ chromosome bivalent from oocyte of F_1 larval female of irradiated *C. pomonella* females with dispersed sex-chromatin body Bar= 1μm.

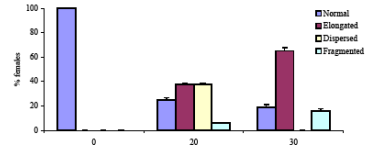

Fig. 6. Relationship between gamma irradiation and the percentageC. pomonella females with normal, elongated, dispersed and fragmented W sex chromatin in F_1 lines.

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SECRETARIA DE AGRICULTURA, GANADERIA, DESARROLLO RURAL, PESCA Y ALIMENTACION

THE AUGMENTATIVE BIOLOGICAL CONTROL COMPONENT IN THE FRUIT FLIES MEXICAN CAMPAIGN

IAEA-CN-131/023P

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The Mexican campaign against fruit flies incorporates different strategies under an area-wide approach. These strategies are the use of specific lures to detect and monitoring fruit fly populations, the application of specific toxic bait through aerial or ground aspersions, the use of mechanical control, the release of sterile *A. ludens* and *A. obliqua*, the establishment of quarantine procedures, and the releases of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hym.: Braconidae) (Fig 1), in specific zones and periods.

Fig. 1 *Diachasmimorpha longicaudata*, a fruit fly parasitoid

Fig. 2 Densities of *D. longicaudata* mass releases in different work zones in Mexico

Parasitoid releases are made by air or ground, focused to *Anastrepha* spp. host fruits located in marginal areas previously identified as reservoirs (i.e., backyard orchards; Fig. 3).

RESULTS

The case of the Michoacan State. In this zone it is possible to find fruit fly populations all year around because of its great ecological diversity. In level of importance, *Anastrepha ludens* is the most common species with an 89% of total individuals in traps. The second one is *A. obliqua* (6%), followed by *A. striata* (3%) and *A. serpentina* (2%).

Diachsmimorpha longicaudata is released by ground at a density of 1500 wasps/ha, over 1500 ha. The percent parasitism reached can be observed in Fig 4. The FTD index reduction was nearly 39% in 2004, and for the current year it is around 43 % (Table 1).

Fig. 3 Backyard orchards showing different host fruit flies

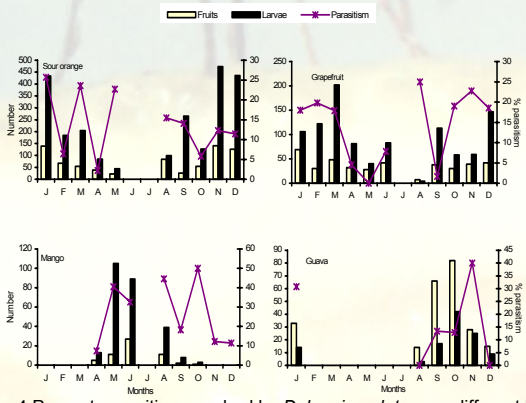

Fig. 4 Percent parasitism reached by *D. longicaudata* over different kind of fruits in the transition zone of Michoacan, Mexico, 2004.

Table 1 FTD reductions associate to *D. longicaudata* mass releases in different work zones

Zone of work	Year	FTD Reduction (%)
Michoacan	2005	43
Michoacan	2004	39
Mazatlán Sin	2004	41
Tepic Nay	2004	42
Tepic Nay	2003	46
Chiapas	2002	69

DISCUSION

The effect of released parasitoids has been similar in most zones under control (Table 2), showing a good relationship between the FTD indices reduction and parasitisms.

The conditions where parasitoid releases could offer a best performance are: 1) areas with fruit organic agricultural practices, 2) areas as canyons and barracks with high numbers of host fruits, and 3) marginal areas where growers are not going to implement control actions. These circumstances are commons in tropical countries where commercial exploitations of fruits are carried on.

CONCLUSIONS

These data show the impact that Augmentative Biological Control (ABC) can have on fruit fly populations, when releases are focused to host fruits in marginal areas. Under specific circumstances the benefits of ABC are broad, and its application against fruit flies could be highly advantageous.

CAMPAÑA NACIONAL CONTRA MOSCAS DE LA FRUTA

Cotton
IAEA
CN
131/24P

IPM at the Cross Roads with Realistic Approach: A Success Story of IPM on Rainfed Cotton in Tribal Areas of Maharashtra, India on a Whole Village Approach System

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1. Objectives of this IPM programme

i) To conserve bio-diversity
ii) To demonstrate the IPM module in whole village involving maximum number of farm families of a village
iii) To train youth and women in all aspects of IPM and to involve them actually in each operation in tribal areas
iv) To popularise Indigenous crop protection practices in tribal areas
v) To reduce the cost of plant protection
vi) To develop sustainable crop production technology
vii) To develop model IPM village in tribal areas

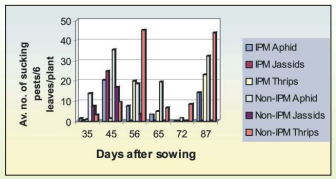
2. Village, season and area of IPM

i. Village : Budki, Tal :- Shirpur Dist :- Dhule Maharshtra, India
ii. Season : *Kharif,* 2001 *Kharif,* 2002
iii. Area under IPM : 64.4 ha (161 acres) 202 ha(505 acres)
iv. Hybrids : NHH-44= 44.4 ha (111 acres) NHH-44 H-8 = 20 ha (50 acres)
iv. No. of cultivators involved in IPM : 61 181

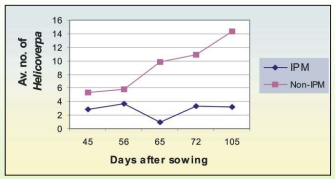
3. IPM module operated

i) Seed treatment : Imidacloprid (Gaucho 70 WS) 10g/kg seed
ii) Sowing period : 12 to 15 June
iii) Inter & mix cropping :
a) Border one row of maize
b) Between two maize plants, one plant of cowpea
c) At every 9th row of cotton, a line of *Setaria*
v) Use of pheromone traps : 5 each /ha (*Helicoverpa*) on 50 DAS
vi) Trichocards : Twice at 45 & 72days @ 1.5 lakh/ha
vii) Collection of larvae : As and when
viii) NSKE 5% : 4 sprays
a. 11 to 15th August b. 2 to 10th September
c. 15 to 20th September d. 2nd week of October on cotton of heavy soils
ix) HaNPV Spray : 250 LE HaNPV /ha on 105 DAS

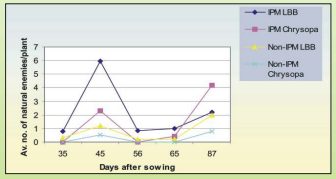
Sucking Pest Population



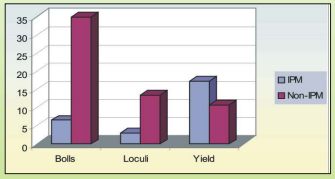
***Helicoverpa* larval population**



Natural Enemy Population



% bollworm incidence and yield (q/ha)



Av. no. of parasitoids / 25 damaged bolls

Treatments	*Rogas aligarhensis*	*Apanteles angleti*	*Bracon sp.*	others	Total
IPM	**22.6** (12 - 31)	**15.40** (8 - 21)	**9.70** (5 - 15)	**11.30** (6 - 16)	**59.00**
Non-IPM	**5.60** (2 - 8)	**2.40** (0 – 4)	**1.80** (0 - 3)	**2.20** (2 - 3)	**12.00**

Figures in parentheses are range values.


Imidacloprid seed treatment


Maize, *Setaria* & Cowpea mix crops


Pheromone trap & maize in cotton


NSE Spraying Demonstration

Economics of IPM & non-IPM plots

Particulars	IPM	Non-IPM
1. Yield (q/ha)	17.33	10.75
2. Cost of plant protection (Rs./ha)	1039	1116
3. Yield increase over non-IPM (q/ha)	6.58	--
4. Net income over non-IPM (Rs/ha.)	13160	
5.Total yield increase	1329 q (202 x 6.58)	
6. Net returns (National gain) (13160 x 202)	Rs 26.58 lakh	

4. Results:

- Sucking pests *viz.,* aphids, jassids & thrips reduced.
- *Helicoverpa* larval population was quite low.
- Bolls & loculi damage due to bollworms was low.
- Predator & parasitoid population was quite high.
- Yield of good kapas was high.
- Imidacloprid residues at harvest were BDL 0.01 ppm in both seed & kapas.

IPM Cotton Crop


Group meeting with farmers


Picking

5. Impact of IPM

- Full confidence about IPM among cotton growers
- Lateral spread of technology from farmer to farmer and village to village
- Collection of neem seed from their own villages, forest, surrounding areas and adopt whole IPM technology in a whole village
- Formation of group of IPM farmers
- Development of resource person for IPM in the village
- Reduction in cost of plant protection
- Awareness about the ill - effects of chemical pesticides on pest outbreak, health, environment etc.
- Visits of farmers from other villages to IPM plots
- Improved socio-economic status of IPM farmers in the village

6. Large Scale Demonstration of IPM on Rainfed Cotton

Tal. Shevgaon, Dist. : Ahmednagar Year -2003

Village	Hybrid	Area (ha)	No. of Cultivars involved
Varur	Ajeet-33	415	345
Kharadgaon	Ajeet-33	40	75
	3 hybrids	61	20
Akhegaon	Ajeet-33	112	108
	3 hybrids	501	250
Sonvihir	4 hybrids	45	31
Bodhegaon	3 hybrids	40	21
Balamtakali	4 hybrids	35	21
Hatgaon	5 hybrids	251	201
Kambi	8 hybrids	551	406
Total		2100 (5250 acres)	2092

IPM module operated : Sowing within a week
: Seed treatment with imidacloprid
: Inter / mix crops ize, cowpea, *Setaria,* sorghum, bajra
: Pheromone traps
: NSE 5% sprays (2)

Results :
Pests :
- Aphid and jassid population was quite low
- Thrip population was high due to long dry spell
- Bollworm complex – negligible, *Helicoverpa* and other bollworms were very low

Yield : 8 to 30 q/ha , Av. 17 q/ha, Non-IPM=10 q/ha,
Net gain = Rs. 17500/ha
Total gain = Rs. 3.68 crores

Influence of juvenile hormones and protein on male Caribbean fruit fly sexual success.

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Introduction

Polyphagous tephritid fruit flies often have complex mating systems in which aggregated males hold individual territories from which they emit chemical, acoustic and visual signals. Females arrive at these leks in order to choose mates and the variance in male reproductive success is typically high, i.e., relatively few males obtain the majority of copulations. Male competitiveness and attractiveness play important roles in sexual selection and consequently in this unbalanced sexual success.

Exposure to juvenile hormones (JH) and protein consumption during the adult pre-sexual maturation period accelerates male development and may lead to greater sexual success through increased pheromone production. However, accelerated maturity could have nutritional consequences since there is less time for adult flies to acquire reserves. Thus the addition of a protein rich adult diet may have particularly important consequences when hormone titers are manipulated.

The effects of JH application, protein adult diet and their interactions on the sexual success of male *Anastrepha suspensa* represents the main goal of this study. Experiments conducted in laboratory and in field cages offer a broad perspective in how males perform in both situations when submitted to different combinations of JH application and protein supply.

Material and methods

Insects. The Caribbean fruit flies used in the study had been in a laboratory colony at the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) USDA-ARS, at Gainesville, Florida, USA, for less than 2 years and were produced according the Caribbean fruit fly mass rearing protocol.

Treatments. The study compares male *A. suspensa* performance under the following four treatments:

A. Males with application of juvenile hormones (JH) and sugar and hydrolyzed protein (P) as adult food (JH+/P+)
B. Males with application of JH and sugar as adult food (JH+/P-)
C. Males with no application of JH and sugar and hydrolyzed protein as adult food (JH-/P+)
D. Males with no application of JH and sugar as adult food (JH-/P-)

Sexual success in laboratory. The experiment was conducted in cages with 20cm by 20cm by 20cm. The test was run with 12 replications (different days) with 15 cages per replication for a total of 180 cages. In each cage, 4 males (1 per treatment) competed for a female.

Sexual success in field cage. The experiment was conducted in a standard field cage (2.0m high and 2.9m diameter) used for the study of male compatibility and competitiveness in tephritids. In this experiment 2 cages were run per day during 6 days, for a total of 12 cages (replications). In each cage a potted guava was moved inside the cage to serve as a lekking site for calling males. Sixty males (15 per treatment) competing for 30 females were released per cage.

Sexual performance in a life-time basis. This experiment was conducted in laboratory in individual cylindrical cages (10cm high and 7cm diameter). Eighty cages with individual males (20 per treatment) were observed daily for 35 days. At 17:00 one female was released in each cage and observed until 19:00.

Sexual performance in a daily basis. This experiment was conducted in laboratory in individual cylindrical cages (10cm high and 7cm diameter). Eighty cages (20 per treatment) were observed with the adult males at age 5, 10, 15, 20, 25, 30 and 35 days old. One female was released per cage at 16:00, other at 17:00 and other at 18:00.

Results

Sexual success in laboratory. From a total of 180 cages, 131 successful matings pairs were recorded. Of those, 55% were performed by JH+/P+ males, which is significant higher when compared with all the other treatments. JH-/P- males had significant fewer matings than other treatments (Fig.1).

Sexual success in field cage. A total of 104 matings were observed in the field cage tests. JH+/P+ males accounted for 61 matings (59%) and had a significantly higher mating competitiveness when compared with the other treatments. The treatment JH-/P- with only 3 matings (3%) had a significant lower male competitiveness (Fig.1).

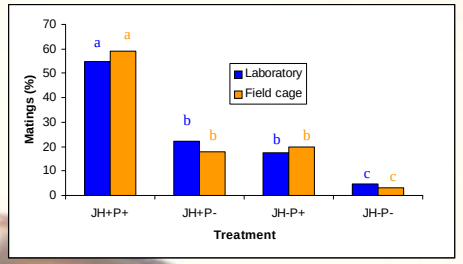

Fig.1-Percentage of matings per treatment of Caribbean fruit fly males in laboratory and field cage experiments when juvenile hormones (JH) is applied and protein (P) is supplied (bars with the same letter present no significant differences at 95% confidence).

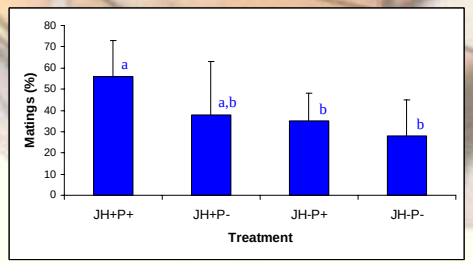

Fig.2-Percentage of matings per treatment of Caribbean fruit fly males from day 4 to the end of experiment (day 35) when juvenile hormones (JH) is applied and protein (P) is supplied (bars with the same letter present no significant differences at 95% confidence).

Sexual performance in a daily basis

The number of matings at adult age 5, 10, 15, 20, 25, 30 and 35 days is presented in Fig. 3. In addition to JH+/P+ males being more likely to mate these males were the only ones capable of mating 3 times consecutively in the same day (in 10% of the cases). As in the previous experiment the JH+ males started to mate earlier (see adult age 5 in Fig. 3).

Sexual performance in a life-time basis

JH+/P+ males were responsible for a greater proportion of mating from day 4 (earliest mating) until the end of the experiment (day 35) (Fig.2). These males were also more likely to mate on 3 or more consecutive days. Treatments with JH+ started to mate on day 4 and the JH- only on day 6. Male survival was higher in treatments with protein (P+).

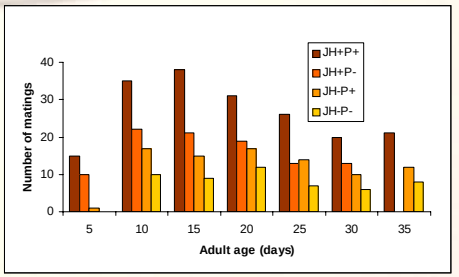

Fig.3-Number of matings per treatment at different adult ages, when juvenile hormones (JH) is applied and protein (P) is supplied.

Conclusions

- Male Caribbean fruit fly sexual performance and sexual success is favored by:
  - JH application
  - Protein supply
  - Interaction of JH and food supply
- JH application causes earlier male Caribbean fruit fly maturation
- Protein supply increases male Caribbean fruit fly longevity

A Modified “easy trap” Could Be a Good “Bait Station” against Fruit Flies.

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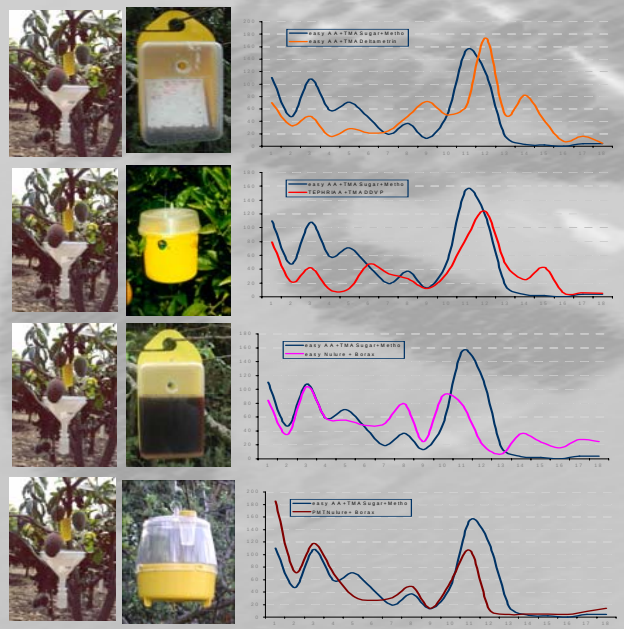
INTRODUCTION

Recently a new trap “easy trap” was launched into the fruit flies market. Easy trap showed a good performance when it was tested against PMT and Tephri traps all baited with Nulure or synthetic attractants.
The way of “bait stations” to control fruit flies is being introduced nowadays. Its efficiency will depend on the amount of flies that it can kill versus the number of beneficial insects killed.
Easy trap can be transformed into a “bait station” by discarding the clear half and replacing it with a yellow one. The effect is a yellow rectangular box with two holes and a hanger.
The position and size of the holes in the above trap have acted as a good mechanism for dispersion when baiting synthetic attractants (AA,TMA,PT) or Nulure. This aspect can be useful when painting the whole surface of the trap with a sugar base syrup plus Methomil. The flies will be attracted by the synthetic attractants inside the trap. They land on the syrup-coated surface, they eat it and die.

MATERIAL and METHODS

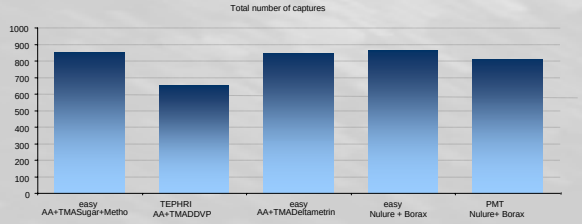
The first tests with this “easy bait station” were run in conjunction with the Standard research protocol 2004 of RCP of IAEA “develop of improved attractant Systems for Trapping and Sterility assessment” from 11 Sep to 26 Oct 2004 in a mango orchard located in Málaga in southern Spain
In order to count the died insects the “Bait Station” is completed by mounting a 30 cm. diameter white funnel at a distance of 10 cm. from it lower part. At the funnel exit a vial of approximate size with water is mounted to collect its.
Three blocks (replicates) of 10/11 treatments each. Twice a week the traps were checked and rotated one position. Two period were considered Sep/Oct with high temperature and high medfly population and Oct/Nov with cold temperature and low population.



RESULTS

The results have been positives. The “easy bait station” have killed approximately so much flies like the best treatment (see the total captures obtained by each treatment in the down table). The evolution of the number of killed flies is compared with the captured ones by the other treatments (left part)

TRAP	ATTRACTANT	RETENTION	WARM TEMP	COLD TEMP	TOTAL
TEPHRI	TMA+AA	DDVP	292	374	657
PMT	NULURE/B	LIQUID	629	217	816
EASY	TMA+AA	DELTAMETRIN	409	492	853
EASY Bait St.	TMA+AA	SUGAR+METHOMIL	508	351	859
EASY	NULURE/B	LIQUID	597	326	866



CONCLUSIONS

Easy “Bait Station” have demonstrated a very good efficiency attracting and killing medflies. It could be a good tool to control fruit flies but we must develop other insecticides compatibles with IPM and organics fruit productions. In our mango orchard this “bait station” have not killed other insects except black flies. It will be necessary to study the incidence of this Lure & Kill method on the beneficial insects of the different ecosystems where fruit trees growth. A roof it would necessary to protect the coated surface of the rain.

Biochemical Genetic Studies on Genotype Strains of Med-fly *Ceratitis capitata* (Wied.)

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Four genotypes of Med-fly, i.e. two laboratory strains (dark and yellow pupae), genetic sexing line (G.S.)and wild strain were examined biochemically in larvae, pupae and adult stages.

Isozymes of Alkaline phosphatase, Polyphenol oxidase and Esterase enzymes were chosen for their controlling of physiological reaction. The laboratory studies of the Med-fly genotypes exhibited variations in the strains as well as (G.S.) line according to the biological characters [1],[2].

The results of isoenzymes were genetically different from one genotype to another and from one stage to others. These results appear to be consistent over polymorphic loci effect . The functional genes of alkaline phosphatase isoenzymes of larvae were higher in band numbers and active than in pupae and adult extractions in all materials. No variations were found between the adult males and famales of all materials except the males of genetic sexing line which were more active. Fig.(1).

Concerning the isoestrases, the genes controlling the activity of isozymes were different from strain to another according to the stages of development. The isoestrases band numbers were low in larvae, but high numbers were found in adult male of genetic sexing line and female of yellow pupae. Fig.(2).

The results of this work were supported by Sabrah *et. al.*(1995) who studied the genic polymorphism and ontogenic variation of esterase isoenzymes in Med-fly collected from different geographical zones in Egypt [3].

Electrophoresis and spectrophotometer enzymatic analysis of polyphenol oxidase indicated that the larval stage was more active than the other stages. In addition,the larval polyphenol oxidase activity of yellow pupae strain and (G.S.) line were more active than the others Figs.(3 and4).

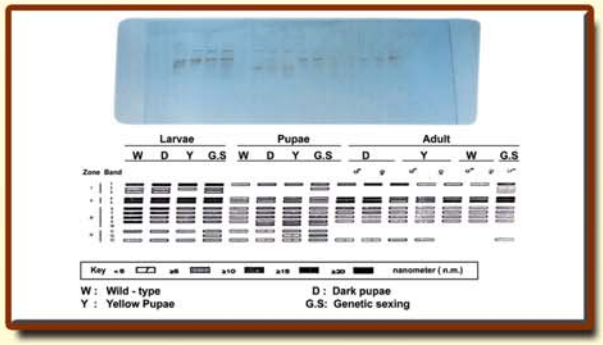

Fig.(1): Electrophoretic patterns of Alkaline phosphatase isozymes extracted from larvae, pupae, and adult individuals of Med-fly.

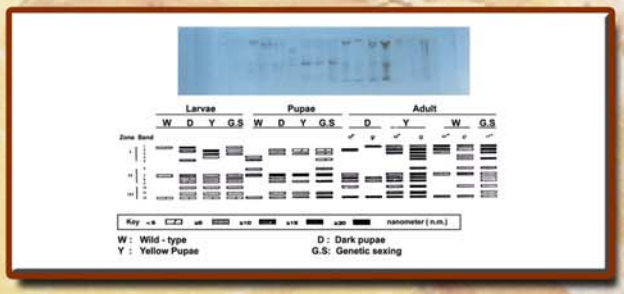

Fig.(2): Electrophoretic patterns of Esterases isozymes extracted from larvae, pupae, and adult individuals of Med-fly.

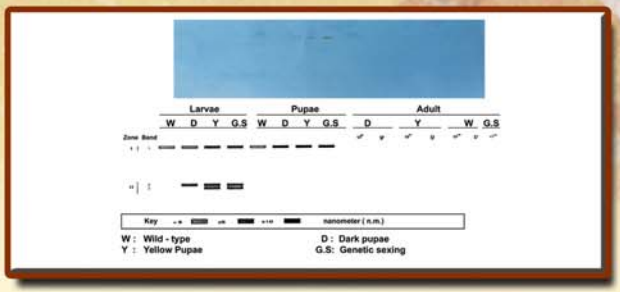

Fig.(3): Electrophoretic patterns of Polyphenol oxidase isozymes extracted from larvae, pupae, and adult individuals of Med-fly.

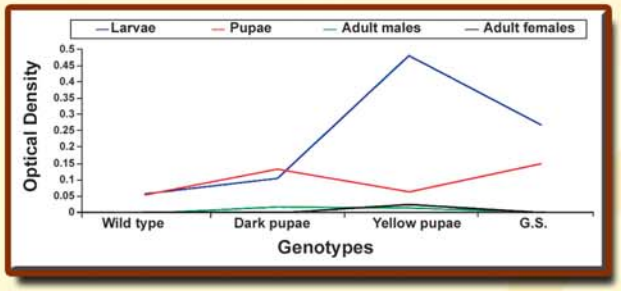

Fig.(4): Polyphenol oxidase activities as optical density units of larvae, pupae, and adult of the three genotypes and genetic sexing line.

[1] EL-Minshawy; A. M., N. S. Sabrah, A. I. Awad and M. B. Hafez (2000).Biological Genetic Studies on the Mediterranean Fruit Fly, *Ceratitis capitata* (Wied.). Minufiya, Journal of Agricultural Research.25 (2): 483-498.
[2] EL-Minshawy; A. M., N. S. Sabrah, and A. I. Awad (2003). Some Biological aspects on genetic sexing line of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wied.). treated by Gamma irradiation. The First Int.Egyptian-Romanian conf., Zagazig, Egypt Dec., 6-8, 2003.
[3] Sabrah; N. S., A. M. EL-Minshawy, M. B. Hafez, and A. I. Awad (1995).Genic polymorphism and ontogenic variation in the Med-Fly *Ceratitis capitata* (Wied.), collected from different geographical zones in Egypt based on esterase isozymes. Egypt, J.Appl.Sci.10 (4): 571-587.

Perspectives on fruitfly expansion: a lesson from a global invader *Ceratitis capitata*

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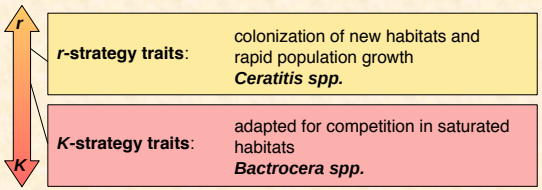
Fruitflies are present worldwide, although the major pests such as species of the genera *Ceratitis*, *Bactrocera*, *Anastrepha* and *Rhagoletis*, each have a limited natural distribution.

Mankind has played an important part in altering the natural distribution of some of the more polyphagous species, as well as certain oligophagous species. However, the question remains why only a few species have become or are becoming major pests.

Ceratitis	Afro-tropical regions
Bactrocera	Oriental and Australian regions
Anastrepha	South and Central America and the West Indies
Rhagoletis	Americas, Europe and temperate Asia

Life history traits as predictors of biological invaders?

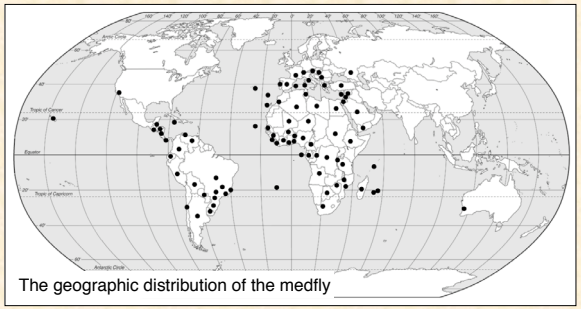
It has been suggested that the *r-K* gradient could be used as a predictor of the potential invasive capacity of a species (Malacrida *et al*, 1996 *Heredity* 76: 592-602; Duyck *et al*, 2004).



Species further along the *r-K* gradient, such as *B. dorsalis*, have invaded over *r*-selected species, never the reverse.

The medfly as a global invader

The medfly is highly polyphagous, very adaptable and has a high reproductive potential. Being an opportunistic species, the medfly took advantage of the new habitats that became accessible through the increased global trade in tropical fruits during the 19th century.



The geographic distribution of the medfly

A global invader at home: South-East Africa

Establishing a fine scale map of the genetic variability of the medfly revealed that the amount of genetic variation is not homogeneously distributed throughout the species range.

All the independent markers were coherent in revealing a vast reservoir of polymorphism in East African populations, which have the attributes of ancestral populations.

This high genetic variability may reflect the genetic plasticity of the medfly.

This genetic variability will be subjected to, and in turn influence, the response to evolutionary processes such as gene flow, genetic drift and selective pressure.

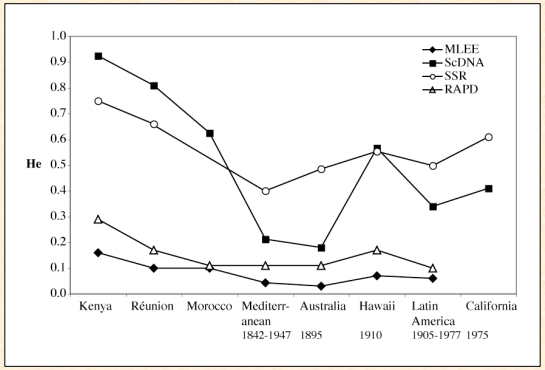
The medfly has a genome size of 540 Mb, three-fold greater than that of *Drosophila melanogaster*, suggesting that the genome may be rich in repetitive DNA including transposable elements (TEs). Indeed, the genome has been shown to contain a rich assortment of Class I and II TEs (Gomulski *et al,* 2004, *Insect Biochemistry and Molecular Biology* 34: 139-148).

That potentially active TEs are present in the genome of African medflies has been deduced by their ability to induce hybrid dysgenesis phenomena and other genetic instabilities (Torti *et al,* 1994, *Journal of Heredity* 85: 92-99).

The reservoir of variability together with the mutations arising from TE remobilisation may contribute to the genetic plasticity of the medfly increasing its ability to colonise and survive in new habitats.

Medfly population genetics: an indirect approach to dispersal.

The loss of variability in the derived populations reflects their demographic history.



The high estimates of gene flow, *Nm*, between populations reflect their recent common ancestry

Secondary colonisation events and outbreaks

Do fruitfly infestations represent outbreaks or established populations?

Most studies have focused on restricted areas in California, Florida and Australia to characterise the demography of invasions and to identify the source of invading individuals.

These studies relied on a combination of shared allele, phylogenetic, assignment and Bayesian analyses applied to highly polymorphic markers such as SSRs or mitochondrial DNA sequence variation to determine the origin of genotypes.

California: a case study

The first reported appearance of the medfly in California occurred in 1975 and medfly infestations have been reported in various localities throughout California since 1980.

The sporadic detection of medfly in California may be the result of new infestations originating from endemic populations such as Hawaii or Latin America or the reappearence of an endemic population with a density below sustainable levels.

Bonizzoni *et al,* (2001, *Molecular Ecology* 10: 2515-2524) suggested that some of the flies captured in California are derived from independent invasion events. Furthermore, analyses of specimens from the Los Angeles Basin supported the hypothesis that an endemic population, probably derived from Guatemala, has been established. Meixner *et al,* (2002) using SSRs and mtDNA also found evidence of multiple introductions into California, perhaps overlaying localised persistent medfly populations.

Is medfly invasion genetics a paradigm for other fruitflies?

The data indicate that different life-history strategies not only influence the invasive potential of species but also the way they interact with each other and with other non-tephritid pest species.

This will make it difficult to predict the potential economic risks associated with each species.

The mass of knowledge acquired on medfly population genetics and colonisation processes may prove to be untypical of tephritids in general given the medfly's cosmopolitan distribution and broad host range are probably exceptions in the Tephritid family.

However, studies of the invasive processes of these other tephritid species are quickly gaining momentum, thanks partly to the application of the techniques and analytical methods perfected for the medfly.

These studies will almost certainly provide alternative and contrasting examples of bioinvasion processes.

Comparison of sodium citrate with defibrination for the processing of blood for tsetse mass rearing

IAEA-CN-131-029P

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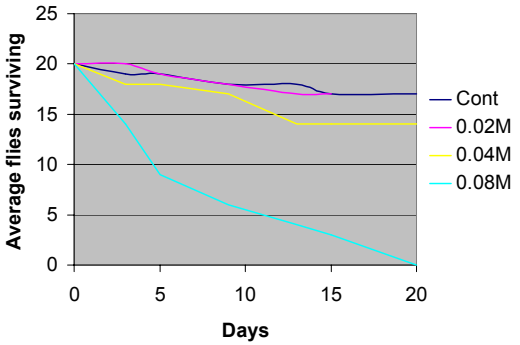
[b] Department of Medical Chemistry, Veterinary University of Vienna, Austria

Toxicity test of sodium citrate



Traditional blood collection procedures for tsetse diet employed either defibrination or heparin anti-coagulant. Heparin is expensive and thermolabile, and defibrination is time consuming, requires special equipment, trained personnel and results in additional bacterial contamination of the blood.

Previous work indicated that citrate might be mildly toxic, but the results were inconclusive. We therefore decided to revisit citrate as an anti-coagulant. Other anti-coagulants, such as EDTA and polyphosphate, had already been shown to be toxic to tsetse at anti-coagulant concentrations.

Various experimental bioassays comparing the use of sodium citrated bovine blood to defibrinated bovine blood were performed in order to reach an alternative anticoagulant procedure.

For the blood collection special care was taken to rule out biological bias by using the same donor animal for both citrated and defibrinated collections. Contamination was avoided by sterile collection from the jugular vein. As the Veterinary University, Vienna, owns these animals their medical history is recorded and could be inspected prior to collection in order to rule out any recent antibiotic treatments which could influence the results of the experiment. Both blood treatments were transported under refrigeration, irradiated in a Gammacell 220 cobalt-60 irradiator with 1 kGy, stored at -20°C and bacteriologically tested (nutrient agar plate test). All flies were kept under standard condition of temperature (24.5 ± 0.5°C) and relative humidity (75 ± 5%).

An initial acute toxicity test showed no significant toxicity at 0.02 or 0.04M citrate. 0.08 M citrate proved to be significantly toxic.
For the bioassays flies from the *Glossina pallidipes* colony Uganda strain, Entomology Unit, Seibersdorf, were used. Two tests were conducted, the first experiment lasting for 37 days (20 flies per cage) and the second 93 days (80 flies per cage). In each test, the flies were divided into 2 groups of 4 cages. One group received defibrinated bovine blood and the other group received citrated bovine blood (0.02M final concentration). Survival, mortality and fecundity rates of the flies and pupal size category and weight were recorded for both groups and were analyzed and compared.

Test of sodium citrate as an anti-coagulant for tsetse feeding

Pupal production per cage (64 females / 16 males per cage)

	n	mean	variance	F	df	p
0.02M citrate	4	122.75	322.25	0.286	1, 6	0.61
Defibrinated blood	4	129.75	364.25			

Pupal size

	A	B	C	D	E	TOTAL	Average size
0.02M	55	200	188	39	9	491	2.48
Control	61	237	189	30	2	519	2.36

CONCLUSIONS:

- An acute toxicity test indicated that tsetse flies can tolerate sodium citrate concentrations up to 0.04M.
- As 0.01M citrate is anticoagulant and the tsetse can tolerate a wide range of concentrations, an initial target concentration of 0.015M citrate can be used for blood collection, so that with variable collection volumes the final concentration will remain between 0.01 and 0.02M.
- There was no significant difference between citration and defibrination for survival, mortality and fecundity and that the pupal size category and weight for the citrated group had slightly better results than the defibrinated group.
- The use of citrate as an anticoagulant for the large-scale collection of blood was tested in trials at 2 different slaughterhouses. Collection using citrate is quicker and cleaner than using defibrination.

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Secretaría de Agricultura, Ganadería, Desarrollo Rural,
Pesca y Alimentación

THE GENETICS OF COLOUR-EYED MUTANTS OF THE FRUIT FLY
Anastrepha ludens (Diptera:Tephritidae)

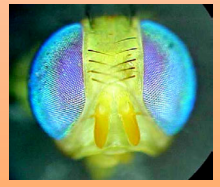
S. ZEPEDA, J. S. MEZA, S. GÁLVEZ, G. BETANZOS, A. ESCOBAR, P. MONTOYA

Campaña Nacional Moscas de la Fruta DGSV-SAGARPA, México.

WILD TYPE FLY

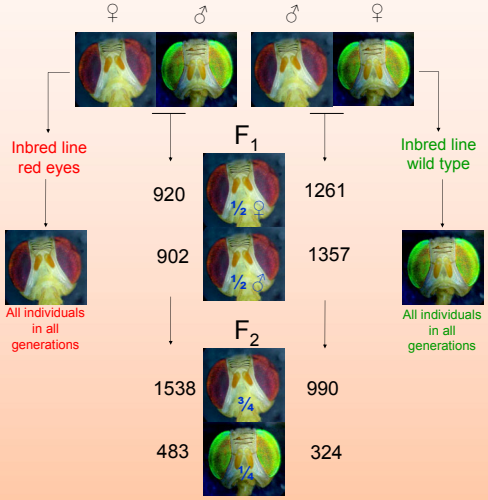
Mutant isolated

Red eyes

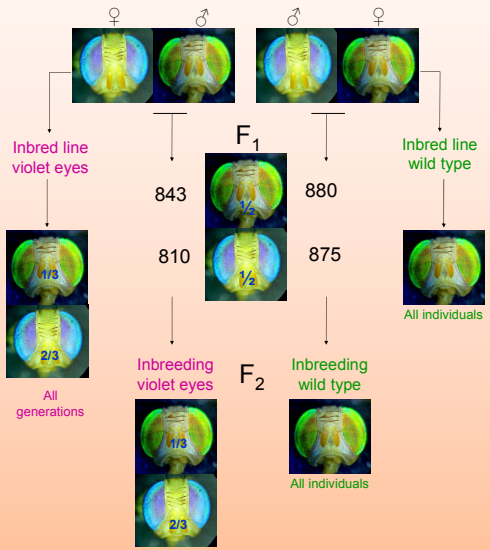


Violet eyes

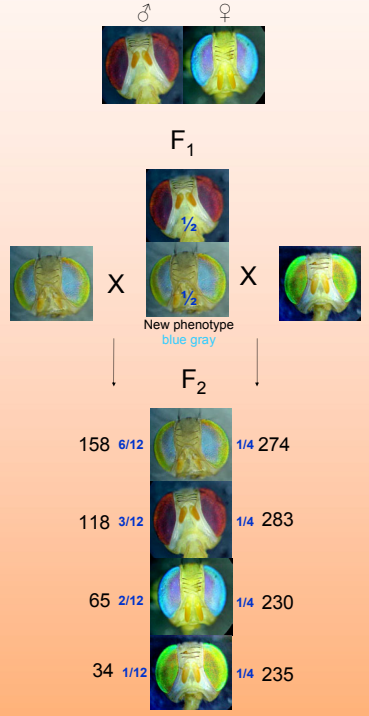
Red-eyes inheritance pattern



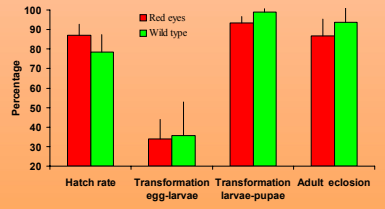
Violet-eyes inheritance pattern



Linkage analysis



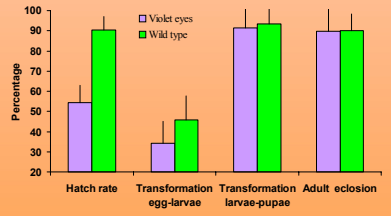
Biological attributes



Inherited as dominat and autosomal

Genotype (*RR*)

Biological attributes



Inherited as dominat, autosomal and lethal as homozigote

Genotype (*VeVe*$^{+}$)

The violet-eye gene and red-eye gene are either located in different chromosomes or far away enough from each other to permit independent recombination

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PLANTA MOSCAFRUT

Present status of the Old World Screw worm *Chrysomya bezziana* Diptera: Calliphoridae) in the Middle Region of Saudi Arabia.

Alahmed, A. M. and Kheir, S. M.

Plant Protection Dept., College of Food and Agric. Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia.

Abstract:

During the period Oct. 2001-Jun 2003, a survey for larval myiasis in sheep due to *Chrysomya bezziana* was carried out in Riyadh Region. 3712 sheep were examined, and only 73 (2%) were found infested with *C. bezziana* larvae. Among these infested sheep, 44 (60%) were young (less than 6 months old) and 29 (40%) were adults; 48 (66%) were females and 25 (34%) were males; and the overall mortality rate was 0.5%. The infestation rates with *C. bezziana* larvae were highest during Mar-May (2.6%) and Sept-Nov. (2.7%), when the temperature and humidity are optimum; while during the dry hot summer and cold winter seasons the infestation rates were very low (0.7% and 0.2% respectively). The seasonal activity of *C. bezziana* adults were also investigated during the same period using sticky traps baited with Swormlure-4. The baited traps were used twice a month, and only 19 adult flies were caught during the worm seasons, but no flies were caught during summer or winter. From these results it is clear that *C. bezziana* is not of great economic importance in the Middle Region of Saudi Arabia, because of the long dry hot summer which is unfavorable for larval development and adult survival. Further studies on the biology, distribution and economic importance of *C. bezziana* and other fly causing myiasis in Saudi Arabia are required.

Introduction:

Saudi Arabia lies in the southwestern part of Asia, with an area of 2000000 km ². Most of the country is an extremely arid area, except for some coastal zones. The weather is very hot during summer (May-Aug) and very cold during winter (Nov.-Feb.). The active rainy season falls between Dec. and March, with an annual rainfall of 100-500 mm. *Chrysomya bezziana* has been reported in many Gulf Countries such as Bahrain, Qatar, United Arab Emirates, Kuwait, Oman, Iraq and Iran (cited in Alahmed, 2002). In Saudi Arabia, Ansari and Oertley (1982) reported a single case of myiasis due to *C. bezziana* in a 14 years old female. Recently, Alahmed (2002) reported for the first time in Saudi Arabia 12 cases of myiasis due to *C. bezziana* in sheep.

Myiasis incidence and distribution in Saudi Arabia:

During a recent survey for larval myiasis which was conducted during the period Oct. 2001 to Sept. 2002, in the Middle Region of Saudi Arabia, 3712 sheep were examined and only 73 (2%) were found infested with different dipterous larvae (Alahmed, 2002). Out of the 115 larvae recovered, 100 (87%) were *C. bezziana*, 10 (8.7%) *Chrysomya albiceps* and 5 (4.3%) *Wohlfahrtia nuba.* Among the 73 infested sheep, 44 (60%) were young (less than 6 month) and 29 (40%) were adult; 48 (66%) were females and 25 (34%) were males and 19 (0.5%) died of myiasis. Alahmed (2004) also showed that myiasis prevalence rates were high during the worm seasons (Oct-Nov. and Mar-May), where the incidences were 2.7% and 2.6% respectively (Fig.1); and low during the dry hot summer (0.7%) and cold winter (0.2%). The prevalence and mortality rates of myiasis due to *C. bezziana* larvae, were higher in the north of Riyadh city than in the south (table1). In Al Haeir, which is the only site in the south of Riyadh city, only 5 cases of myiasis were reported, while in Al Ammariya, Al Waseel and Al Dirriyah (north of Riyadh city) 26 (2.1%), 25 (2.2%) and 17 (2.1%) cases of myiasis were reported respectively. This might be due to the better methods of animal husbandry adopted in Al Haeir south of Riyadh city. The seasonal activity of adult *C. bezziana* was also investigated in the Middle Region using sticky traps baited with swormlure-4, and during the two year study period, only 19 *C. bezziana* adults were attracted in worm season (Mar-May and Sep-Nov) and no flies in summer or winter months (Alahmed *et al*, 2004).

Conclusion:

From these results, it is clear that myiasis caused by *C. bezziana* larvae is not of great economic importance in the Middle Region of Saudi Arabia, because of the very long dry hot summer and very cold winter seasons which are unfavorable for larval development and adult survival. Further studies on the biology, distribution and economic importance of *C. bezziana* and other fly causing myiasis in Saudi Arabia are required.

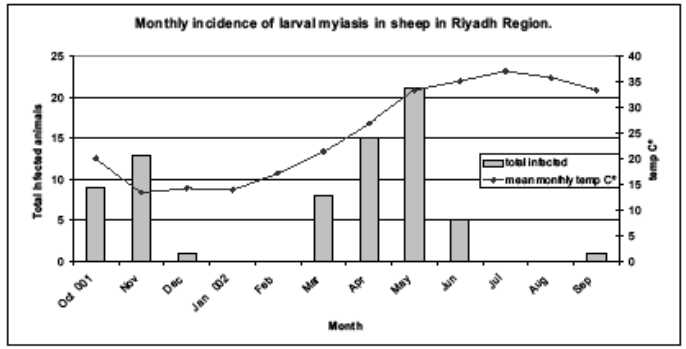

Fig. 1 Monthly incidence of larval myiasis in sheep in Riyadh Region.

Table 1 Prevelence and mortality rates of larval myiasis in sheep in the Middle Region of Saudi Arabia

site	Animals examined	Animals infested	% Prevalence rate	Animals died	% Mortality rate
El Ammariya	1217	26	2.1	6	0.5
El Waseel	1063	25	2.4	6	0.6
El Dirriyah	814	17	2.1	5	0.6
El Haier	618	5	0.8	2	0.3
Total	3712	73	2	19	0.5

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Ansari, M. A. and Oertley, R. E.(1982). Nasal myiasis due to *Chrysomya bezziana* blowfly (screw worm). Case Report. Saudi Medical Journal, 3, 275-278.

Influence of Sterilisation on Sound Production in the Tsetse Fly, *Glossina pallidipes*

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For applying the Sterile Insect Technique (SIT), large numbers of male tsetse flies are reared and sterilised by irradiation, either in air or in nitrogen.

Irradiation in nitrogen atmosphere is supposed to cause minor somatic damage to the flies than irradiation in air. The influence of both methods of sterilisation on the sound production was investigated in the tsetse fly, *Glossina pallidipes*.

As sounds play an important role in communication and physiological activities of feeding, mating and larviposition, a detailed description of spontaneous calls produced by male *G. pallidipes* is presented in this study.

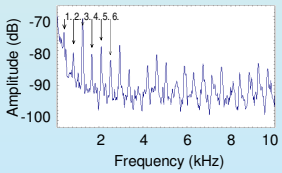
Measuring technique

Recordings of sound activity and measurements of sound pressure level were carried out under laboratory conditions in an anechoic room providing low levels of sound reflection and a reduced noise level. A total of 3037 sounds were recorded and analysed.

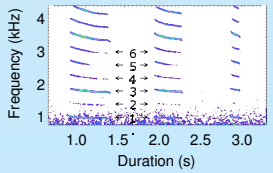
Acoustic characteristics

The tsetse fly call is composed of single sounds of variable duration occurring at unequal intervals, occasionally separated by flight and usually without apparent pattern.

The powerspectrum reveals that the call is built up of a ground frequency (1.) at about 420 Hz and many respective harmonics (e.g. 2.–6.), ranging from 835 Hz to more than 20 kHz.



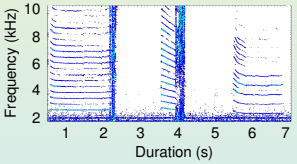
The sonagram shows the characteristic frequency pattern of the ground frequency (1.) and five harmonics (2.-6.), with the maximum level corres-ponding most often to the third harmonic (3.).



Acknowledgements: I thank Helmut Kratochvil for helpful advice in the course of the study. I am grateful to Andrew Parker from the International Atomic Energy Agency (IAEA), Austria, and Anton Lamboj for helpful discussion and comments on the manuscript.

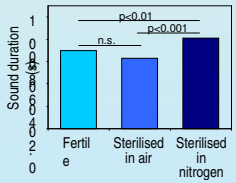
Individual differences

Songs of different individuals are determined by different frequency patterns, resulting in an intra-individual stereo-typy and an inter-individual variation.

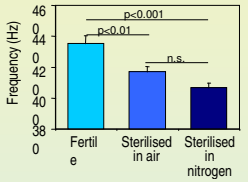


Sound duration

The length of the signals varies widely from 0.03 to 31.47 s.
A Mann Whitney U-test reveals that the median sound duration is influenced by sterilisation in two ways: flies irradiated in nitrogen sing longer than fertile flies ($p < 0.001$), contrary to flies sterilised in air which tend to have shorter songs than non-treated males.

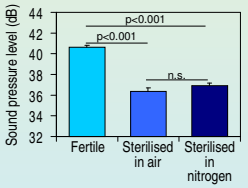


Ground frequency



A significant influence of irradiation on the ground frequency is confirmed by one-way ANOVA. Sounds of flies sterilised in nitrogen tend to have a lower frequency than signals of flies sterilised in air. Fertile flies sing higher than both groups of sterilised males.

Sound pressure level



Sounds of fertile males have a significantly higher amplitude than calls of both groups of sterile flies (one-way ANOVA, $p<0.001$). Flies sterilised in nitrogen tend to sing louder than flies sterilised in air.

Acoustic quality control

Quality control methods are essential to ensure the production of sterilised male tsetse flies of high quality and sexual competitiveness. Tests and standards to evaluate the fitness of flies by measuring certain acoustic parameters are currently being developed.

Conclusions and perspectives

The results of this study confirm that sterilisation has a measurable influence on the temporal pattern, ground frequency and amplitude of the sounds emitted by male *Glossina pallidipes*.

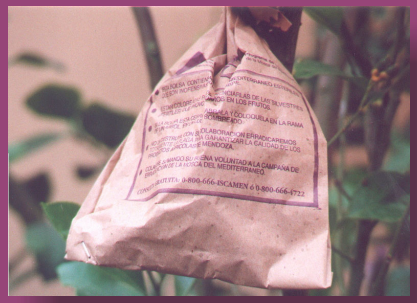
Whether these differences in the acoustic characteristics have an effect on the quality and fitness of the flies, needs to be investigated regarding the behavioural context under natural conditions.

Further investigations on the underlying mechanisms of sound production are necessary with the aim to improve the Sterile Insect Technique and to realise an efficient acoustic quality control.

POST-EMERGENCE HANDLING PROCEDURE FOR PROGRAMS WHICH RELEASED WITHOUT CHILL TREATMENT

Authors: Gabriela Bonpland[(1)] - Sergio Oueyt[(1)] - Oscar De Longo[(1)]
[(1)]ISCAMEN: Institute of Sanity and Agricultural Quality of Mendoza Argentina

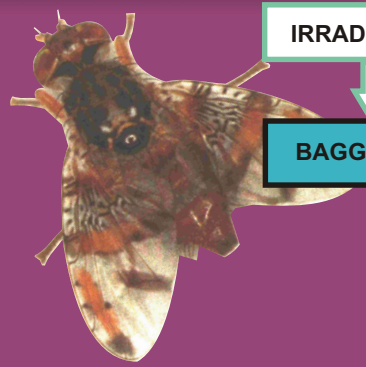
MENDOZA'S MEDFLY PROGRAM (ARGENTINA)

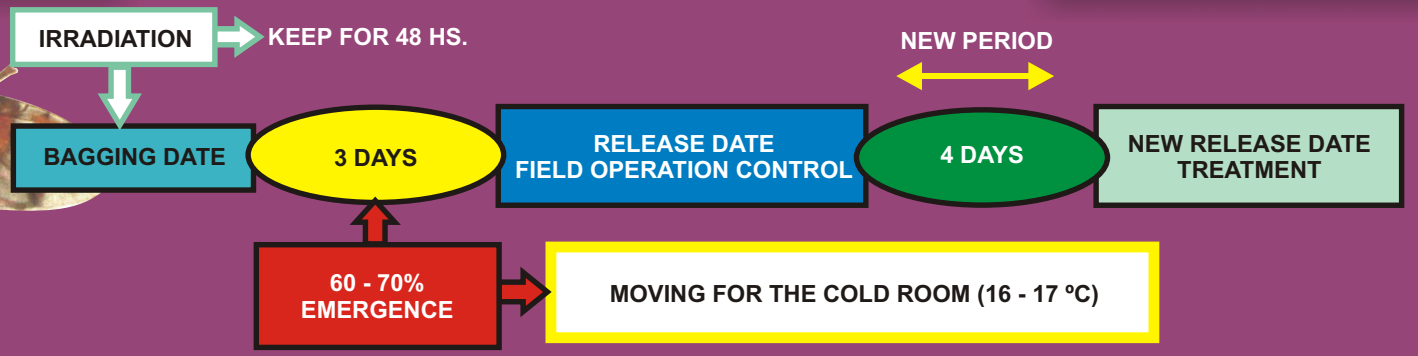


Our release system is achieved by bags without chill treatment. The current procedure's handling consist on releasing our sterile flies 12 to 24 Hs. after the emergence. In that way, these flies cannot survive more than 48 hs without suffering decreased signs in their final quality. (D&R –Bioplant KM 8). Keeping in mind that the best age of mating activity is from 5th - 7th day starting from their emergency, it's supposed that there should be taking place a' certain loss' not quantified.

So, we can improve our procedures increasing significantly the shelf-life of the bagged flies, with the consequent delayed moment for their releasing in field.

The change consists on keeping the routine until the emergence at 25°C-65% Hr. in the package room, approximately at 70% of the total emergence, the bags are placed in other room at 17 °C, for 4 days until reaching the released date, keeping in that way the initial quality.





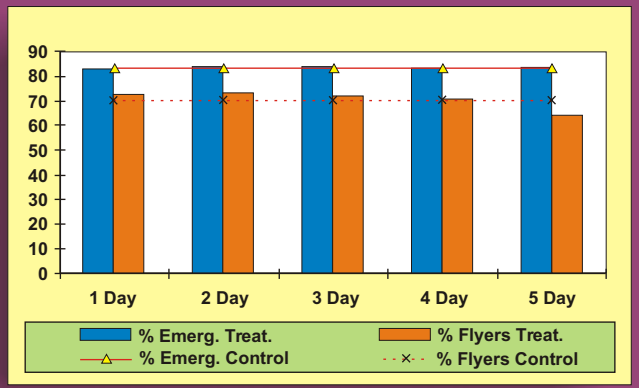
WHY 4TH DAY?

Because:
Is the period during which the flies keep their original quality.

It was carried out seven replications for testing 5 bags for Control in the former release day and 3 bags for each of the 5 following tested days. Each bag contained between 30 - 40 cc. Pupaes, that means it had been tested about 279.000 pupaes. In both parameters, % emergence and % fliers.

Significant differences it have not been found in the results, among days from 1st to the 5th after the emergence inside the bags, with ANOVA (a = 0.05) in the seven times we an the test. Could that new procedure affect the optimal time of mating?.
It was confirmed that it's effectively delayed from 6th to 8th day after emergence from the current one.

With the new methodology, it is got:
Approach the released time with the optimal mating time.
Keep the quality of the insects unaffected for releasing in the field.
Employ an easy and cheap equipment.



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Organization and Expression of a Cluster of Female-Specific Genes in the Australian Sheep Blowfly, *Lucilia cuprina*

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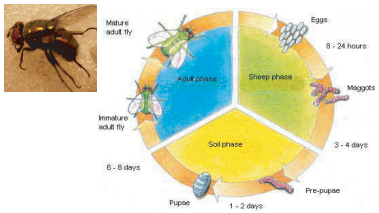
Introduction

The sheep blowfly, *Lucilia cuprina*, is a dipteran (fly) insect belonging to the family Calliphoridae that is a significant pest of the sheep industry in New Zealand and Australia. The female blowfly causes economic damage by laying eggs on living sheep that hatch and parasitize the sheep. While traditionally the sheep growing industry depends on chemical insecticides to control the blowfly, occurrence of resistance to the insecticides and regulatory demand for lower insecticide residues in the products (meat and wool) have led to the search for biological methods of controlling the blowfly. Research in our laboratory aims to develop novel genetic control strategies for the blowfly by using our understanding of the molecular biology of the blowfly to create a genetic sexing strain that can be used for a male-only sterile insect release program. Here we present preliminary data on the analysis of the sequence and female specific expression pattern of a *Lucilia* yolk protein gene cluster.

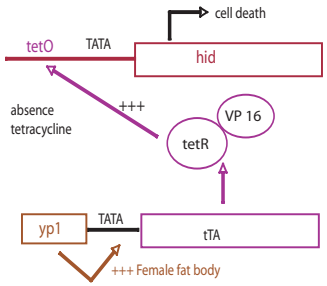
Life cycle of *Lucilia cuprina*

Ref: http://www.dpi.qld.gov.au/sheep/10041.html



A tetracycline-repressible female-specific lethal genetic system

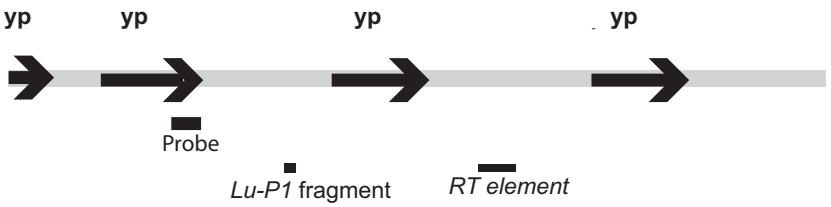
Ref: Heinrich, J.C. and Scott, M.J. (2000) Proc Natl Acad Sci USA 97: 8229-8232.



The expression of tTA is controlled by the female specific regulatory elements of the *yp* gene. In the absence of tetracycline, tTA binds to tetO and activates transcription of the cell death gene ***hid.***

Sequence Analysis of a Cluster of *Lucilia* Yolk Protein Genes

A 14.2 kb genomic DNA clone containing a cluster of yolk protein (*yp*) genes was sequenced by a combination of shotgun sequencing and primer walking strategies. The clone contains three complete *yp* genes; the 3' end of another *yp* gene is present at the 5' end of the clone. The clone also contains fragments of a *Lu-P1* DNA transposable element and a novel retrotransposable element.



Expression Analysis of the *yp* gene cluster by Northern Blotting

Total RNA was isolated from the various stages of the *Lucilia* life cycle and analysed by Northern blotting for the presence of the yolk protein RNA. A 679 nucleotide probe (XbaI-StuI restriction fragment from the 5'-most complete *yp* gene in the clone) corresponding approximately to the exon 3 of the *yp* mRNA was used to monitor the expression of all of the genes in the cluster. (The probe has about 95% sequence identity to all the complete *yp* genes and thus should cross-hybridize to all *yp* mRNAs).

Key

Embryo (E); Larval instars (L1, L2, L3); Pupa (P); Adult male (M) or female (F) unfed (-) or fed with protein cookie (+) post-emergence.

It was observed that the *yp* genes were expressed only in females that had received a protein meal. This demonstrates that the *yp* genes are female-specific in their expression and that their regulatory elements would be useful in driving transgenes in a female specific manner in *Lucilia.*

Sheep Blowfly Genome Project

The Sheep Blowfly Genome Project has been funded by Australian Wool Innovation (AWI) in collaboration with Phil Batterham and University of Melbourne.

The aims of the project are to:

1. **Sequence embryonic and larval cDNAs**
2. **Construct, fingerprint and array a BAC genomic library**
3. **Improve methods for *Lucilia* transgenesis**
4. **Develop methods for controlling gene expression**
5. **Use RNAi to determine the function of some genes in development and host response.**

*** A.S. is supported by a postdoctoral fellowship from the Massey University (Centre for Functional Genomics).**

IAEA-CN-131/36P

FIELD PERFORMANCE OF IRRADIATED CODLING MOTH, *Cydia pomonella* (L.), MALES

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Damascus, Syria

INTRODUCTION

The codling moth (CM), *Cydia pomonella* (L.), is a pest of pome and stone fruits throughout most deciduous fruit growing areas of the world (Barnes, 1991). Chemical control of this pest has many drawbacks (Knight et al., 1994). SIT is used for CM control in some countries (Bloem & Bloem, 2000) and is seriously considered for CM control in Syria. Laboratory studies showed that it is possible to induce high levels of sterility in CM males without causing a significant reduction in their competitive ability (Mansour, 2000). Field performance of the sterile males under orchard condition, however, was not examined.

In this report, we examine the effects of gamma radiation on performance of irradiated CM males under orchard conditions.

MATRIALS AND METHODS

Insects

Insects used in this experiment were reared on a local diet similar to that reported by Brinton et. al. (1969). Rearing conditions were maintained at 27 ± 1°C, 50±10 % RH and (16:8) L:D photoperiod. Following emergence, moths were separates by sex, males were placed separately in Petri dishes and kept under refrigeration till used.

Labeling

In order to distinguish released males from wild ones, a Calco red dye was added to the diet that allowed an easy distinction between wild and lab reared males.

Irradiation

Males, about 24 h-old, were irradiated (250 or 350 Gy), returned to the lab immediately after irradiation and kept under 2-4 °C until they were transported to, and released in the field, the next day.

Experimental orchards

Two adjacent orchards with an area of about 40 ha each were used in this study. One of the orchards was used for releasing irradiated males while control males were released in the other.

Moths release

Males were released in the morning (7-8 A.M.) in a square of 20X20 m in the middle of each orchard. Moths were distributed by hand under trees at a rate of 305-337 irradiated males/square (the same number for the control) and this experiment was repeated in three different occasions for each dose level (250 and 350).

Male dispersion ability

The dispersion ability of released males was monitored using pheromone traps. CM pheromone traps were placed in the 4 main directions around the release square. The traps were placed at a distance of 50-300 m from the release point at 50 m intervals and checked daily for two weeks.

Response to sex pheromone

The response of released males (irradiated and un-irradiated) to female sex pheromone was estimated by measuring the attraction of released males to pheromone traps.

RESULTS

1. Male dispersion ability

Table 1. Dispersion ability of irradiated CM males.

year	Dose (Gy)	Percentage of males caught at different distances (m) from the release point					
		50	100	150	200	250	300
2002	0	61.5 ± 2.0	17.8 ± 0.7	10.4 ± 0.4	7.2 ± 1.6	2.8 ± 1.1	0
	350	76.3 ± 0.1	12.4 ± 1.7	3.8 ± 3.0	2.1 ± 0.1	0	0
2003	0	57.0 ± 9.5	16.3 ± 3.8	11.0 ± 1.7	7.6 ± 1.2	6.1 ± 1.4	0
	250	71.2±11.3	15.4 ± 4.0	8.5 ± 2.3	6.5 ± 1.3	0	0

2. Response to female sex pheromone

Table 2. Response (attraction) of irradiated CM males to pheromone traps.

year	Dose (Gy)	% Caught	Percentage daily capture of males in pheromone traps (day after release)					
			1	2	3	4	5	6
2002	0	14.4	12.3±2.2	18.2±7.1	28.4±4.3	24.3±3.6	11.1±3.4	5.4±2.8
	350	8.6	09.2±2.3	14.7±3.6	36.7±4.5	29.9±2.6	6.7±4.6	2.6±0.9
2003	0	15.9±3.9	10.7±3.8	19.1±4.4	30.7±9.2	21.4±5.4	9.8±2.8	6.6±3.4
	250	14.7±4.8	07.9±2.3	18.3±4.6	39.9±11.6	26.6±7.2	6.5±1.8	2.0±0.3

CONCLUSIONS

These results suggest that irradiating CM males at a dose of 250-350 Gy would cause a negative effect on male dispersion ability under field conditions which is in general agreement with findings by Bloem et al. (1999). Male response to female sex pheromone may also be negatively affected. Therefore, it would be desirable to reduce the irradiation dose as long as the reduced dose would guarantee complete sterility of females.

The Distribution of OWS in I.R. of Iran and the Establishment, Maintenance and Mass Rearing of a Colony in the Laboratory

IAEA-CN-131/37P

International Conference on Area-Wide Control of Insect Pests:
Integrating the Sterile Insect and Related Nuclear and Other Techniques
9 - 13 May 2005
Vienna, Austria
Organized by
the Food and Agriculture Organization of the United Nations *and the*
International Atomic Energy Agency

Gholamreza Shahhosseini
Nuclear Research Center for Agriculture and Medicine (NRCAM),Atomic Energy Organization of Iran - Karaj, I.R. of Iran / E.mail address: gshahhosseini@yahoo.com

Introduction:
The Old World Screwworm fly (chrysomya bezziana) is an external parasite infesting a wide variety of wild and domestic animals and man. Its distribution includes tropical, subtropical regions of Africa, south- east Asia, west Asia (such as I.R. of Iran, Iraq,...). Both the threat to human and animal health and the economic costs associated with the control of c. bezziana are considerable. In nature, screwworm fly larvae can only survive and mature on fresh, healthy tissue, usually a wound sit. The wound becomes larger and deeper through the lacerating action of larva mouth hooks and successive fly strikes. To reproduce similar growing conditions in the laboratory was a challenge. For the first time OWS fly was recorded in south west of I.R. of Iran (Mahshahr city in Khuzestan province) in May 1995 formally. Since then the fly has infected some of farms in South West and South of the country. This paper describes the successful establishment, maintenance and mass rearing of an OWS fly colony in the laboratory and offers some information about the distribution of it in the country briefly.

Materials and Methods :
Some of larvae were collected from some of the farms of the mentioned areas. The samples were transferred from three provinces to the laboratory in Nuclear Research Center for Agriculture and Medicine (in Tehran province). All of the collected specimens were larvae (from the first stage to third stage) and they were usually colleted some sheep and goat's wounds (more than the other livestock). Of course the other larvae were collected too, for example: Chrysomya megacephala (less number – in comparison with Chrysomya bezzianna).

Transferring and Setting Samples:
After collecting Samples, dishes containing larvae and straw transferred by airplane from the mentioned regions to Tehran province where laboratory of Animal Production and Health Section of NRCAM is located. The distance between Tehran province and the mentioned areas is about 1000-1200 km. It was made suitable condition for rearing of the collected OWS and also making live cycle of OWS in the laboratory two times: 1 –May 2003 to June 2003 (First Effort), 2-January 2004 to now (Second Effort).In the first inspection, it was appeared that all of the larvae developed to pupae during 3-5 days. The pupae were separated from straw by sieving. All of the pupa were divided by some groups and put in some cages. The cages were transferred to incubator instrument.

Larvae Collecting – Table 1:

A- First Effort	B- Second Effort
1. Temperature of the Regions: 35-40^{oc}	1.Temperature of the Regions: 18-28^{oc}
2. Humidity of the Regions: 65-80%	2. Humidity of the Regions: 55-65%
3. Kind of Livestock: more goat	3.Kind of Livestock: more Sheep
4.Wounds Location for Sampling: Flanks, Underbelly and Ear	4. Wounds Location for Sampling: Flanks
5. Number of the Collected Larvae: 83 (in 3rd larval Stage)	5. Number of the Collected Larvae: 180 (in 3rd larval stage)
6. Collection Time: During 5 days	6. Collection Time: During 7 days
7. Collection Month: May 2003	7. Collection Month: January 2004
8. Collecting Method: After dropping from the wound, larvae were taken from soil and were put in the dishes containing straw or soft sawdust.	8. Collecting Method: After dropping from the wound, larvae were taken from soil and were put in the dishes containing straw or soft sawdust.

Rearing and Feeding

A-First Effort
1-Temperature of the Incubator Instrument:30^{oc}, 2-Humidity of the Incubator Instrument: 60-80%, 3-Number of Developed Larvae to Pupae: 83 4-Number of Developed pupae to flies: 76, 5-Rearing and Feeding Time: During 30 days 6-Diet: For the Flies:
Crushed liver – often, or Larvae Rearing Media (LRM) – seldom, or Lean Minced Beef Meat – seldom Water, sugar - water

Note :
LRM containing: Whole Dried Blood, Skim Milk powder, Water Lock powder, Egg Yolk powder Also using cattle fresh blood with EDTA and formalin (fixed quantities of them) for adding to the diet

B- Second Effort
1-Temperature of the Incubator Instrument: 28^{oc} 2-Humidity of the Incubator Instrument: 65-75% 3-Number of Developed Larvae to pupae: 180 4-Number of Developed pupae to Flies: 165 5-Rearing and Feeding Time: About 13 months 6-Diet:
A- For the Flies:
Lean Minced Beef Meat-always (almost), or LRM-seldom (it was very little available)
b- Water, sugar and water Note 1:This is same as the First Effort.
Note 2: The information No.1 and 2 are the relation to rearing the flies, but for the larvae:
1-Temperature of the Incubator Instrument: 33oc 2-Humidity of the Incubator Instrument: 65-75% 3-Diet: Lean Minced Beef Meat-always (almost), or LRM-seldom (it was very little available) Note 3: In addition to use the incubator instrument for rearing the flies, when the number of the pupae and flies were increased, it was used a room of the flies separately (with the same condition).
There are some equipments in the rearing room:
a- moist maker, b- vacuum and pressure system, c-cooling and heating system, d-Lighting system, e-racks for the flies cages. The area of the room is 12 square meter.

Results and Conclusions:
A-In the First Effort:
1-In spite of the different activities in order to show a live cycle of OWS fly completely and to see larval, pupal and maturity stages, but no laying was seen in the laboratory. In the sentinel animals was not seen laying in the wounds of them too.
Therefore the first effort in order to establish a laboratory colony of OWS failed. The test of sentinel animal was unsuccessful too.
2-In the Second Effort:
In this effort the laying was seen because the mating was seen males and females very good and very much. Therefore, at the first time in I.R. of Iran a live cycle of OWS fly was produced in the laboratory completely (from the egg to matured fly). The number of the produced pupae were about 85.000 and the number of the produced flies were approximately 72.000 in more than 150 ovipositions during about 1 year.
Also, after the different evaluations in some of infected farms in the most important the infected provinces, it can be explained briefly:
The most affected cases were in farms Mahshahr, Shadegan and Shoush(the region in Khouzestan province), farms of Daier, Genaveh, Dashtestan, Boushehr, Kangan, Ahram,Tangestan and Deilam (the region in Boushehr province) and farms of Minab (the region in Hormozgan province) in comparison with the other regions.
The most infected livestock consisted of cattle, sheep, lamb, goat and calf (in Boushehr province), sheep, goat, calf and kid (in Hormozgan province) and sheep and cattle (in Khouzestan province).
There was the myiasis in the different parts of livestock body as fat, foot, anus, vagina, flanks, underbelly, lip, umbilicus, vulva, gum, tail, ear, hoof, interior maxilla.

Table 2 : OWS Positive Cases in I.R. of Iran

	Year						
Province	1996	1997	1998	1999	2000	2001	Total
Khuzestan	12	7	54	106	2	12	193
Boushehr	0	20	23	79	48	62	232
Ilam	0	1	0	0	0	0	1
Fars	0	7	6	12	7	3	35
Esfehan	0	0	0	4	0	0	4
Kerman	0	2	0	7	20	23	47
Hormozgan	0	10	22	15	53	53	153
Total	12	47	105	223	130	153	665

(April 2002- May 2003) Table 3: Percentage of Affected Cases

Province	Region	Kind of Animal	Affected Animals Average%
Boushehr	Dashtestan	Sheep,cattle,lamb,goat	4.5
	Boushehr	goat	11
	Ahram	lamb	14
	Deilam	Cattle	50
	Daier	goat,calf	58
	Genaveh	Sheep,lamb,goat	18
Hormozgan	Minab	Sheep,goat,calf,kid	27

Table 4: Percentage of Affected Animals Average (in the Different Farms) (September 2003 – December 2003)

Province	Region	Kind of Animal	Affected Animals%	The Infected Parts of Body
Boushehr	Genveh	Lamb	11.4	fat-foot-umbilicus
		Kid	52.5	Anus-hoof
		Sheep	35	hoof-ear-vulva
		Goat	56	hoof- tail
	Deilam	Goat	?	hoof
	Dashtestan	Lamb	1	fat
		Goat	?	fat

Table 5: Percentage of Affected Animals Average (in the different farms) (October 2004- January 2005)

Province	Region	Kind of Animal	Affected Animals%
Khuzestan	Shadegan	Sheep	8
		Cattle	16.5

Table 6: Percentage of Affected Animals Averaage (in the different farms) (May 2004- October 2004)

Province	Region	Kind of Animal	Affected Animals
Khuzestan	Shoush	Sheep	12.6

Note: The most infections of OWS in Khuzestan province was the following regions in March 2004-January 2005:
Shadegan and Mahshahr, 2- Shonsh, 3-Dezfuls, 4-Ahvaz, 5- Ramhormoz, 6-Dashte – Azadegan, 7- Baghe- Malek

References:
1- HALL, M., WALL, R., Myiasis of Human and Domestic Animal (Advances in Parasitology Vol.35) (1995)262-269,280-282
2- HALL, M., EDGE, W.,TESTA, J., ADAMS, Z., READY, P., OWS Fly,Chrysomya bezziana.Occurs as Two Geographical Races (Medical and Entomology) (2001)393-400
3- MAHON, R., Proceeding of the Screwworm Fly – Emergency Preparedness Conference (2001)140-151
4- SPRADBERY, J.P., Australian Screwworm Fly Unit – Manual of Operations (1990)35-149
5- SPRADBERY, J.P., A Manual for the Diagnosis of Screwworm Fly (1991)18-58
6- SUKARSIH, et al., Establishment and Maintenance of a Colony of the OWS Fly, Chrysomya bezziana at Balitvet in Bogor- Indonesia (2000)144-149

Myasis for *Cochliomyia hominivorax:* Development and evaluation of an adult suppression system.

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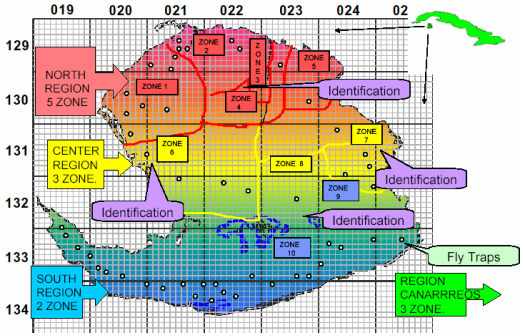
[a] Instituto de Medicina Veterinaria (IMV) La Habana, Cuba. [b] Consultor FAO/IAEA. México. [c] Instituto de Medicina Veterinaria (IMV) La Habana, Cuba.

Results

Preparation of the territory to evaluate the New World Screwworm (NWS) Adult Suppression System.

Figure 1 shows the division of the totality of the Isla de la Juventud in Regions and Zones as well as the situation of Identification Laboratories of the parasite.

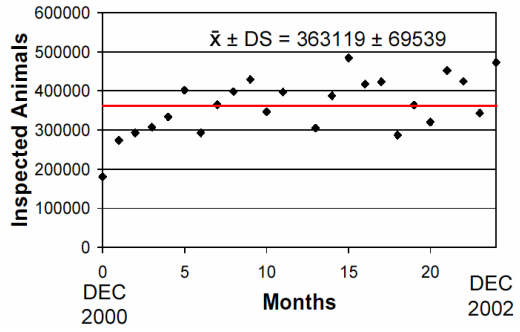
Fig. 1 Geographical Division of the Isla de La Juventud, Cuba for Regions and Zones.



Animal inspection

December 2000 (month 0) was the month taken as starting point for the development and evaluation of the NWS adult suppression system, sites were selected where major presentation of myasis cases they were reported, these places were visited daily and the rest of the sites at least twice week. In the Figure 2 it is observed as the animal inspection was kept during the evaluation time without significant differences existed throughout 2001 and 2002. According to the Livestock Census, a total of 59046 animals are susceptible to be infested by NWS, these were visited several times in the week and the month for the Field Technicians from the Instituto de Medicina Veterinaria from Cuba.

Fig. 2. Inspection of Animals December 2000 to December 2002



Treatment of wounds

Similarly in Figure 3 it express the behavior of the wounds treated with coumaphos 5% (organophorous compound) without significant differences in the time, slight increases in the parturition time exist where the navel of newborn and the vulva are very attractive wounds.

Figure 4 shows the different types of wounds, the most frequent are: Navel 24,81 %, Wired 23.83 %, Bitten 16.47 %, Vulva pos-parturition 11.01 % and Earring with the 8.72 %.

Fig. 3 Treatment of Wounds December 2000 - December 2002

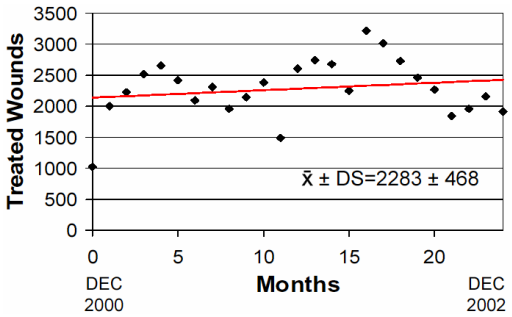
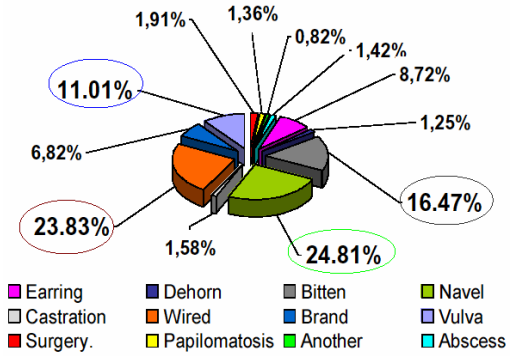


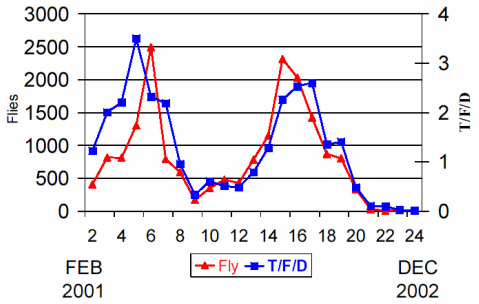
Fig. 4 Cases of NWS for Type of Wound December 2000 - December 2002



Mass Trapping of NWS.

Figure 5 shows the capture levels where we see two big peaks that correspond to the population dynamic of the parasite, an aspect of extreme importance constitutes the fact that 88 % of the captured adults were young females of C. hominivorax. (Nulliparous females)

Fig. 5 ADULT CAPTURE February 2001 – December 2002



Report of cases and behavior of the Suppression.

Figure 6 shows the two most affected species by NWS, the Bovine with 59,78 % and the Porcine with 31,05 % although, incidence exist in the other susceptible species.

The cases reported by this parasite were diminishing in the time, existing significant differences as of the 6 months and between the first and second year of application of the system (Figure 7). The abrupt variations in the behavior is for climatic factors that of course they influence in the seasonal dynamic of the parasite.

Fig. 6 Species affected for NWS December 2000- December 2002

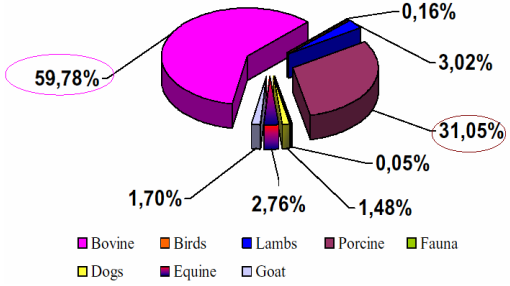


Fig. 7 NWS Cases December 2000 - December 2002

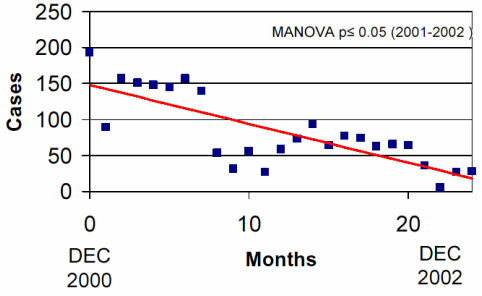
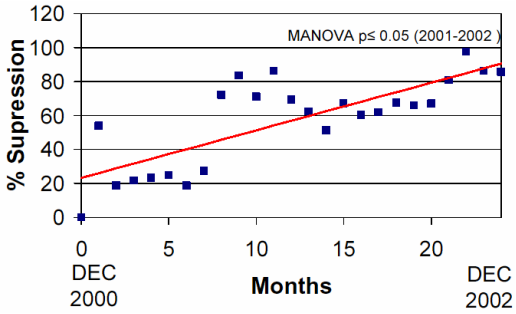


Figure 8 shows the behavior of the suppression where a tendency to the increase is observed in the whole period of the application of the measures of the system, existing significant differences as of the 6 months with respect to month 0 (December 2000).

Fig. 8 % SUPRESSION December 2000 - December 2002



Conclusions

- The division of the territory of the Isla de la Juventud in regions and zones, the situation of several identification laboratories, the selection and daily inspection of sites with greater incidence of NWS cases, as well as the excellent job of the Field and Laboratory Technicians facilitates the prompt response for the application of preventative measures in all the zones with great effectiveness, obtaining to maintain in the time prompt diagnosis and the inspection and treatment of wounds in a high number of animals.
- The use of the Vertical Sticky Trap baited with a specific attractant (swormlure-4) allowed to know the Geographic Distribution, the Migration Routes and the Sites with the highest incidence of NWS. There was demonstrated that with the application of several activities of suppression, the number of NWS cases diminishes in the time, achieving an increase in the suppression of the wild population of *Cochliomyia hominivorax* (Coq) that becomes more evident as of the 6 months.
- With these results, the territory selected is in a very favorable situation for the implementation of a NWS Eradication Program using the Sterile Insect Technique.

The use of a tote-box type irradiator for Sterile Insect Technique Applications

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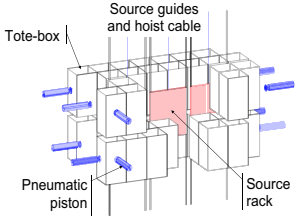
IRASM is a Multipurpose Irradiation Facility (IAEA TC Project ROM /8/011) with a SVST Co-60/B type irradiator (Fig.1) and several laboratories: dosimetry, microbiology, chemistry. The three independent source racks support the "multipurpose" attribute.



Radiation sources: Co-60 ; Source type: CoS-43HH
Source rack: rectangular; Rack numbers: 3
Max. source load: 2 MCi; wet storage
Internal dimensions of totes: (48x 48x 88) cm
Maximum load per tote: 120 kg
Uniformity ratio: 1.3 (200 kg/m^3)
Operation modes: Batch, Continuous, Stationary

Fig.1. IRASM tote-box irradiator

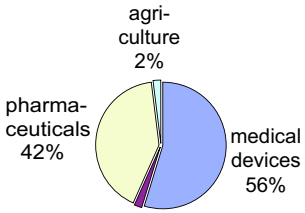


Fig.2. Main applications currently performed at IRASM

Dose distribution for 200 kg/m^3 -full load

(52 tote-boxes, 9 kCi - right side rack)

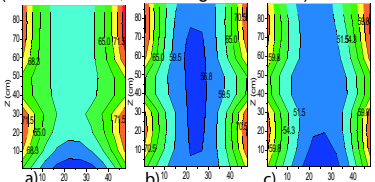


Fig.3. Dose maps a)y=0; b)y=24cm; c)y=48cm
3D-dosimeter array: 5x3x5 (alanine)

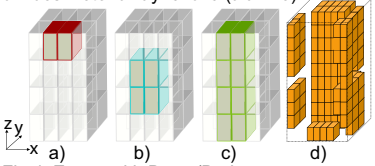


Fig.4. Zones with Dmax/Dmin=
a) 1.12; b) 1.14; c) 1.17;
d) zones excluded for Dmax/Dmin=1.20

Dose distribution in air

52 cycles (empty tote-boxes), 9 kCi - right side rack

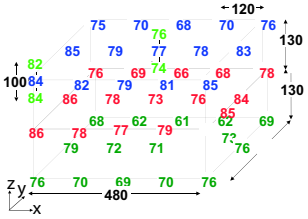


Fig.5 Absorbed dose distribution in air (Gy)
3D-dosimeter array: 5x3x3 (alanine)

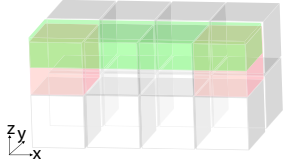


Fig.6 Zones with Dmax/Dmin=1.12 (red) and Dmax/Dmin=1.14 (green)

Dose distribution for 400 kg/m^3

- partial loaded tote-boxes

52 tote-boxes, 12 kCi - right side rack

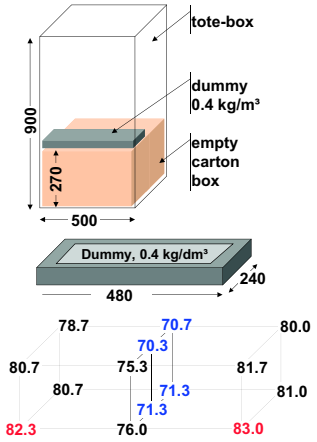


Fig.7 Loading pattern and dose distribution (Gy)

Table 1. Dose diagram for dummy with 400 kg/m^3

Zone	Run 1	Run 2	Run 3	Mean	ST DEV	CV (%)	Min&Max Equivalence
	Gy						
1	82	83	80	81.67	1.53	1.87	Zones
2	80	80	80	80.00	0.00	0.00	
3	83	83	83	83.00	0.00	0.00	MaxZone
4	81	81	81	81.00	0.00	0.00	
5	80	81	81	80.67	0.58	0.72	
6	79	78	79	78.67	0.58	0.73	
7	82	82	83	82.33	0.58	0.70	MaxZone
8	80	81	81	80.67	0.58	0.72	
9	75	75	76	75.33	0.58	0.77	
10	69	70	72	70.33	1.53	2.17	MinZone
11	71	71	70	70.67	0.58	0.82	MinZone
12	76	76	76	76.00	0.00	0.00	
13	70	72	72	71.33	1.15	1.62	MinZone
14	71	71	72	71.33	0.58	0.81	MinZone

Minimum Dose: 70.33 Gy
Maximum Dose: 83.00 Gy
Overall Uncertainty: 0.77 Gy
Minimum Detectable Difference: 1.07 Gy
Uniformity Dose Ratio: 1.18
Uncertainty of the Uniformity Dose Ratio: 0.02

Irradiation Costs

A. 200kg/m^3– full load (fig. 4)

Quantity (kg)		125	250	500	1000
Price (EUR)	a)	120	200	320	560
	b)		120	200	320
	c)			120	200
	d)				120

B. 400kg/m^3– partial load (fig.7)

Quantity (kg)	120	240	360	480
Price (EUR)	120	200	260	320

C. Turn-table (fig.9)

Quantity (kg)	200	400
Price (EUR)	50	80

Advantages	Disadvantages
Large quantity Good /very good uniformity	Special loading pattern (dummy) Difficult dosimetry (d)
Good uniformity	Small quantity
Large quantity (Dose uniformity not evaluated)	Reducing space in irradiation room Electrical wiring

Note1: The data from fig.5 indicates that the quantity for 400kg/m^3– partial load could be doubled with the same dose uniformity.
Note2: for 400kg/m^3– ***full load*** the dose uniformity is worsening and the volumes that could be used are smaller.
Note3: Prices were calculated taking into account the commercial (actual) value of the operation hour at IRASM irradiator. The dose distributions presented in fig.3, 5 and 7 are obtained with an irradiation time of 1h 18m (maximum speed of the tote-box conveyor)

Sample irradiation

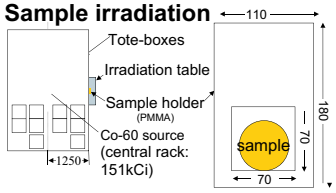


Fig.8 Static arrangement for sample irradiation
Dose rate in reference position: (705±21)Gy/h
(traceable to national standard, 22.03.05)
Transit dose: 1.35Gy

Turntable (project)

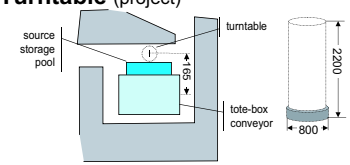


Fig.9 Arrangement for turntable
(rough estimation of the dose-rate: 100Gy/h)

Conclusions

* A tote-box irradiator could be used for SIT, but only with a small quantity of Co-60 sources.
IRASM can maintain a relative long period a small amount of Co-60 in one of the side racks.
* For the production start the best choice is B – irradiation in partial loaded containers. It needs a high production volume for having enough reasons to build a turn-table
* Turntable will become necessary when the source activity will be higher than 10kCi in the side racks.

AREA-WIDE INTEGRATED CONTROL OF ORIENTAL FRUIT FLY (*Bactrocera dorsalis*, Hendel) AND GUAVA FRUIT FLY (*B. correcta*) IN THAILAND USING THE STERILE INSECT TECHNIQUE (SIT)

Watchreeporn Orankanok[1], Suksom Chinvinijkul[1], Sujinda Thanaphum[2] Manon Sutantawong[3] and Walther R. Enkerlin[4]

The effective cooperation between Thailand's Department of Agricultural Extension (DOAE), Office of Atoms for Peace (OAP) and the International Atomic Energy Agency (IAEA), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, started in 1991. An area-wide integrated pest management (IPM) programme with a sterile insect technique (SIT) component is under operations in two distinctive relatively small pilot areas in the provinces of Ratchaburi (34 km^2) and Pichit (37 km^2) located in the west and north of Bangkok, respectively. Before the application of an IPM approach including releases of sterile flies, Oriental fruit fly (*Bactrocera dorsalis*; OFF) and guava fruit fly (*B.correcta*; GFF) infestation levels were 82% and 43 % infested fruit in the mentioned areas, respectively. Based on data collected in year 2002 from captured flies in Methyl Eugenol baited Stainer traps it was found that the OFF : GFF ratio in Ratchaburi Province was 1:3.4 and 1:1.5 in Pichit Province (figure 1) . Therefore DOAE has resolved to control GFF together with OFF by integrating SIT with other monitoring and control methods since year 2003.

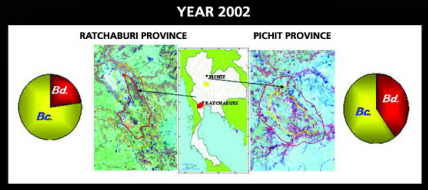


Figure 1 The map of release area at Paktor district, Ratchaburi province and Sak Lak district, Pichit province and ratio of captured wild male Bactocera dorsalis and Bactocera correcta in year 2002.

Mass rearing

Both species are being mass reared and sterilized at the facility located in the Pathumthani province following standard operation procedures. For OFF amount of larval diet prepared was, 109, 107, 36,128 and 190 tons/year during years 2000-2004. A total of ca.715 , 810, 280 , 782 and 1092 million pupae were produced per year, respectively. The *B. dorsalis* and *B.correcta* pupae were select with age of 2 days before emergence and were marked with 2.0 gram fluorescent dye powder per liter of pupae. The pupae were sterilized using 90 Gy and 80 Gy for OFF and GFF, respectively, using gamma radiation from a ^{60}Co source. The 400 ml. of pupae were close-packed in narrow polyethylene bags and kept in polystyrene containers with ice packs during shipping to target areas. The percent of sterility for OFF in year 2002-2004 was 98.06 % , 99.37 %, 90.90 %, respectively and for GFF in years 2003 and 2004 was 99.0% , 99.84 %, respectively. The high variability in pupae production is a result of not being able to maintain stable conditions throughout the rearing process. The average production in mango season was kept in 20 million of *B. dorsalis* pupae per week. The percentage of pupae recovery in year 2003 and 2004 was very low . The facility has renewed colony strain once in two years in order to assure competitiveness under field conditions. (figure 2,3)

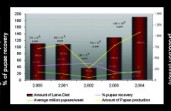


Figure 2 Data of sterile Bactocera dorsalis production for SIT release areas in Ratchaburi Province and Pichit province, show amount of pupae production, % of pupae recovery, amount of larvae diet and average of million pupae per week.

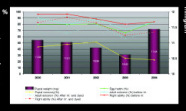


Figure 3 Show quality of OFF pupae at the Rathumthani production facility during 2000-2004. Adult eclosion and flight ability in the shipments arriving at Paktor was variable between shipments.

Sterile Fly Release

Weekly sterile pupae shipments using a refrigerated truck are conducted and pupae are delivered directly to sterile adult holding and emergence facilities. Upon arrival at distribution facility the bags of pupae are taken out of the container and pupae are placed in plastic boxes 800 cc./each, with around 32,000 pupae per box. Pupae are incubated for 3-4 days at 26-27 ^{0}C room temperature. After emergence sterile adults are taken to the field for release. Stationary release sites are uniformly distributed in the release area using GPS/GIS system. The amount of 3,000 sterile flies per hectare were release during the critical months (October to March).

Figure 4 a) The adult holding and emergence facility in Wang Tob Sai sub-district, Pichit province b) Weekly transport sterile adult to release sites using air-condition truck. c) Stationary release site.

Surveillance activities

The ratio of sterile males to wild males was set considering that the aim of the programme is population suppression and not eradication.

The ratio of GFF: OFF in core area (i.e. mango orchards) of Ratchaburi province was reduced from 3.4:1 in 2002 to 0.7:1 and 0.5 in 2003 and 2004, respectively. In Pichit province from 1.5 in 2002 to 1.1 and 0.9 in 2003 and 2004, respectively as showen in Table 1.

Figure 5 Data in year 2002 before releasing of sterile fly and during integrated area-wide SIT application in years 2003-2004 in Pichit province.
a) Fly / trap / day of wild OFF and GFF
b) S/W ration year 2003-2004.

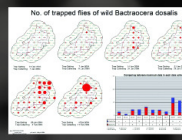


Figure 6 Location of the captured flies in georeferenced steiner traps.

Table 1 Ratio of average captured wild male *Bactocera correcta* and *B. dorsalis* year 2002-2004

Ratio Bc/Bd.	year 2002		year 2003		year 2004	
	Core area	Buffer zone	Core area	Buffer zone	Core area	Buffer zone
Ratchaburi	3.4/1	NO DATA	0.7/1	0.5/1	0.3/1	0.3/1
Pichit	1.5/1	1.4/1	1.1/1	1.0/1.0	0.9/1	1/1

Mangoes with infestation symptoms were sampled in the sterile fly release areas during mango season (around March-May) to assess OFF and GFF damage. After years of effort, percent of infestation in Ratchaburi province was reduce to an avearge less than 3.6 % in the past five years (2000-2004). The effort has paid good dividends to a small number of mango growers. And with two years under the control programme in Pichit province the percent of infestation fruit has been reduced from 43 % in 2002 to 15 % in 2004 as shown in the Figure 7 .

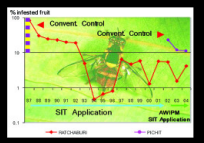


Figure 7 Percent of mangoes infested by the OFF and GFF in 2000-2004.

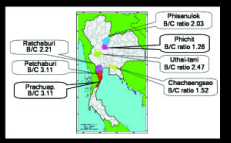


Figure 8 Map of Thailand showing location of selected provinces and B/c ratio of each province.

The growers in project area , Paktor , Ratchaburi province have been exporting mango production to countries that do not require a fly free certificate such as Canada, Hong Kong, Malaysia, Singapore, China etc, but discriminate against pesticide residues and fruit quality in year 2003 and 2004 approximately 240 and 300 tons respectively. And mango production from Pichit area have been also exported more than 2,000 tons to those said countries and Japan but only after attaining a compliance with a post-harvest treatment and a Federal Phytosanitary Certificate. This achievements to convinced mango producers in others area to request using SIT under an integrated approach.

Economic assessment

An economic analysis projected over 14 years indicates a benefit to cost ratio of 7.5 to 1 and a net-benefit of US$ 7.5 million for the mango growers of Paktor, Ratchaburi province. Furthermore, the economic assessment for current areas and areas purposed for expansion was conducted by IAEA expert. Results are shown in Figure 8 .

Conclusion

This project is one of few examples of the routine use of the SIT for effective suppression of fruit flies. Nevertheless, substantial improvements are needed in order to cost-effectively apply the SIT at a larger scale. The basic requirements for scaling up the project from a pilot level to a nationwide project is a complex procedure that requires government comitment and international technical cooperation for integration of multidisciplinary research. We also address the public information targeted to mango growers to obtain support for the operational programme.

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IAEA-CN-131/41P

EFFECTS OF GAMMA IRRADIATION ON MORTALITY AND THE INTERACTION BETWEEN THE AGE OF PUPAE AND IRRADIATION ON STERILITY OF GUAVA FRUIT FLY, *Bactrocera correcta* (Bezzi)

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SUMMARY

Mature eggs, larvae and pupae of *B. correcta* (Bezzi) were irradiated at the doses of 0, 100, 200, 300, 400, 500 and 600 Gy for eggs; 0, 30, 50, 70 and 90 Gy for larvae; 0, 100, 200, 400, 600, 800 and 1000 Gy for pupae. Mortality was scored 3 days after irradiation for eggs, 7 days for larvae and 14 days for pupae. LD_{50} and LD_{99} were 157 and 293 Gy for eggs,19 and 42 Gy for larvae, 567 and 994 Gy for pupae. Brown eyes and blue eyes pupae of *B. correcta* (Bezzi) were irradiated at the doses of 50, 60, 70 and 80 Gy. Treated males were crossed with virgin females, and treated females were crossed with virgin males. Sterility and survival were observed 17 days after adult eclosion. Complete sterility was found at the dose of 60 Gy and the difference in irradiation doses did not significantly cause a difference in survival.

1. INTRODUCTION

Guava fruit fly, *B. correcta* (Bezzi) (Tephritidae) is an important pest of economic crops. In Thailand, it frequently infests guava, jujube, mango, rose apple, carambola and tropical almond (Terminalia catappa). It is widely distributed in North, East-North and Central part of Thailand. The purpose of the current study was to measure the effect of gamma radiation on mortality, as well as the interaction between the age of pupae and gamma irradiation on sterility of *B. correcta* (Bezzi) for SIT program.

2. MATERAILS AND METHODS

1). Mature eggs, larvae and pupae of *B. correcta* (Bezzi) were irradiated with gamma radiation Mortality was scored

2). The experiment was designed using the 2x5 factorial design and 3 replications. Brown eye and blue eye pupae of *B. correcta* (Bezzi) (2 and 1 days before adult eclosion, respectively) were irradiated. Treated males were crossed with virgin females, and treated females were crossed with virgin males. The mean of sterility and survival percentages were analyzed by DMRT.

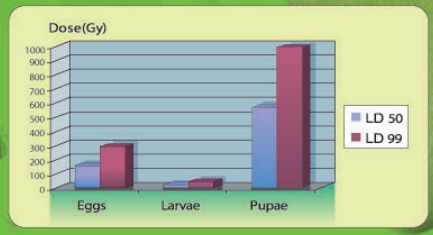

Table 1. Means of sterility percentage of **B. correcta** *(Bezzi) 17 days after adult eclosion*

Dose (Gy)	Sterility (%)			
	Treated males x Virgin females		Virgin males x Treated females	
	Brown eyes	Blue eyes	Brown eyes	Blue eyes
0	0a	0a	0a	0a
50	100b	100b	92b	88.9b
60	100b	99.7b	No eggs laid	No eggs laid
70	99.8b	100 b	No eggs laid	No eggs laid
80	99.7b	100b	No eggs laid	No eggs laid

Table 2. Percentages of survival of **B. correcta** *(Bezzi) 17 days after adult eclosion*

Dose (Gy)	Survival (%)			
	Brown eyes		Blue eyes	
	Males	Females	Males	Females
0	91.6a	99.6a	91.6a	99.6a
50	75.0a	66.6b	80.0ab	66.6a
60	83.3a	55.5b	66.6b	90.0a
70	75.0a	80.0ab	86.6a	98.3a
80	81.6a	83.3ab	95.0a	93.3a

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

3. DISCUSSIONS

The results of effect of gamma radiation on mortality of *B. correcta* (Bezzi) showed that the larval stage was most sensitive to gamma radiation than egg and pupal stage, respectively. These observations on the mortality of *B. correcta* will be applied for other objectives in the future. The differential sensitivity between males and females might be an advantage if a single dose of irradiation would sterilize males but cause a loss in fecundity in females. Nevertheless, the dose of 60 Gy was considered because it gave a high percentage of sterility in males and caused no eggs laid in females. However, we will study their sexual behavior and ability to mate with female. Sterile males are the key players in our SIT program. It's efficiency depends on the mating success of sterile males compared to that of wild males. The negative effects of irradiation on sexual competitiveness of fruit flies are well documented. In addition, increasing the irradiation dose beyond the 99 % egg sterilizing dose greatly reduces the mating competitiveness of males. However, most program managers still demand doses of irradiation giving 100% egg sterility resulting in reduced quality of irradiated males. Previous study showed that the effective of irradiation dose for *B. correcta* (Bezzi) was 80 Gy. Our results suggest that a dose of 60 Gy is sufficient to induce sterility. In addition, the results indicate that what changes tend to reduce the acceptability of age of pupae and doses of irradiation on *B. correcta* (Bezzi) for increasable quality insects and easy to handle in SIT program.

IAEA-CN
-131/43P

AREA-WIDE INTEGRATED PEST CONTROL OPERATION IN THAILAND HAVING TWO INTERACTING CLOSELY RELATED SPECIES *Bactrocera dorsalis* sensu stricto AND *Bactrocera correcta* WITH POTENTIAL OF SPECIES COMPLEXITY

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Working group

Since 1987, we have set up a pilot project initially for the control of the Oriental fruit fly (OFF; *Bactrocera dorsalis* (Handel), and recently also for the control of Guava fruitfly (GFF; *Bactroceral correcta*. The effective cooperation between Thailand's Department of Agriculture Extension (DOAE), Office of Atoms for Peace (OAP) and the International Atomic Energy (IAEA), Joint FAO/IAEA, Division for Nuclear Techniques in Food and Agriculture, started in 1991. Fruit fly molecular genetic laboratory at the Department of Biotechnology, Mahidol University (MU) has joined to this cooperative group since 2001. This jointing aims to cooperate several biotechnological R&D in order to enchance the cost effective SIT/AWIPM for a larger scale. The MU is recently under a co-ordinated research project (CRP) entitled "Molecular Technologies to Improve the Effectiveness for SIT".

AWIPM/SIT small pilot program with two similar fruit fly species

An area-wide integrated pest management program involves two different small areas in the provinces of Ratchaburi (34 km²) and Pichit (37 km²) located in the west and north of Bangkok, respectively. There were OFF and GFF infesting fruits in the area of mangoes orchards and the surrounding area having multiple tropical fruits orchards. The infestation levels were as high as 83% to 43%, respectively before the control program. It was found that the OFF:GFF ratio were 1:3.4 and 5:1, respectively from methyl eugenol lure the OFF population control was successful. Therefore,DOAE has resolved to control GFF together with OFF by integrating GFF in SIT and other control methods.

Details about the mass rearing, sterile fly release, surveillance activities, economic assesment of the AWIPM/SIT are described in our seperate poster presentation entitile "AREA-WIDE INTEGRATED CONTROL OF ORIENTAL FRUIT FLY (*Bactrocera dorsalis*,Hendel), AND GUAVA FRUIT FLY (*Bactrocera*) IN THAILAND USING THE STERILE INSECT TECHNIQUE (SIT)" by Orankanok, *et al.* in this meeting.

ORIENTAL FRUIT FLY

GUAVA FRUIT FLY

Why should we compare the OFF and the GFF?

Both OFF and GFF are systematized as Diptera: Tephritidae: Dacinae: of the genus *Bactrocera*. They are both widely distributed pests of Asia. The geographical distribution of the OFF seems to be wider in range from India, Sri Lanka, Nepal across Southeast Asia countries toward the Far East such as southeastern china, Hong Kong, Okinawa and was introduced to Hawaiian. Whereas, the GFF shows overlapping distribution with the OFF mostly in Southern Asia such as India, Pakistan, Sri Lanka, Nepal and Thailand (only the northern and central regions) and not present at all in the Far East countires. The GFF could be differentiated from OFF by their relatively smaller size. The ovipositor of the GFF are relatively shorter and show obvious dark spot at the wing tips. OFF and GFF are both polyphagous pests. The infestation is often mixed between these two species. It was reported that they shared more than 30 types of commercial fruit hosts. However GFF prefers softer fruit skin against hard ones. GFF usually infests fruit hosts at both young and ripen periods so that the outbreak season of the GFF may be longer than the OFF.

B. dorsalis sensu stricto,(OFF) is a member of a large *Bactrocera dorsalis* complex species. It represents a small number of polyphagous members within this complex which are pest of international significance. On the other hand there is no report on similar species complexity status with the *B. correcta*; (GFF). Also, the GFF has not yet become a pest of international significance at the same extent. Thus, comparative ecological studies of the two species may reveal understand about fundamental natural history which may implicate the pest control program.

How are the OFF and GFF density distributions within an AWIPM area?

We have investigated the OFF and GFF population density distributions in a control area in Pichit province during the fruit fly season in March 2004. (Figures 3 and 4) It appears that high population density distribution of the GFF are located within the area of mixed fruit orchards

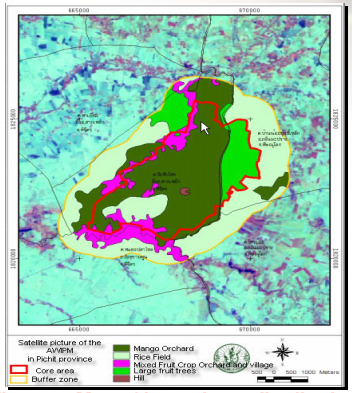

Figure 1. Map of host plants distribution

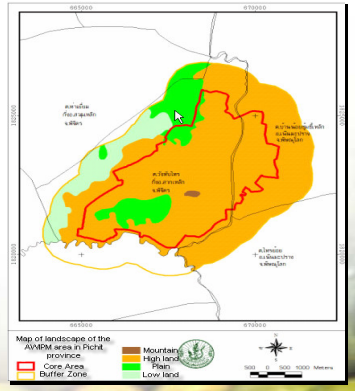

Figure 2. Map of land elevation

On the contrary, the high population density of the OFF appears to be located in the center of the industrial mango orchard area and in the area of large trees fruits which are mono-crop. The GFF:OFF ratios in figure 5 also suggest a consistent pattern that the representation of GFF

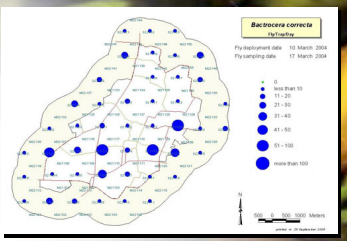

Figure 3. Map of GFF density distribution

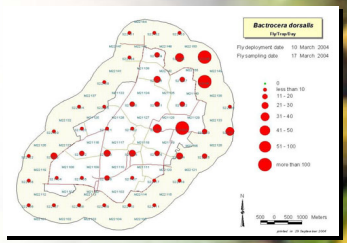

Figure 4. Map of OFF density distribution

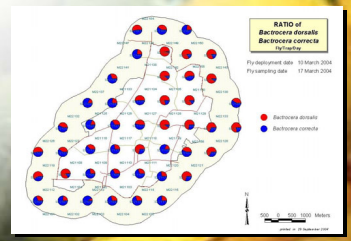

Figure 5. Map illustrating OFF:GFF ratios

Both GFF and OFF show high population densities on highland when compare to plain and low land. (figure 2)

Conclusions. Although, there are two similar fruit fly species present in the same AWIPM area. They may show certain degree of differences in microhabitat and host preferences. Multidisciplinary research approaches are needed and invited to study

DEVELOPMENT OF THE GENETIC METHOD OF CODLING MOTH CONTROL IN VARIOUS CLIMATIC REGIONS

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INTRODUCTION

Codling moth (*Cydia pomonella* L) (Lepidoptera: Tortricidae) is a cosmopolitan insect pest of deciduous fruits in Armenia. The potential loss of crop from codling moth makes it the most important pest management of pome fruits. In case of non-control, codling moth can annually destroy about 90 % of apple croup and 50–70 % of other fruit crops in Armenia.

Different methods of control have been used against codling moth. The most popular method was chemical control for many years. About seven decades, the chemical applications were the main way for codling moth control, but insecticide usage has been failed on worldwide scale due to evolution of pescicide-resistant strains.

Earlier we developed the genetic method of codling moth control (Sarkisyan S., A. Azizyan, 1977, 1978, 1988), based on the phenomenon of inherited sterility (Charmillot *et al.*, 1973, Azizyan et al. 1996, Bloem *et al.* 1999, Carpenter *et al.* 1993, La Chance 1985, North 1975, Proverbs 1978). This method is very simple and the application introduction of hereditarily sterile (HD) diapausing larvae into pest natural populations by synchronizing the development of introduced and wild insects.

The development of new genetic methods of pest control is a multiplane character and includes creation of economically acceptable technology of its development. For successful genetic pest control of the codling moth using the method of synchronized flying out of natural and inherited-defective moths with a lethal burden, it is necessary to forecast the population and phenoecology of the pest correctly, based on the knowledge of its biological peculiarities in specific localities. Moreover, it is of great importance to develop a biotechnological process of an irradiated, breeding, and introduction of defective insects into wild populations.

The complex mountainous relief of Armenia and large variation in altitudes has created a huge variety of climatic conditions. In connection with this, the necessity has arisen to study the biology and ecology of the natural population of the codling moth in various sharply differing climatic regions. Preliminary results obtained suggest the genetic diversity of codling moth populations adapted to different living conditions, i.e. climatic ones and host plants. The results obtained suggest genetic differences between codling moth populations inhabiting diverse conditions and host plant. The adaptive potential of the codling moth is restricted by the fact that it must adapt to the seasonal nature of the fruits as well as to the climatic rhythm (Azizyan et al. 1998, 2000, 2001).

The objectives of the researches are development of genetic control of codling moth populations in various climatic regions. The goal of this study is comparative analysis of the resistance to ionizing radiation in codling moth populations inhabiting various geographical regions having various level of natural irradiation.

RESULTS

The embryonic death in F1 was shown to correlate positively with irradiation dose in all three populations studied. At the same time pests from these geographically isolated populations were demonstrated to have different radiosensitivity. The insects from plains are the most sensitive, from mountain zone - the most resistant, and pests from foothills express intermediate radiosensitivity (Fig.1). The same pattern is specific also for the progeny of F1-males and wild females (Fig.2), or, in other words, for the level of the inherited sterility.

In geographical regions studied the variations in annual sum of solar radiation are as high as 145 (plains), 152 (submountain), and 160 (mountain zone) kcal/cm2. Together with altitude rise the ionizing UV irradiation intensity is known to increase as well. It may be a reason of the revealed radiosensitivity modulations in the populations inhabiting these zones. So, we can consider the phenomenon described as a genetically fixed consequence of adaptive reactions of the insect.

As the intensity of pest irradiation is determined by organism radiosensitivity the results obtained suggest that sterilizing irradiation doses for insects inhabiting different climatic zones have to be various. The highest dose studied (15 Krad) may be recommended to induce the inherited sterility in mountain zone population, the dose 10 Krad seems to be sufficient for foothills population and, finally, as low dose as 8 Krad sterilizes 94,6% of insects from plain zone.

It may be a reason of the revealed radiosensitivity modulations in the populations inhabiting these zones. So, we can consider the phenomenon described as a genetically fixed consequence of adaptive reactions of the insect.

Our previous study had shown that mountain population inhabiting the area of high natural ionization has developed resistance to irradiation. However, it is widely known that sex cells show different radiosensitivity to irradiation at various stages of the development. In this respect, of particular interest would be to study the comparative genetic radioresistance at various stages of gametogenesis in individuals from mountain populations compared to those in the planes.

The comparative studies of sterility and inherited sterility at the time of irradiation of individuals from mountain and plane populations at various stages of development. Each irradiated male in the early stage of pupal and moth development in both populations was sequentially paired with three females (each day per female) which was followed by a genetic and cytological analyses of their generations. Therefore, an opportunity was offered to study each portion of the sperm of an irradiated parent male separately in relation to each female.

The comparative genetic analysis of male sterility in irradiated insects from both populations has shown (Table 1 and Table 2) that the number of fertile eggs decreases in a progeny of each successive female paired with same irradiated male, in plane (pupae 41.2 %, 25.4 %, 20.8 % and moth 45.1 %, 40.5 %, 37.8%) and mountain populations respectively (pupae 59.1 %, 58.3 % , 38.4 % and moth 64.3 %, 60.2 %, 41.6 %). This suggests that the number of genetic damage causing dieback of zygote increases with each consecutive portion of the sperm.

When comparing the level of sterility in irradiated parents at their pupal and moth stages, the sterility in pupae had shown to be higher than in moths. This is particularly evident in the last portions of the sperm that was used to fertilize the second and third females.

It is widely known that sex cells in early pupa are at the earlier stage of the spermatogenesis, i.e. spermatogonium, primary and secondary spermatocyte and are much less at the stage of maturity of the sperm. Whilst, in contrast to pupa the ratio of post-mejosis to pre-mejosis sexual cells increases in moths. Probably, the pupal sex cells found at the pre-mejosis stage at the time of irradiation lead to formation of greater number of dominant lethal mutations (Lyon, 1970, Russel, Spear 1955), which causes the dieback of the zygote.

The comparative genetic analysis of F1 males from irradiated parents (Table I and Table 2), shows that the number of fertile eggs in individuals received from the last portion of the sperm is greater. When comparing the irradiated pupae and moths, the number of fertile eggs in F1 males from the second and third portions of the parents sperm is greater (plane populations 23.5 % and 22.4 , mountain populations 42.7 % and 53.7%) than in moths (plane populations 4.3 % and 11.4% , mountain populations 29.4 % and 28.5 %).

This points to the fact that all successive females were paired using the sperm which was largely at the pre-mejosis stage during irradiation, and those cells that survived the mejosis cell fission proved to be genetically undamaged. Consequently, the inherited sterility decreased compared to the first portion of sperms. And since the majority of pupal cells was at pre-mejosis stage of the fission, the level of inherited sterility in F1 males is lower than in irradiated parent moths.

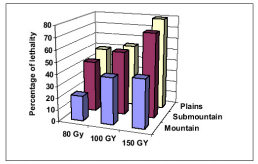

Fig.1. The sterility level of irradiated codling moth males derived from various geographical zones

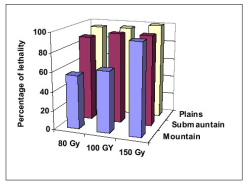

Fig.2. Inherited sterility of F-1 males derived from various geographical zones

This data is supported by a model proposed by a number of authors (Cattanach, 1974, Preston, Brewen, 1976), which maintains that the cells in a population of spermatogonium are heterogenic towards the radiosensitivity. The dieback of spermatogonium results in interim sterility after irradiation. The survived spermatogonium multiply, the number to their populations is restored and they attain the stage of mature sperms, and their sterility terminates. No degeneration of spermatides and mature sperms takes place after irradiation and they maintain the ability for fertilization (Mandl, 1964), however, their genetic damage are observed at successive stages of progeny, which leads to formation of imbalanced gametes in F1 generation and causes inherited sterility (Fig 3).

The comparison of the two populations clearly indicates that on all portions of the sperm individuals from the mountain populations (Table 2) display a much lower lethal effect during irradiation both at the pupal and moth stage than in the individuals of the plains.The statistical difference the percentage of fertility between plane and mountain populations for all portion of sperm and between pupae and moths is confidential P >0.01.

This suggests that the number of genetic damages at all developmental stages of sex cells in populations from the plain zone is higher than in populations from the mountain areas. Hence, the sex cells in the population from the mountain zone proved to be of a higher radioresistance at all developmental stages than those from the plains. This can be probably accounted for by the fact that genetic damages in the last portion of the sperm in the mountain population were less, owing to increased level of resistance in cells at the pre-majotic stage of fission.

Table 1. Genetic analysis of consecutive portions of the sperm in codling moth males irradiated with 100 Gy at various stages of development and paired with sequentially untreated females (plane population)

Sequential number of paired female	Fertility, % M±m					
	(P) treated male x untreated female		Control	F_1 male x untreated female		Control
	2 day old pupae	2 day old moths		2 day old pupae	2 day old moths	
N. 1	41.2±1.5	45.1±2.4	82.6±2.6	13.1±2.5	5.1±0.98	81.4±3.2
N. 2	25.4±3.1	40.5±1.8	80.1±2.1	23.5±3.6	4.3±0.82	78.1±2.9
N. 3	20.8±2.9	37.8±3.4	69.3±1.5	22.4±2.1	11.4±1.8	79.4±3.1

Number of pairs n =15
The statistical difference the percentage of fertility between the first and the last portion sperm of (Student's one-tailed test) is confidential P >0.01.
The statistical difference the percentage of fertility between the first portion of sperm of irradiated pupae and moths is confidential
P >0.2 and last of portion of sperm of irradiated pupae and moths is confidential P >0.01.

Table 2. Genetic analysis of consecutive portions of the sperm in codling moth males irradiated with 100 Gy at various stages of development and paired with sequentially untreated females (mountain population)

Sequential number of paired female	Fertility, % M±m					
	(P) treated male x untreated female		Control	F_1 male x untreated female		Control
	2 day old pupae	2 day old moths		2 day old pupae	2 day old moths	
N. 1	59.1±4.4	64.3±4.2	86.4±3.9	28.3±2.3	21.9±2.4	88.9±3.2
N. 2	58.3±2.0	60.2±2.9	85.2±2.7	42.7±3.4	29.4±3.1	84.7±4.1
N. 3	38.4±1.8	41.6±3.0	75.4±3.4	53.7±3.3	28.5±2.7	86.1±2.9

Number of pairs n =15
The statistical difference the percentage of fertility between the first and the last portion sperm at irradiated pupae and moths is confidential P >0.01.
(Student's one-tailed test) is confidential P >0.01.

The genetic analysis was confirmed by the cytological analysis of F1 males. It showed that the inherited sterility was correlated with the number of chromosomal aberrations in F1 males received from irradiated pupae and the last portions of the sperm is lower than in those from irradiated moths and the last portions of the sperm of an irradiated parent (Fig. 3 and 4). Concurrently, it is noteworthy that the total of percentage of inherited sterility and number of chromosomal aberrations in F1 males from the mountain zone is lower than in those from the planes.

Acknowledgements

The authors are grateful to the FAO/IAEA, Insect and Pest Control Section for supporting this work.

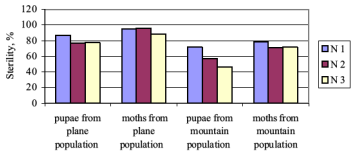

Fig 3. Inherited sterility in progeny F1 males depending on portions of the sperms of males irradiated at various stages of development and paired with sequentially untreated females N 1 –first female, N 2 –second female and N 3 – third female

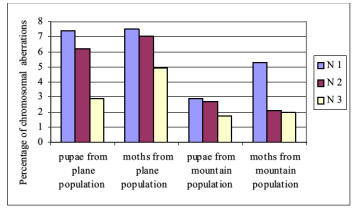

Fig 4. Frequency of chromosomal aberrations in F1 males progeny of males irradiated at various stages of development and paired with sequentially untreated females (50 testicle F1 males examined in each treatment)

ADVANCES IN AREA-WIDE CONTROL SINCE 1ST INTERNATIONAL CONFERENCE – ZAMBIA POSITION AND EXPERIENCES IN TSETSE CONTROL

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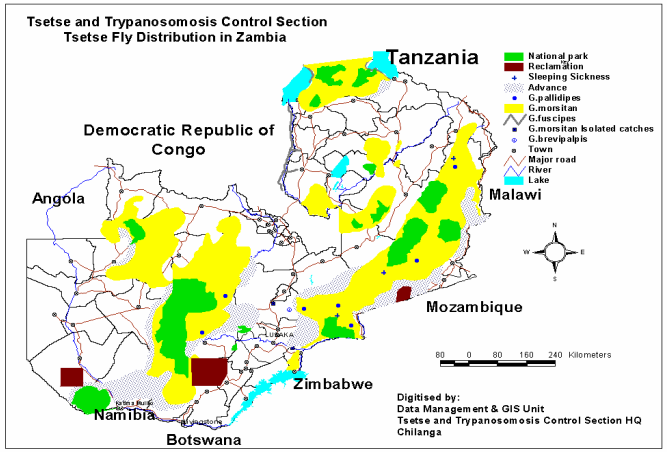
INTRODUCTION

In Zambia trypanosomosis is one of the major constraints to sustainable agricultural development particularly in the traditional sector, which accounts for about 80% of the national livestock (cattle) herd in the country. The remaining 20% commercially managed herd is located in tsetse free areas. Coetzer *et al.*,(1994), reported that more than five-eighths of Zambia is tsetse infested, and the boundaries of tsetse infestation are not constantly monitored, indicated boundaries are only estimates. In the late 1980's it was reported by Chizyuka and colleagues, that two thirds of the country was infested with tsetse flies and 25% of the traditional herd was at risk of trypanosomosis. Currently Zambia accounts some 2.8 million cattle, a million goats, a marginal number of sheep and 0.5 million pigs.

PAST EXPERIENCE

During the last 15 years Zambia had achieved a lot in terms of tsetse control in the country. Tsetse densities and the disease prevalence were brought down to almost fly density as high as 7 fly/trap/day to as low as 0.05 fly/trap/day and trypanosomosis prevalence as high as 20% to as low as 0% in the tsetse controlled areas. Approximately 50,000 Km2 had been controlled of tsetse under projects funded by different donors in Western, Southern, Lusaka and Eastern provinces. In recent years the country has been experiencing re-invasion of the areas that where once cleared.



Map 1. Tsetse distribution in Zambia

The main specific problems experienced from past control operations under the support of donors (EU, Belgium, Dutch) include among others:

- Re-invasion of tsetse flies in controlled areas when maintenance activities relaxed due to insufficient funding and inconsistent release of funds.
- High costs of keeping controlled areas free of tsetse indefinitely. Small areas are difficulty to maintain free of tsetse.
- The areas of concern got re-infested with flies from neighboring areas that are not under control.
- Minimal regional cooperation and collaboration due to varying priorities along border areas.

It is against this background that Zambia is advocating for area-wide control of tsetse flies in the country and the region. One of the areas where this concept is being applied is the Kwando-Zambezi region where Namibia, Botswana, Angola and Zambia have common boundaries. All the four countries have agreed to eradicate/control tsetse in this region using integrated user friendly methods (e.g. odoured baited targets, aerial spray, SIT). Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) has ear-marked this area as a start point for tsetse eradication in the Southern African Region and is encouraging member countries to use the concept of Area-Wide control of tsetse flies in a coordinated manner. Zambian component that has been earmarked in the first phase is about 27,000 Km2 divided into in two blocks.

Work done that has been done in readiness for tsetse control/eradication in the Kwando- Zambezi Region on the Zambian side

Zambia has already started procurement of materials to clear invaded tsetse, holding sensitization and consultative meetings with other countries.

Target barrier servicing

A target barrier of 6,000 targets preventing invasion of tsetse into the controlled area **(Map 2)** of Shangombo (11,500km2) was serviced in December 2004. Disposable Blue/black 1 m x 1 m stick targets were used. They were treated with FASTAC 10% SC at 1% concentration.

Deployment of targets in the Shangombo district, Western Province.

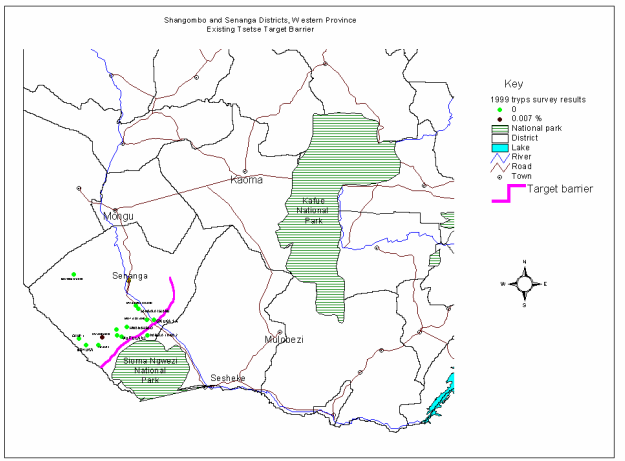
From December 2004 to the first quarter of the year 2005, a total number of 3150 targets were deployed to mop flies that had invaded the controlled area of Shangombo **(Map 2)**.

DISCUSSION

The once tsetse cleared areas in Zambia have been re-invaded by tsetse flies from adjacent areas. This urgently calls for an area-wide control of tsetse at international/Regional levels as called for by Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC).

CONCLUSIONS

Zambia fully support the area-wide control/eradication of Tsetse flies from this region has it will solve the problem for ever. This fly-belt in the Western province of Zambia seems to be an isolated pocket extending into Nambia, Botswana and Angola.



Map 2. Target barrier in the Shangombo Area

Effect of X-ray Irradiation on the male moths of two voltine groups of the silkworm *Bombyx mori* and inheritance of induced sterility

CN131-046P

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Lepidopteron insect *Bombyx mori* an eukaryotic bisexual higher organism have since long been used as a material of choice for radiobiological studies and they have biological advantage of exhibiting sequential events of spermatogenesis in the homogametic males (XX) and oogenesis in heterogametic females (XY). The present study is an attempt to understand the inheritted sterility in the progenies of X-ray irradiated male moths of silkworm *B.mori* belonging to two voltine groups of Indian origin.

Male moths were irradiated with two doses of 5kR and 10 kR X-rays with 8mA, 2.5 mm Aluminium filter and a dose rate of 200R/minutes. Eggs laid by the untreated bivoltine female moths crossed to treated males and its progenies were acid treated to get continuous hatching. The non-irradiated batches were used as control. The rearing was conducted at rearing house of the department providing standard laboratory conditions and two important parameters of embryonic lethality and unfertilized eggs were recorded following the procedure of Tazima (1956). Appropriate statistical procedure was applied to analyse the data.

Results and Conclusion

The results of the experiment presented in table 1 and summarised in Fig-1 showed a typical case of embryonic death and unfertilized eggs in the parental generation. The percentage of unfertilized and unhatched eggs (28-42%) are significantly higher in the treated batches compared to those of control batches. From the results presented in table 1 it is clear that spermatozoans invariably present in adult moths are equally sensitive to two doses of X-rays at 5kR and 10kR in the induction of sterility and unhatched eggs in the parents.

The experimental results related to inherited sterility is summarised in table-2 and is shown in Figs 2-4. As seen from the tables it is clear that there is a significant reduction in the number of unfertilized and unhatched eggs from F_1-F_3 generations and there is marked increase in the hatchability. Although mechanism causing inherited sterility in silkworm was not apparent, in a similar experiments Sugai and Mirumachi (1973) in *Bombyx* attributed the cause of sterility to reciprocal translocations, which could be transmitted to the progenies. Murakami (1974) using silkworms proposed that X-ray treatment results in delayed lethal effects in the progenies and reciprocal translocation may not be an obstacle and individuals without genetic damage results in normal reproduction. The results of the present experiment is in support of the findings of the above authors. The X-rays have induced functionless spermatozoan in the male moths (cytological observation of spermatozoan by the authors). It is opined that the functionless spermatozoa is transmitted to the gametes resulting in sterility and embryonic death.

Table-1: Hatchability and Sterility in the eggs laid by female silk moths crossed with x-ray irradiated male moths in two voltine groups of silkworm, *Bombyx mori*.

Race and voltinism	Dose rate (kR)	No. of moths Irradiated	Irradiated moths mated with untreated females (No)	No. of eggs laid	No. of unfertili-zed eggs	No. of unhatc-hed eggs	* No. of hatched eggs
Pure Mysore I (Multivoltine)	5	20	12	3000	840(28)	1100(37)	1060(35)
-do- II	10	20	10	2200	660(30)	812(37)	728(33)
Kalimpong- A I (Bivoltine)	5	20	11	3300	990(30)	1386(42)	924(28)
-do- II	10	20	09	2800	1008(36)	1120(38)	672(26)
Control Pure Mysore		20	-	7200	-	-	90
Kalimpong-A		20	-	8140	-	-	92

* Values are significant at 5% level through student "t" test.
Figures in the parentheses are percentage values.

Table –2. Egg hatchability and sterility in the F1, F2 and F3 progenies derived from a cross between X-ray irradiated male moths with untreated females at parental generation.

Generation	Race and Voltinism	No. of Pairs	%	Unfertilized %	*Hatched %
F1 progenies of Irradiated males X untreated females	Pure Mysore- I (Multivoltine)	20	60	30	10
	-do- II	20	65	28	07
	Kalimpong-A - I (Bivoltine)	20	64	28	08
	-do- II	20	67	26	07
Control	-	20	-	-	90
F2 progenies of Irradiated males X untreated females	Pure Mysore- I (Multivoltine)	20	67	18	15
	-do- II	20	67	19	14
	Kalimpong-A - I (Bivoltine)	20	65	22	13
	-do- II	20	67	21	12
Control	-	20	-	-	89
F3 progenies of Irradiated males X untreated females	Pure Mysore- I (Multivoltine)	20	67	5	28
	-do- II	20	68	7	25
	Kalimpong-A - I (Bivoltine)	20	65	9	26
	-do- II	20	65	10	25
Control	-	20	-	-	88

Note: I=Moths derived from progenies exposed to 5 kR
II=Moths derived from progenies exposed to 10 kR
• Values are significant at 5% through "t" test.

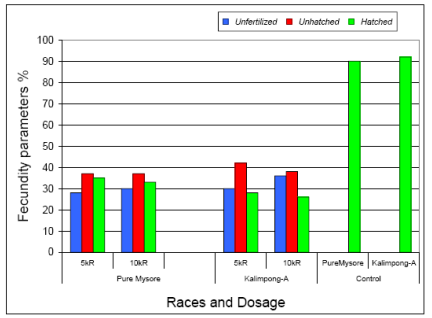

Fig-1. Effect of X-rays on the male moths of two races of silkworm *Bombyx mori* on hatchability and sterility in the parental generation.

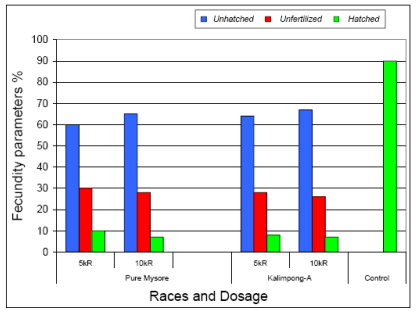

Fig-2.Hatchability and sterility in the F_1 progenies derived from irradiated male moths of *Bombyx mori*.

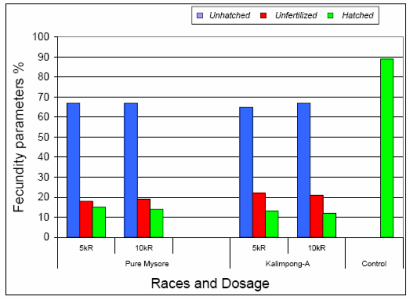

Fig-3.Hatchability and sterility in the F_2 progenies derived from irradiated male moths of *Bombyx mori*.

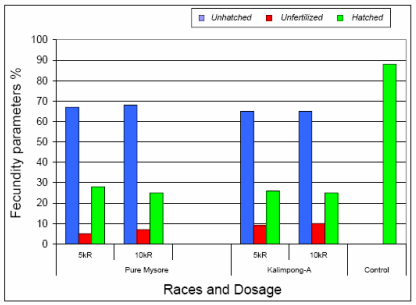

Fig-4.Hatchability and sterility in the F_3 progenies derived from irradiated male moths of *Bombyx mori*.

Use of radiation in extending the duration of host suitability for managing *Ephestia kuehniella* and *Sitotroga cerealella* by egg-parasitoid, *Trichogramma evanescens*

AYDIN S. TUNCBILEK, ULKU CANPOLAT, & FAHRIYE SUMER
Erciyes University, Faculty of Arts & Sciences,

Objective:
The aim of this study were to evaluate the effects of gamma radiation, storage temperature, and duration of host egg parasitization by *T. evanescens* and to determine the role of these factors on the induction of diapause in *T. evanescens,* as a way of facilitating extended storage.

Results

Storage experiments

When the hosts' eggs have previously been irradiated with gamma radiation to prevent development and than stored, two way anova analysis show that parasitization and adult emergence of *T. evanescens* were not affected by irradiation doses for both eggs of *E. kuehniella* and *S. cerealella* (Fig. 1). However, parasitization of *S. cerealella* eggs irradiated with 50 Gy was significantly affected In general, parasitization and adult emergence of *T. evanescens* were not influenced by gamma radiation for both host eggs.

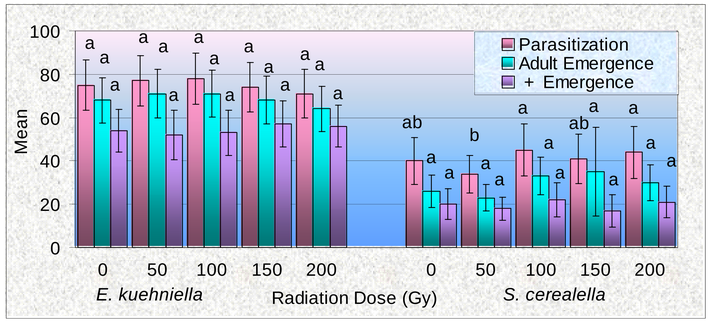

Fig. 1. Effect of irradiation dose of stored eggs of *E. kuehniella* and *S. cerealella* on parasitization and adult emergence of *T. evanescens*.

In the same way, there was no significant differences on parasitization and adult emergence of *T. evanescens* between control and stored eggs for up to 30 days, and but it was significant difference (87 and 84%, respectively) at 60 days when reared on *E. kuehniella* eggs. At 90 day no parasitization as well as emergence were observed . On the other hand, parasitization and adult emergence of *T. evanescens* from *S. cerealella* eggs was significantly reduced for up to 30 days, very less parasitization was observed, and no wasp emergence was recorded at 60 days of storage (Fig. 2).

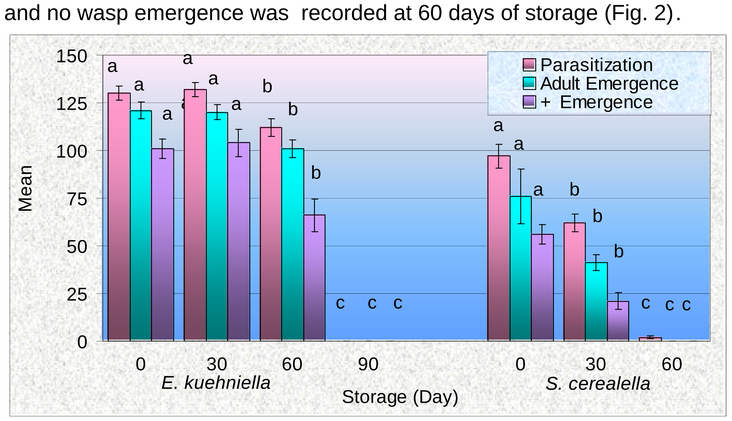

Fig. 2. Effect of storage time of irradiated eggs of *E. kuehniella* and *S. cerealella* on parasitization and adult emergence of *T. evanescens*.

Diapause experiments

Data obtained from diapaused *T. evanescens* regarding prestorage and storage temperatures are given Fig. 3 for host eggs of *E. kuehniella* and *S. cerealella*. Results indicated that prestorage temperatures affected the induction of diapause. It was possible induce diapause in developmental stages of *T. evanescens* by exposing the preimaginal stages (prior to the prepupal stage) to 10 and 12ºC for 30 days.

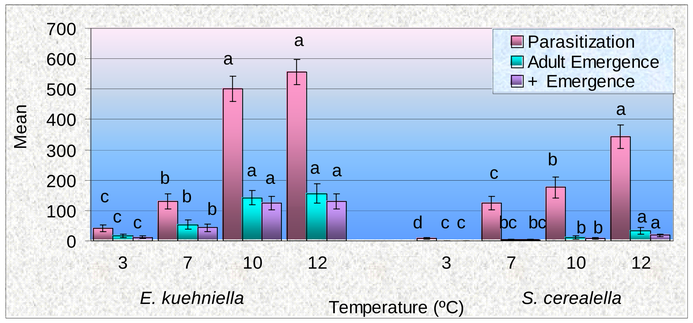

Fig. 3. Influence of storage temperature of *E. kuehniella* and *S. cerealella* eggs on parasitization, adult and female emergence ratios of *T. evanescens*' diapause.

The number of parasitization and adult emergence at the prestorage temperatures of 3 and 7ºC decreased considerably. In contrast, the development of parasitoids that were held at 10 and 12ºC for 30 days was entered diapause, tolerating storage at 3ºC for a period achieving 150 days. The number of parasitization and adult emergence of *T. evanescens* reared on the egg of *E. kuehniella* were meaningfully higher than *S. cerealella* (Fig. 4).

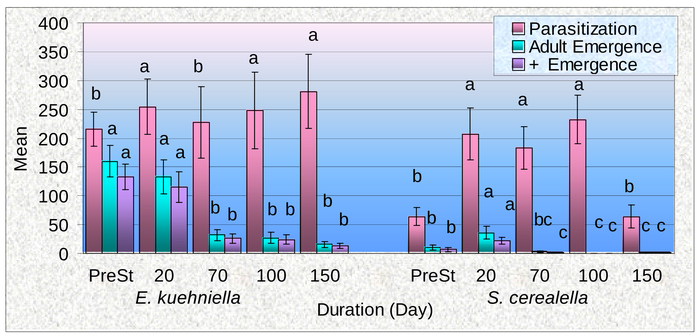

Fig. 4. Influence of storage duration of *E. kuehniella* and *S. cerealella* eggs on parasitization, adult and female emergence ratios of *T. evanescens*' diapause.

* Means followed by the same letter between treatment groups are not significantly different at the 5% levels of confidence by an analysis of variance and Tukey HSD.

Conclusions

Parasitization and adult emergence of *T. evanescens* were not influenced by gamma radiation for both host eggs. Thus, these eggs could be used both for mass rearing program and for warehouse releases to avoid any problem posed by the hatching of host eggs.

The access production of the eggs of *E. kuehniella* can be stored for up to 30 days at 4ºC after irradiation at the dose of 200 Gy without any loss in parasite production number and quality. Nevertheless, there was significant difference in number of emergence for up to 30 and 60 days for eggs of *S. cerealella* and *E. kuehniella*, respectively.

Data obtained from diapaused *T. evanescens* indicate that prestorage temperatures affected the induction of diapause. The parasitoid could be stored at low temperature for a period of 50 days, without adverse affects on emergence. Thereafter the emergence of *T. evanescens* appeared to decrease with an increase in the duration of storage at low temperature, for a period achieving 150 days

The long-term storage of parasitoids in diapause allows an enlargement in the mass rearing potentialities of these species for future biological control releases by allowing procedures to stockpile the parasitoids for release in the suitable time.

In our study egg of *E. kuehniella* is more suitable host for *T. evanescens* than egg of *S. cerealella*.

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Different paired male & female of *Campoletis chlorideae* Uchida parasitised on chickpea pod borer *Helicoverpa armigera* Hübner in Myanmar

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Pot Experiment

Different pairs of parasitoid *C. chlorideae* affected significantly the survival of different stages of *H. armigera* larvae ($p = 0.0001$, $R^2 = 0.963$, CV = 10.98) (Table 1 & 2).

Table 1 Comparisons of different pairs of parasitoid *C. chlorideae* affect on different stages of *H. armigera* larvae (df = 17, MSE = 1.309. All comparisons are significant at the 0.05 level).

Different population of parasitoid	Means population of larvae *H.armigera*	Difference between Means	Tukey's grouping
5 pairs	12.83	1.83	A
4 pairs	11.00	1.33	B
3 pairs	9.67	1.50	B
2 pairs	8.17	-	C

Table 2 Comparisons of different stages of *H. armigera* larvae affected by parasitoid *C. chlorideae* (df = 17, MSE = 1.309. All comparisons are significant at the 0.05 level).

Different population of parasitoid	Means parasitization	Difference between Means	Tukey's grouping
2nd stage	15.38	3.88	A
1st stage	11.50	7.12	B
3rd stage	4.38	-	C

Two pairs of parasitoid *C. chlorideae* affected highly the survival of 30 larvae of *H. armigera* (Figure 1). Parasitism rate (%) of *C. chlorideae* on the larvae of *H. armigera* was found to be the highest (55 %) in 5 pairs of male and female and *C. chlorideae* affected the 2nd stage of larvae of *H. armigera* in the pot experiment of chickpea in 2002 – 2003 Winter Season at Paleik (Figure 2).

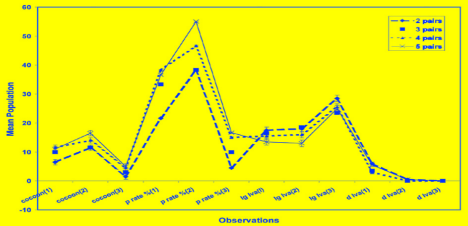


Figure 1 The host preference of *Campoletis chlorideae* on the larvae of *Helicoverpa armigera* in the pot experiment of chickpea in 2002-2003 Winter Season at Paleik.

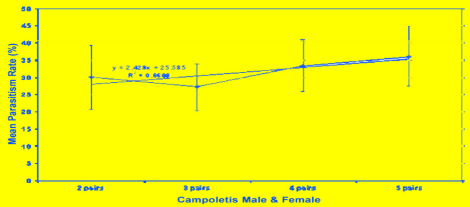
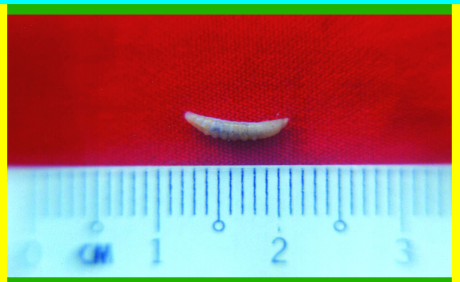


Figure 2 Parasitism rate of *Campoletis chlorideae* on the larvae of *Helicoverpa armigera* in the pot experiment of chickpea in 2002-2003 Winter Season at Paleik.



Field Experiment

Different pairs of parasitoid *C. chlorideae* significantly affected the mean survival of population of *H. armigera* larvae. Five pairs of parasitoid affecting on the larvae of *H. armigera* was the lowest when compared with all utilized different pairs released (Table 3). The parasitoid *C. chlorideae* affected highly the 2nd stage of *H. armigera* larvae (Table 4).

Table 3 Tukey's studentized range test for comparisons of different pairs of parasitoid *C. chlorideae* affect on different stages of *H. armigera* larvae (df = 17, MSE = 1.309. All comparisons are significant at the 0.05 level).

Different population of parasitoid	Means population of larvae *H.armigera*	Difference between Means	Tukey's grouping
5 pairs	10.83	0.83	A
4 pairs	10.00	1.33	A
3 pairs	8.67	2.17	B
2 pairs	6.50	-	B

Table 4 Tukey's studentized range test for comparisons of different stages of *H. armigera* larvae affected by parasitoid *C. chlorideae* (df = 17, MSE = 1.309. All comparisons are significant at the 0.05 level).

Different population of parasitoid	Means parasitization	Difference between Means	Tukey's grouping
2nd stage	13.38	3.63	A
1st stage	9.75	6.25	B
3rd stage	3.50	-	C

Parasitism rate (%) was found to be highest on the 2nd stage of *H. armigera* larvae (Figure 3). Four pairs of released parasitoid were highest on the larvae of *H. armigera* in the field experiment of chickpea in 2002 – 2003 Winter Season at Paleik (Figure 4).

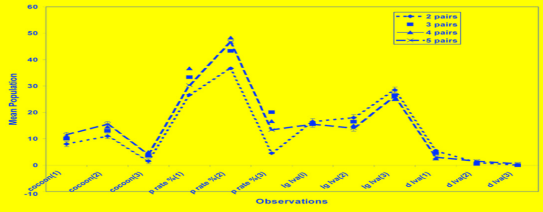


Figure 3 The host preference of *Campoletis chlorideae* on the larvae of *Helicoverpa armigera* in the field experiment of chickpea in 2002-2003 Winter Season at Paleik.

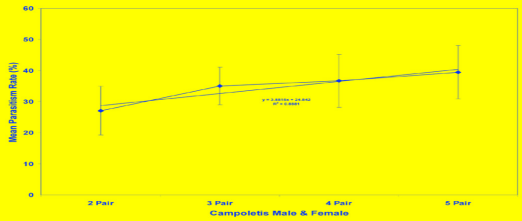


Figure 4 Parasitism rate of *Campoletis chlorideae* on the larvae of *Helicoverpa armigera* in the field experiment of chickpea in 2002-2003 Winter Season at Paleik.

Parasitization was highest on the 2nd instar of *H. armigera* in both laboratory and field experiments.

The highest parasitization (55%) was found in 5 pairs of released parasitoid in laboratory experiment, but (48.33%) in 4 pairs of released parasitoid in the field experiment.

Generation of microsatellite markers in the medfly, *Ceratitis capitata* and their use in physical and genetic mapping

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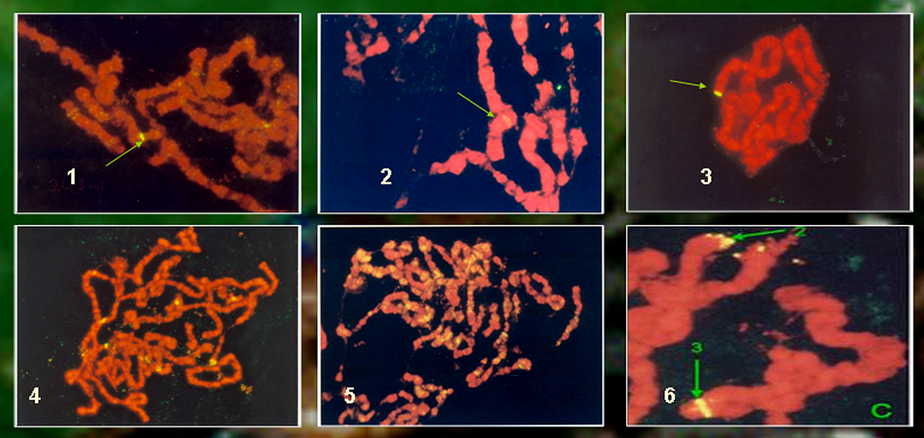
Generation of microsatellites. Eight different small-insert genomic libraries were constructed from genomic DNA extracted from about 100 flies of the wild type strain *Benakeion*, digested separately with *AluI, RsaI, MboI*, *HpaII* or *Sau3A* and cloned into *pBS* or *pUC18* plasmid vectors. Clones containing microsatellites were identified by filter hybridization, mainly with radiolabeled $(TC)_{15}$ and $(TG)_{15}$ oligonucleotide probes. Following DNA sequencing of the positive clones a total of 132 microsatellite bearing clones were identified. A number of clones were found to contain more than one distinct tandem repeat, thus raising the number of microsatellites to 223. The most common microsatellite motif was the dinucleotide $(TG)_n/(CA)_n$ occurring in 105 out of the 223 microsatellite loci (Table 1). Primer pairs were designed in unique sequences flanking each microsatellite for uniform amplification conditions. Amplification products for each primer pair was screened against genomic DNA of the *Benakeion* strain to examine product size and quality. Those microsatellites exhibited the expected size and sufficient quality (108 out of the 118 pairs tested) were in turn used for genotype analysis.

Table 1

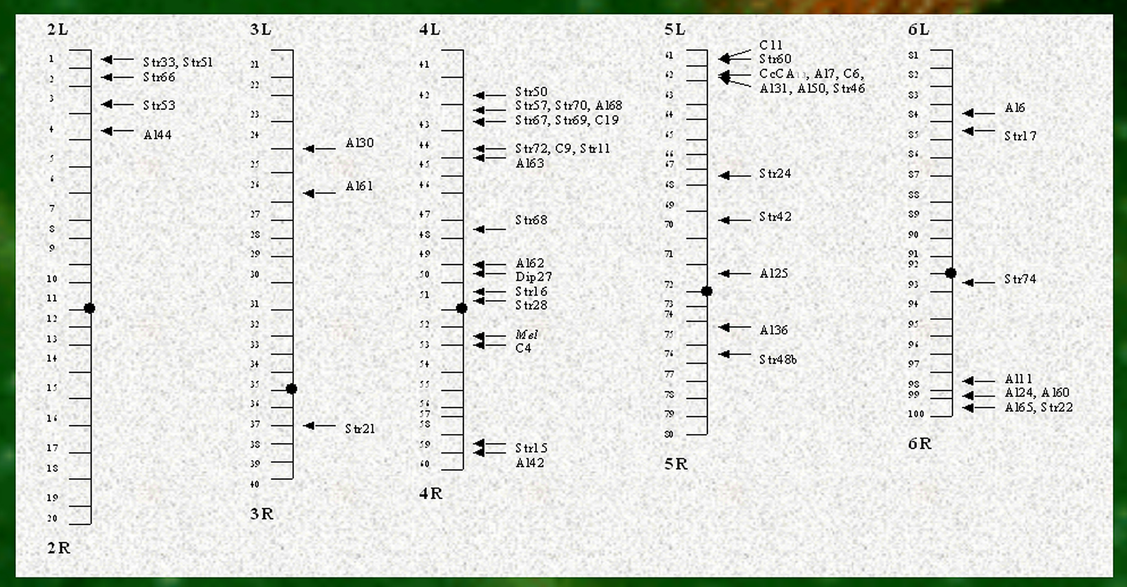
Repeat unit (bp)	Repeat motif	Number of microsatellites
1	A / T	41
2	**TG / AC**	**105**
	TC / AG	32
	TA / AT	21
3	GTT / CAA	8
	GAA / CTT	4
	ACG / TGC	1
	AGG / TCC	1
	ATT / TAA	1
4	CCTT /GGAA	1
	TTGG /AACC	1
	TATG / ATAC	4
	TTAA /AATT	1
5	AAATTT	1
8	GAAGAAGT	1
TOTAL		223

Figures 1- 6



Physical mapping. Fluorescence *in situ* hybridization (FISH) was performed on medfly salivary gland polytene chromosomes for localization of the isolated clones bearing microsatellites. From the 111 clones tested, 49 showed a unique cytological position (Figure 1-3), 31 showed multiple hybridization signals (Figure 4-6) and 31 did not show any signal at all. The localization of the 49 microsatellite clones on polytene chromosome maps is shown in Figure 7. As it is shown, their distribution is neither analogous to the size of the chromosomes nor uniform throughout each chromosome. It should be pointed out that medfly sex chromosomes are heterochromatic and do not form polytene elements.

Figure 7



Genetic crosses and Genotyping. Primer pairs were evaluated for polymorphism on several available wild type strains in an effort to discriminate the most polymorphic strain for linkage analysis. Following this, crosses were set up *en masse* between the *Benakeion* and *Madeira* strains and the F1 females were individually backcrossed with one of the parental strains. The F1 parents were genotyped by PCR amplification of individual microsatellite loci. Finally, 175 F2 progeny from two single pair backcrosses were genotyped for 69 polymorphic microsatellites. The genotype of each fly was determined by electrophoretic analysis of the PCR products followed by autoradiography. Furthermore, primer pairs of those loci physically localized on chromosomes, were tested on F1 parents of single pair backcrosses between the *Benakeion* and multiple visible marker strains for the four out of the five autosomes (2^{nd}, 3^{rd}, 4^{th} and 5^{th}). Pairs showing the appropriate polymorphism were subsequently genotyped on F2 progeny of the respective crosses in order to map the microsatellites in relation to the visible markers. A total of 745 progeny derived from seven backcrosses corresponding to the second, fourth and fifth autosome respectively, were genotyped for 9 microsatellite loci. No one polymorphic microsatellite was found in the crosses for the third chromosome.

Linkage analysis and map construction. Linkage analysis was performed using the JoinMap® 3.0 program with a LOD score ≥3. This analysis resulted in six linkage groups based on 69 microsatellite markers genotyped in two backcrosses between *Benakeion* and *Madeira* strains. Based on the cytological localization of several microsatellites, four of these groups were assigned to their respective chromosomes, corresponding the 2^{nd}, 4^{th}, 5^{th} and 6^{th} autosomes. The other two, probably correspond to autosomes based on the absence of sex-linked inheritance of the respective markers. However, due to the lack of cytogenetic data they cannot be assigned to a specific chromosome. In these linkage groups three additional microsatellites as well as ten visible markers were included from the second type of single pair backcrosses. Each linkage group was further analyzed with JoinMap in order to construct the final genetic map. Although 72 microsatellite loci were analyzed, only 48 were finally incorporated into the maps (Figures 8-11). This may be due to the limitations in the data and the pooling of information from several families, each analyzed for only partially overlapping subsets of markers. In addition, maps which correspond to the same chromosome/ linkage group can not be integrated. This would be explained by the number of markers analyzed in combination to their non-random distribution in relation to the insect's genome size.

Concluding remarks: In the present work we display the first "microsatellite based" genetic maps for the medfly in which several visible markers have been incorporated and orientated, specifically for the 2^{nd} and 4^{th} chromosome respectively. Although these maps do not cover the entire genome, the sex chromosomes and the 3^{rd} autosome are not included; they constitute the first step towards the construction of high density genetic maps and represent a significant advance in the genetics of this important species.

Figure 8

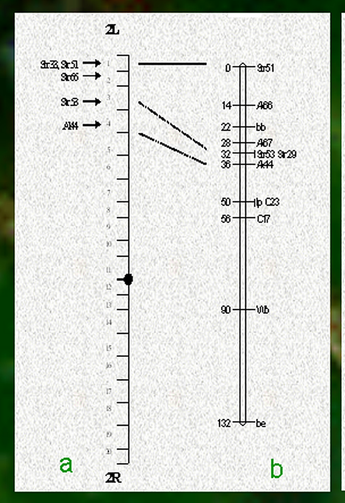


Figure 9

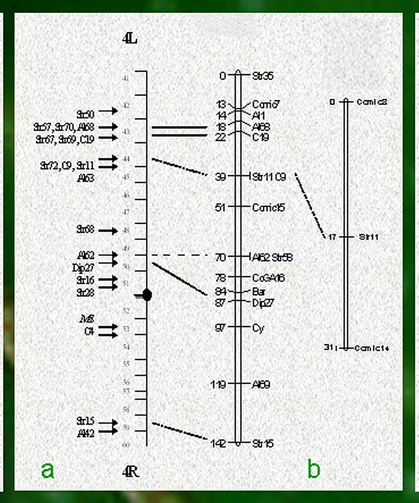


Figure 10

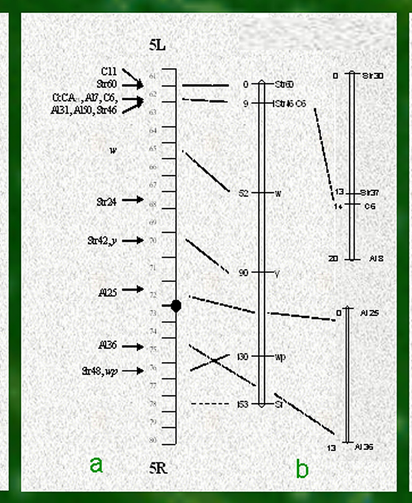
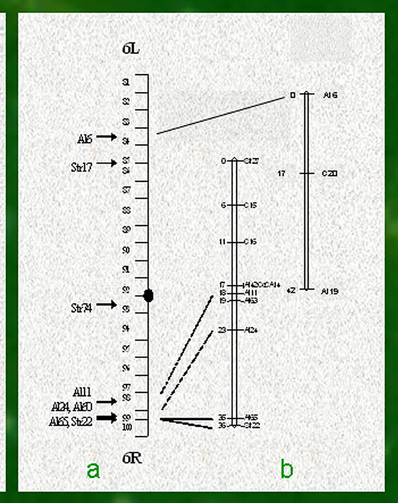


Figure 11



a: Polytene chromosome map b: genetic map

Outcomes-based Planning and Implementation Led to the Success of the Hawaii Fruit Fly Suppression Program

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UH CTAHR Plant and Environmental Protection Services[1] and USDA Agricultural Research Service[2]

Abstract

A critical component of successful area wide pest management (AWPM) programs are organized, coordinated and comprehensive outreach educational programs. The Hawaii Area Wide Fruit Fly Pest Management (HAW-FLYPM) program's educational program, a part of a USDA AWPM program in Hawaii, utilized the "logic model" approach to organize, plan, execute and evaluate farmer and community educational programs statewide. The logic model approach was an outcome-driven rather than activity based method that employed a linear sequence that developed relationships between program inputs, outputs and outcomes.

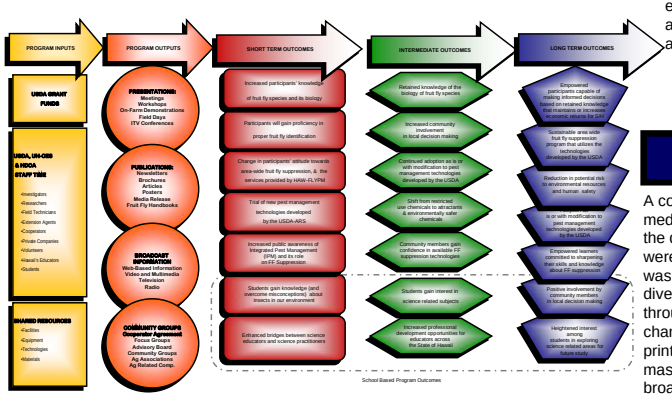
This model was utilized extensively to transfer sustainable, science-based technologies to suppress tephritid fruit fly pests. HAW-FLYPM's educational program targeted growers and community door yard growers, three teaching curricula aimed at elementary through high school students, and a statewide awareness program for the public at large. Additional key components of the HAW-FLYPM education program was the development of implementation schedules used to track program progress, a comprehensive media matrix was developed to ensure educational materials met the needs of target audience groups, and a sustainability calculator to assess the likelihood of program sustainability after the initial five year funding cycle. Program impacts include empowered learners with the knowledgebase to make informed decisions, development of sustainable fruit fly control techniques, reduction in fruit fly populations, decrease in infestation levels, reduction in organophosphate usage, increased community involvement in local decision-making, improved attitude towards fruit fly suppression efforts, enhancement of food safety with the reduction of high risk chemical, and heightened level of satisfaction with the services provided by UH CES.

The model served as a "blue print" for ensuring program elements were planned, delivered and executed on a timely basis. Utilization of the logic model to organize efforts and manage diverse, multi agency programs such as the HAW-FLYPM program has shown to be a successful method of program advancement and outcome achievement.

Logic Model Approach

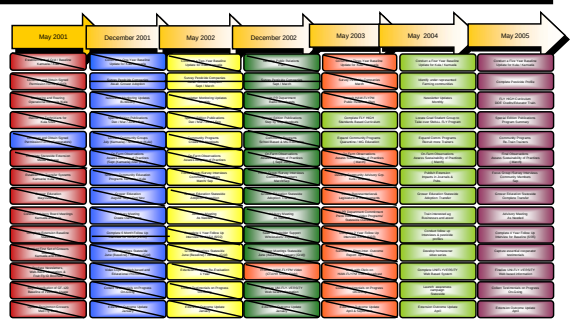
Designing an organized and relevant program plan can lead to effective extension educational programs. The HAW-FLYPM outreach education program utilized the logic model planning approach because it provided a sound outcomes-oriented framework for organized statewide outreach in an easy to convey format for industry and funding stakeholders.

The logic model subdivides goals and objectives into attainable short, intermediate and long term outcomes. It provided a "road-map" to plan, execute and evaluate short, intermediate and long term effects and was flexible in allowing changes to be made along the way.



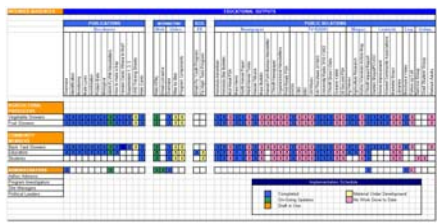
Implementation Schedule

A comprehensive implementation schedule was created to track and assure orderly implementation of educational programs at the three demonstrated areas. The schedule was needed to assure that infrastructure, educational, and evaluation activities were completed in a timely manner.



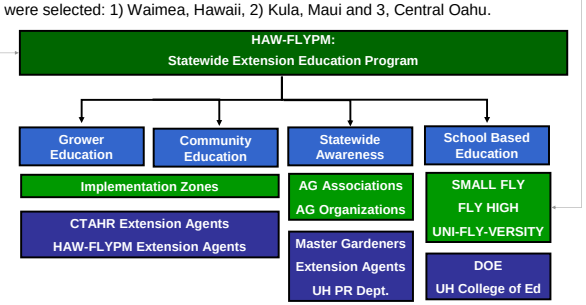
Comprehensive Extension Material Matrix

A comprehensive outreach educational media matrix was developed to ensure that the diverse needs of the intended audiences were being met. The matrix's main purpose was to ensure educational materials met the diverse needs of targeted audience groups through appropriate information delivery channels. Educational material included printed material, video, web pages, printed mass media articles, radio and television broadcasts.



Targeted Audience Groups

Four audience groups were targeted by the HAW-FLYPM outreach education program: 1) Commercial Growers, 2) Backyard Growers, 3) Community Members, and 4) Hawaii's Educators and Students. Three implementation sites were selected: 1) Waimea, Hawaii, 2) Kula, Maui and 3, Central Oahu.



Program Impacts

Oahu
Acres under Integrated Pest Management: 4200 acres
Grower and Community Cooperators: 100
Melon fly infestation rates down from 30% to less than 1%
Reduction in organophosphate usage: 90%
Estimated economic benefit of program on bitter melon: $7,273/a

Maui
Acres under Integrated Pest Management: 450 acres
Grower and Community Cooperators: 84
Melon fly infestation rates down from 40% to less than 5%
Reduction in Mediterranean fruit fly: 95%
Reduction in organophosphate usage: 50%
Estimated economic benefit of program on zucchini: $7,321/a

Hawaii
Acres under Integrated Pest Management: 1500 acres
Grower and Community Cooperators: 250
Melon fly infestation rates down from 20% to less than 2%
Reduction in organophosphate usage: 100%
Estimated economic benefit of program on citrus: $2,843/a

- ✓ Adoption of a sustainable suppression program utilizing an "area-wide" approach by most growers
- ✓ Heightened knowledge and competencies among grower and community groups
- ✓ Advanced adoption of environmentally acceptable, cost-effective fruit fly suppression technologies
- ✓ Shifted pesticide use to environmentally friendlier alternatives
- ✓ Enhanced bridges between science educators and practitioners
- ✓ Established 'certified' trainers throughout the state
- ✓ Increased fruit fly suppression technologies available to the general public
  - ✓ Advanced worker and environmental safety
  - ✓ Heightened level of food security
  - ✓ Increased contribution to state's economy

National Preventive Campaign Against Cactus Moth *(Cactoblastis cactorum)* in Mexico

IAEA-CN-131/51 P

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Mexico has the highest genetic diversity of cactus (*Opuntia* spp.), with 107 species of this genus (51 species of Platyopuntia and 56 of Cilyndropuntia; 38 native of Mexico). It is estimated that from cactus growing areas 150,000 ha are for forage, 60,000 ha for fruit production, 10,500 ha for green vagetable and 100 ha to mealybugs for making dye. In addition, wild cactus areas in the country cover aproximately 3,000,000 ha. Cactus in Mexico is an important resource for human, and livestock food diet. It is used to dye and as mechanism to prevent soil erotion. It's a great source to generate employments, it is the most important component of genetic biodiversity and host for a wide variety of wildlife. The cactus moth (*Cactoblastis cactorum*) in its larval stage feeds on opuntias, therefore, it has been used worldwide as a control agent againts cactus species considered as invasive. *Cactoblastis cactorum* was introduced in countries where some cactus especies were considered as invasive species (Australia 1925, South Africa 1933, Hawaii 1950 and West Indies 1960).

Since its introduction either voluntary or accidentally, in the Carribbean, Nevis Inlands, Monserrat, Antigua, Haiti, Bahamas and Virgin Islands, the native population of opuntias have been seriously affected (Zimmermman *et al.* 2003). The insect was found in Florida in 1989 (Habeck and Bennet, 1990), and it could be spread to the southwest of the USA moving towards Texas, Arizona, New Mexico, and threatening areas with a vast presence of opuntias, and as a consequence the potential entry to Mexico. Since the cactus moth would wreak have to Mexico, it is a quarantine pest for Mexico. Therefore the Mexican Government through the Plant Health General Directorate (SENASICA-SAGARPA), has implemented phytosanitary measures through a National Campaing to Prevent the entry of the cactus moth.

Actions Plans:

A) To prevent the introduction of cactus moth in Mexico:

- Risk analysis of the potential impact of cactus moth on the ecological, economical and social aspects.
- Public awareness campaign.
- Training on identification and detection targeted to quarantine inspection personnel, academical and growers.
- Establishment of an advisory group (national and international).
- Surveillance of possible pathways and surveillance of susceptible host areas.
- Multilateral cooperation of the countries where cactus moth.

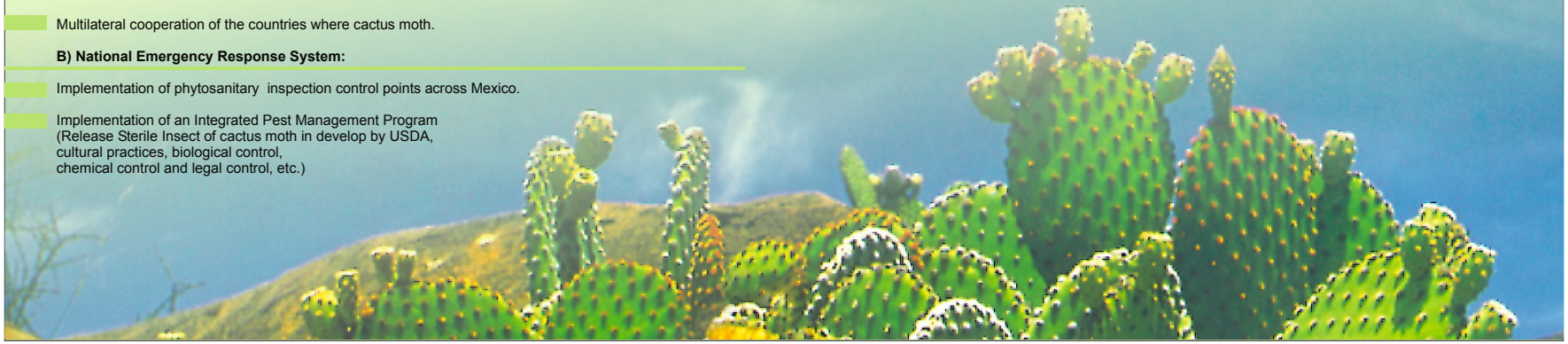
B) National Emergency Response System:

- Implementation of phytosanitary inspection control points across Mexico.
- Implementation of an Integrated Pest Management Program (Release Sterile Insect of cactus moth in develop by USDA, cultural practices, biological control, chemical control and legal control, etc.)

Results and Conclusions

Update field sampling has taken in 18 of the 20 states with the highest potential for the establishment of the pest. These were choosen based on the host economic, enviromental or social importance and considering the probability of its establishment in relation to similar climates. Results until now indicate that the cactus moth is not in Mexico. Methodology sampling includes geological references from the sampling points as well as the production areas (commercial and wild) which allows for a regular risk evaluation. It is necesary to increase research activities that help understand the insect biology, host preferences, diagnostic methods earlier detection and emergency response.

The Plant Health General Directorate, has been organizing meetings, workshops, simposium, National and International Forum Joint Universities, Agencies, and the International Atomic Energy Agency. 3,100 books titled “Biology, history, threat, surveillance and control of the cactus moth Cactoblastis cactorum“ and 160 videos titled “ Cactus moths, an economic, social and ecological threat” were distributed to more than 100 National and International, public and private institutions, financed between International Atomic Energy Agency and SAGARPA.



FORECASTING OF TWO MAIN MANGO FRUIT FLIES PESTS IN MEXICO USING TIME SERIES MODELS.

V. M. Pinto , A. Delgado-Matus. Universidad Autónoma Chapingo. México

INTRODUCTION

Mexico annually produces fruits by 3.35 billions with the mango (*Mangifera indica L.*) being one of the most exported. The fruit flies (*Diptera:Tephritidae*) are the main pest problem for mango farmers causing direct damages up 25% of the production aside from the cuarentenary restrictions for exportation. This work was carried out to evaluate the time series models performance for fruit flies populations forecasting and eventually to include them within the monitoring programs of the Mexican campaign against these pests.

METHODOLOGY

Field samples in mango orchards growing in Actopan region of Veracruz, México were taken weekly during a 79 weeks period to capture adults of two of the main fruit flies pests of mango in tropical México: *Anastrepha obliqua* (Macquart) and *A. ludens* (Loew) (Figs 1 and 2).

By CNMF-SAGARPA. MÉXICO

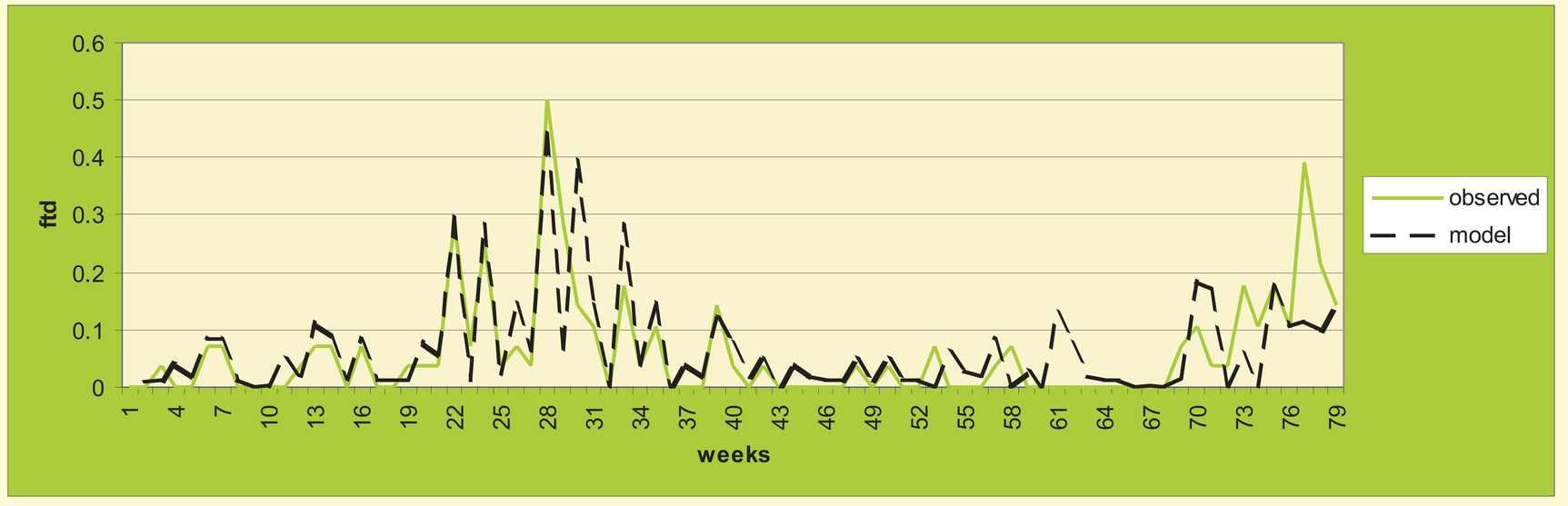
The data were analyzed using Time Series models under the Box and Jenkins methodology [1] to attempt predicting the population behaviour of the pests populations as basic knowledge for integrated pest management. Flies captures were estimated by the ftd (flies trap day) method = ((number of flies captured x number of traps used)/ number of days of traps exposition). We used McPhail glass traps for the two different species of fruit flies captures during 79 weeks, each one represented the two time series using in this work.

By CNMF-SAGARPA. MÉXICO

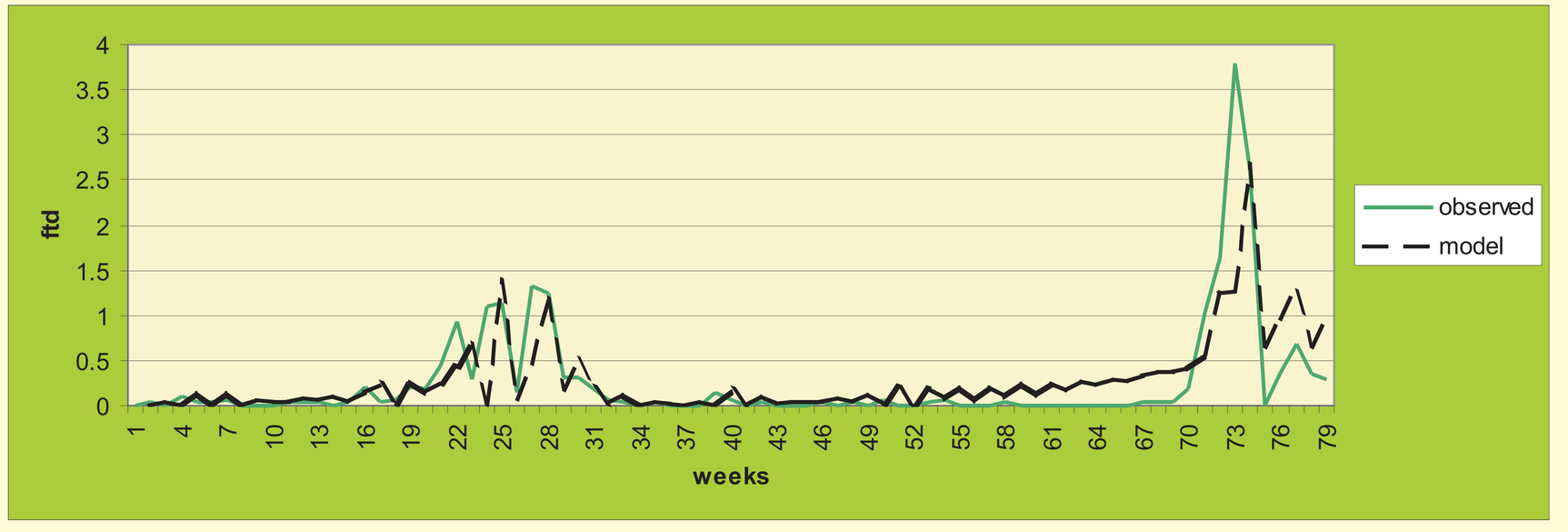
[1] BOX, G.E.P., JENKINS, G.M., Time Series Analysis, Forecasting and Control. Holden Day. San Francisco (1976) 575 p

RESULTS

After running all the four main steps (identification, parameters estimation, verification and forecasting) indicated in the Box and Jenkins methodology to find the adequate time series models, the ARMA (1,2,2,1) and ARMA (2,1,0,1) models (Figs 3 and 4), both with a seasonal behaviour, full fitted the field observed behavioural patterns for *A. obliqua* and *A. ludens*, respectively, and gave good predictions of its future values up four weeks in advance, information that could help to take the management strategies in an opportune and accurately way when the fruit flies populations surpassing its innocuous level numbers. On the comparative predictions with other different mathematical models, the sixth degree polynomial model was able to made good forecastings on the two studied series.



ARMA (1,2,2,1) output compared with *A. ludens* population observed data.



ARMA (2,1,0,1) output compared with *A. obliqua* observed data.

Gene flow among some Tanzanian *Glossina pallidipes* and *G. swynnertoni* populations

E.S. Krafsur[1], I.I. Malele[2], J.G. Marquez[1], J.O. Ouma[1], A.R. Msangi[2]

[1]Iowa State University, Ames, Iowa, USA and [2]Tsetse and Trypanosomiasis Research Institute, Tanga, Tanzania

G. pallidipes is widely but discontinuously distributed throughout much of East, central and southern Africa. *G. swynnertoni* is mostly confined to northern Tanzania. Both species are highly significant economically. It is important to learn how strongly populations are isolated from each other. ***The principle question to be asked is, how much dispersion exists among the populations of each species?***

We used genetic methods to estimate dispersion rates indirectly (Krafsur, 2003). The index of dispersion is in terms of the average number of reproducing flies per generation. Thus, it is an historical measure, and is not instantaneous or necessarily predictive.

Genetic measurements used include mitochondrial variation, which follows a matrilineal mode of inheritance, and microsatellite variation, of co-dominant alleles which follow biparental inheritance.

G. pallidipes

Mitochondrial variation was assessed in four Tanzania populations and a sample from Nguruman, Kenya.

34 variable sites were observed among 666 nucleotide base pairs sequenced. A sum of 33 haplotypes were recorded. Mean population diversity was 0.86, indicating the odds that two randomly chosen flies in a population had different haplotypes.

These populations were highly differentiated from each other (G_{ST} = 0.19) and the estimated mean rate of gene flow was 2.1 reproducing females per generation.

It has been claimed that Nguruman populations were subject to very high rates of immigration from the escarpment that borders Tanzania (e.g. Brightwell et al., 1997). We found 8 haplotypes in Nguruman, but none was shared with Tanzania.

Microsatellite variation and diversities were consistent with the mitochondrial.

Gene flow among Tanzanian *G. pallidipes* is comparable to that estimated among East and southern African populations.

G. swynnertoni

Flies were obtained from five populations as shown on the map.

Nineteen variable sites were found among a mitochondrial 668 nucleotide base-pair sequenced. Only 11 haplotypes (strains) were detected. Mean diversity in populations was 0.66, meaning a 66% chance two random flies in a population had different haplotypes. These diversities suggest that *swynnertoni* populations survived in large numbers after 20 years of insecticidal programmes.

Their lesser diversities than *pallidipes* however indicate much lesser population densities historically than *pallidipes*.

Sampled populations were highly differentiated from each other (G_{ST} = 0.29) .

The estimated mean rate of gene flow was only 1.2 reproducing females per generation.

Tanzania and Kenya, showing Tanzanian sampling sites and the approximate range of *G. swynnertoni*.

Further information

Brightwell, R. Dransfield, R.D, Stevenson, P, & Williams, B. (1997). Bull. Entomol. Res. 87, 349-370.

Krafsur, E.S. (2003). Tsetse fly population genetics: an indirect approach to dispersal. Trends in Parasit. 19, 162-166.

Conclusions

1. Gene diversities are directly proportional to effective population sizes suggesting much smaller *G. swynnertoni* populations than *G. pallidipes*.

2. Tsetse populations do not breed randomly. Most seem to be local and dispersion among populations is quite limited.

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Potentialities of development of *Ceratitis capitata* (Diptera : Tephritidae) on the argan fruits (*Argania spinosa*, Sapotacea) in Essaouira region (Morocco).

IAEA-CN-131/54 P

Naamani K.[1], Bakri A. [1] & Herbaut C. [2]

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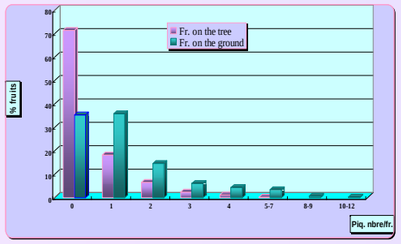
I- General :

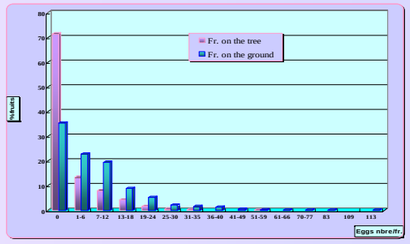
- ✓ The surface occupied by the argan tree in Morocco is 828.000 ha (Ayad, 1989).
- ✓ The argan fruit is on of the favorite host for Medfly in Morocco.
- ✓ The bioecology of this fly wos studied during 3 years.

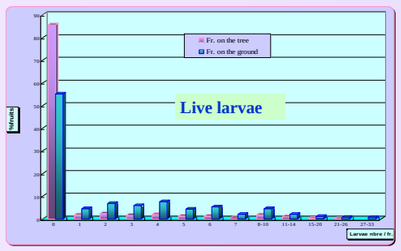
Argania spinosa L. (Skels)

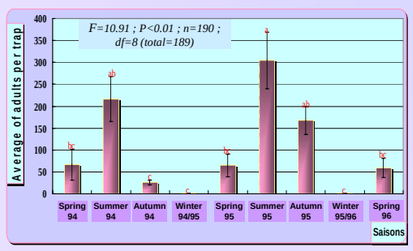
II- Results :

1- infestation of argan fruits :





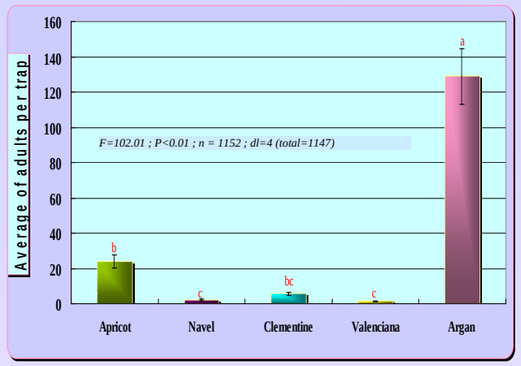




Biological characteristics	Fruits on tree	Fruits on the ground
Average of the punctures by fruit	0.44 ± 0.02	1.25 ± 0.04
Egg average per fruit	2.63 ± 0.17	7.75 ± 0.32
Average of live larvae per fruit	0.77 ± 0.07	2.45 ± 0.13
Average of larvae dead by fruit	0.38 ± 0.04	1.12 ± 0.09
% of Hatching of eggs	43.73 %	46.06 %
% average of hatching of eggs in the two layers	44.89 %	
Egg average per fruit having generated larvae	1.15	3.57
% of live larvae	67 %	68,63 %
% average of live larvae in the two layers	67.82 %	
% of larvae dead by layer	33 %	31.37 %
% average of larvae dead in the two layers	32.18 %	

Dynamics of development of Medfly by argan fruit in Essaouira region.

2- Comparison of the capture of Medfly between the Cultivated areas in Haouz of Marrakesh and the argan forest.



Dates	Total pupes	Pupes dead	Pupes parasitized by *Opius Concolor*
18/VII/1994	109	26.60 %	2.75 %
15/VII/1995	688	9.30 %	3.19 %
17/IV/1997	190	7.36 %	1.05 %
7/V/1997	634	11.67%	3.15 %
28/V/1997	1304	10.50%	3.52 %
18/VI/1997	1164	19.75%	12.62 %
8/VII/1997	827	30.47%	18.13%

Mortality and parasitism by *Opius concolor* (Hymenoptera Braconidae) of the pupes of Medfly collected per Kg of argan fruits.

Argan fruits

Ceratitis capitata Wiedmann

III- Conclusion :

- ✓ Approximately 65% of fruits fallen on the ground and 30% still on the tree were infested with Medfly during the period of fructification.
- ✓ More than 30 eggs per fruit were recorded in 59% of fallen fruits and 28% of the fruits on the trees. However, the hatching rate was 45%.
- ✓ 10 larvae per fruit were found in 41% of fallen fruits and 13% of fruits on the the trees.
- ✓ Current rate of parasitism by *Opius concolor* in argan forest can reach 18%.
- ✓ The period of infestation of the cultivated fruits corresponds to that of their maturation. Among the cultivated fruits, it is on the apricot orchard that high number of adults were captured, however, this capture number remains low compared with the argan.

IV- Reference:

- Ayad A., 1989 : Présentation générale de l'Arganeraie. Formation forestière continue, thème ¨l'arganier¨, station de recherches forestières, Agadir - Maroc, 13 – 17 mars, pp : 9-17.

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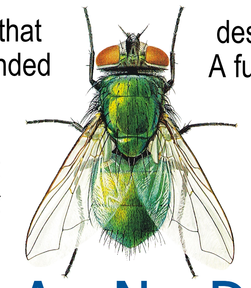
Assessing genetic variation in natural populations of the new world screwworm fly *Cochliomyia hominivorax*: evidence from mitochondrial DNA

I N T R O D U C T I O N

The New World screwworm fly, *Cochliomyia hominivorax* (Coquerel 1858 -Diptera: Calliphoridae), is an obligate ectoparasite that causes myiasis in warm-blooded vertebrates throughout the Neotropical region **(1)**. Historically, the distribution of *C. hominivorax* extended from the southern United States to Argentina. However, this species has been successfully eradicated from North and most of Central America using the Sterile Insect Technique (SIT), but still occurs in the Caribbean islands and South America, except for Chile **(2)**. In Uruguay, as in other South American countries, *C. hominivorax* is one of the most important insect pests and represents a significant health problem for livestock industry often causing great economic losses **(3)**. Because of the economic importance of *C. hominivorax* and its influence on the trade of live animals among infested and not-infested countries, international efforts have been aimed at designing a program to control and eventually eradicate this species from endemic areas and to prevent invasions into screwworm-free areas. A fundamental component of a successful pest management strategy, such as SIT, is a good understanding of the genetic diversity and structure of the target populations.



Mitochondrial DNA (mtDNA) is a suitable marker for studying micro-evolutionary processes in animal populations and can is a suitable marker for estimating the genetic variability within populations. The aim of this study was to examine the genetic variability among geographically distinct populations of *C. hominivorax* from Uruguay, at the southern limit of this species' distribution, using mtDNA PCR-RFLP analysis.

M A T E R I A L A N D M E T H O D S

SAMPLES

Larvae of *C. hominivorax* were obtained from wounded sheep, dogs or cattle in January 2003. Samples from seven localities, including important Uruguayan livestock areas, were analyzed. The larvae were fixed in 100% ethanol and genomic DNA was isolated using Phenol: Chlorophormio extraction, modified for Calliphoridae species **(4)**. A total of 175 larvae were obtained from 48 wounds. The geographic locations of the seven areas sampled are shown in **Figure 1.**

Figure 1 Geographic locations of the screwworm populations sampled in this study.

PCR-RFLP

Two specific mtDNA regions were amplified (**Figure 2**). Amplifications reactions were conduced using previous described conditions **(5)** and universal primers described for these regions **(6)**. The PCR products of A+T-rich/12S were digested with the endonuclease *Dra I,* and the PCR products of *cox*1/*cox*2 were digested with the enzymes: *Ase I* and *Msp I.* Digestions were done according to the enzyme supplier's protocols (Gibco-BRL and Pharmacia, Peapack, NJ). The digested fragments were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide (EtBr) and photographed using the Kodak EDAS 290 software in an ultraviolet trans-illuminator. The size of the fragments was estimated using appropriate marker (DNA Ladder Plus 1Kb, Gibco-BRL).

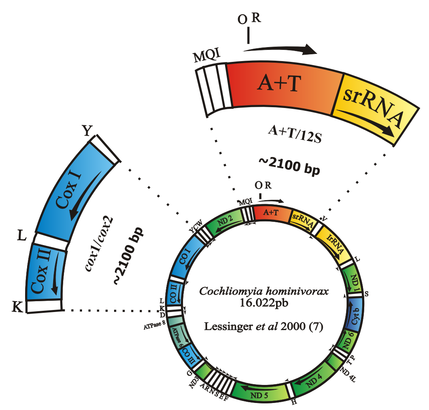


Figure 2. Mitochondrial DNA regions amplified.

DATA ANALYSES

To avoid a bias in the haplotype frequency estimations and in the genetic comparisons caused by sampling the same mitochondria, each haplotype found in a wound was considered only once. Using this approach the number of *C. hominivorax* considered in the analysis of genetic variation was 65 (**Table 1**) and was sufficient to provide information at the hierarchical level of the population.

Haplotype analyses: The different restriction patterns for both regions obtained with each enzyme were designated with capital letters, according to the order in which they were detected. For each individual, these letters were compiled into a composite haplotype designated by numbers (**Table 1**). The evolutionary distance (*d*) between all pairwise comparisons of haplotypes was estimated **(8)** using the REAP software **(9)**.

Diversity: The genetic diversity within each the population analyzed was interpreted using the estimate of haplotype diversity (*Hs*) **(10)** and nucleotide diversity (p) **(11)**. The indices were estimated using the REAP software **(9)**.

Population differentiation: The variation among the populations analyzed and the population differentiation were interpreted using different indices. The similarity index (*F*) or the proportion of shared fragments between populations was calculated for each possible pairwise comparison of populations **(8)**. The nucleotide divergence (d) was estimated according to Nei and Tajima (1981) using the REAP software **(9)**. To study the distribution of genetic variation within and among populations an AMOVA was done using ARLEQUIN **(12)**. The degree of isolation of the populations was interpreted using the F_{ST} parameter. This analysis was done by considering the number of pairwise differences and the evolutionary distance (*d*) between haplotypes.

R E S U L T S A N D D I S C U S S I O N

Table 1 Distribution of mtDNA haplotypes in Uruguayan populations of *C. hominivorax*. The haplotypes are designated by a number and a combination of the three restriction enzymes patterns (*Dra I, Ase I, Msp I*). The numbers in parentheses indicate the total number of individuals found with a haplotype and those without parentheses indicate the number of individuals used for population analyses. Freq: Frequency.

Haplotype	J. Suarez	B. Medina	Colonia	Florida	P. Muñoz	Dayman	S. Antonio	Total	Freq (%)
1 AAC	2 (7)	5 (14)	3 (12)	6 (15)	3 (12)	7 (17)	7 (10)	33 (87)	51
2 AAD		1 (3)						1 (3)	2
3 ABA	3 (17)	4 (11)		3 (5)	3 (8)		1 (1)	14 (42)	22
4 ABB		2 (8)	2 (10)	6 (10)	1 (2)		1 (4)	12 (34)	18
5 BAC						1 (1)		1 (1)	2
6 ABE				1 (2)				1 (2)	2
7 CAC							1 (3)	1 (3)	2
8 AAA						1 (2)		1 (2)	2
9 ABC						1 (1)		1 (1)	2
Total	5 (24)	12 (36)	5 (22)	16 (32)	7 (22)	10 (21)	10 (18)	65 (175)	100
Nº of wounds	5	8	5	10	4	9	7	48	

PCR-RFLP analysis of the two amplified regions of the *C. hominivorax* mitochondrial genome using three restriction endonucleases revealed high genetic variability. We examined approximately 25% (~ 4.2 kb) of the *C. hominivorax* mitochondrial genome and nine haplotypes were identified in the seven Uruguayan screwworm populations sampled (**Table 1**).

The PCR-RFLP analysis revealed marked polymorphism in the *cox*1/*cox*2 region at the intra- and inter-populational levels and was useful for defining different haplotypes. However, the A+T/12S region revealed lower PCR-RFLP polymorphism than expected. In any case, the size variation in the amplified fragment of A+T/12S region is an indication of length polymorphism as a molecular marker for screen intra-specific variation.

The overall estimates for the indices of genetic variation within and among populations are shown in **Table 2**.The nucleotide diversity estimated for *C. hominivorax* in this study is the highest value obtained for this species. The average value of gene diversity estimated here indicates a high level of genetic variation and reinforces the result obtained for nucleotide diversity.

Table 2 Estimates for the indices of genetic variation within and among populations. : Nucleotide diversity; *Hs*: Haplotype diversity; *F*: Similarity; : Nucleotide divergence; $_{st}$ (pd): **F**-statistic based on pairwise differences (= $_{w}$); $_{ST}$ (nd): **F**-statistic based on Nei's evolutionary distance (*d*).

Genetic variation within populations		
	π	*Hs*
	0.0229 (+-2.09 10^{-5})	0.6355 (+- 1.17 10^{-3})

Genetic variation among populations			
F	δ	Φst (pd)	Φst (nd)
0.967	0.000555 (+-1.2 10^{-6})	0.0762 (p = 0.0865)	0.1449 (p = 0.8768)

Comparisons among the Uruguayan screwworm populations clearly indicated that there was no evidence of subpopulation differentiation. The presence of the common haplotype 1 at a high frequency in all populations and the wide distribution of haplotypes 3 and 4 suggested that the populations were very similar (**Table 1**). The high value of the similarity index (**Table 2**) confirmed this observation. There were six local haplotypes, but the divergence between each of them and the common haplotypes was very low. For this reason, the estimates of nucleotide divergence between populations was very low, indicating that the populations analyzed were very similar.

The AMOVA showed that the genetic variability was distributed mainly within populations. This finding and the Φ_{ST} estimates provide evidence that there was no genetic differentiation by natural forces, such as drift and selection, and that screwworm populations from Uruguay are a unique panmitic population **(13)**.

Microssatellite data also suggest that little differentiation exists among these population **(14, IAEA-CN-131/93P).**

The lack of genetic structure among the screwworm populations in Uruguay may reflect the effect that there are no geographical barriers or important climatic differences among the regions studied. The large livestock population and the trading of animals in the country, possibly infected with *C. hominivorax*, could contribute to the dispersal of this species and results in more homogeneous populations.The results obtained for New World screwworm populations in Uruguay suggests that this country could be a candidate for testing the efficiency of SIT in South America.

Sterile insect release programs, either as a holding buffer zone or an eradication campaign, requires knowledge of the composition of the target species in order to determine optimal strategies. Further analysis with mtDNA markers and microssatellites involving other populations of *C. hominivorax* from South America are been conducting in our laboratory. The information, such patterns of genetic structure throughout the current geographic distribution, will be valuable for resolving evolutionary genetics and will contribute as a guideline for control strategies for this livestock pest in tropical habitats.

R E F E R E N C E S

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Medfly Terminal Velocity: Implications for Dispersal During Air or Ground Release

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Why do we need medfly TV?

- **It is, for practical purposes, the speed the medfly falls to the ground.**
- **Knowing this speed means we can better understand how far flies are blown by the wind.**
- **Knowing the TV of a fly will:**
  - Aid planning for improved accuracy of aerial release flights and ground releases.
  - Improve understanding of fly dispersal from air or ground continuous release systems.
  - Allow for fewer flies to be released, potentially reducing the size of production and eclosion facilities.
  - Allow understanding of the importance of the height from which sterile flies are released.
  - Contribute to Aerial / Ground release Cost-Effectiveness calculations.

Results:

- Asymptotic model fitted to data
- $v = 3.0553(1-e^{-0.6092h})$
- Terminal velocity of inert male flies = 3.1 m/s
- Model explains 52% variation in velocity
- Residual variation caused by unmeasured covariates (e.g. mass, spinning etc.)

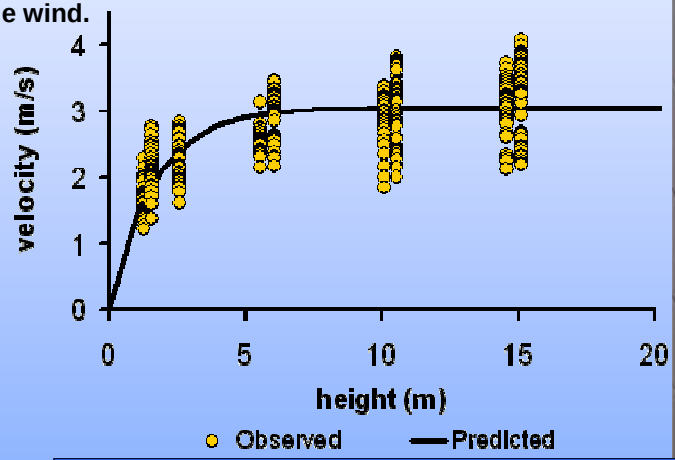


Table 1: Drift (metres) of medfly depending on height released, wind speed and a terminal velocity of 3.1 m/s (ignores speed of the plane and turbulent effects on the flies).

Height (m)	Wind Light (1.4m/s)	Wind Moderate (3.6ms)	Wind Strong (7.0ms)
100	45	116	226
300	135	348	677
600	271	697	1355

Implications:

- The effect of even a slight transverse breeze has been underestimated with the result that many areas may not be covered by the sterile blanket.
- The importance of releasing in low or no wind conditions is illustrated (Table 1).
- Release flight patterns that are parallel to the wind direction will improve dropping accuracy.
- For ground release, release height influences the effective range of the release system.

Acknowledgements: Thanks to the droppers. The Clean Fruit Project is funded by the European Community's Sixth Framework Programme. See "The Clean Fruit Consortium" at http://www.cleanfruitsit.org/.

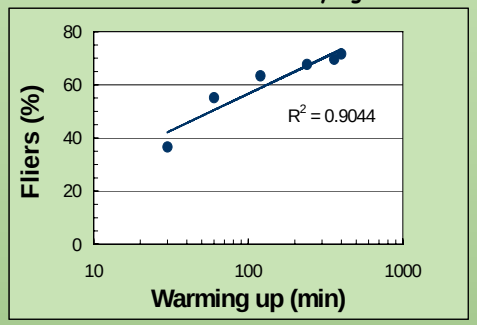
Inertifying Medfly

Yoav Gazit, Ruti Akiva, Michal Aviv and Sagi Gavriel

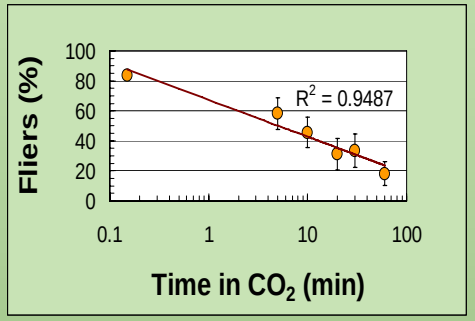
The Israel Cohen Institute for Biological Control
PPMB – Citrus Division
Bet-Dagan, Israel

www.cleanfruitsit.org

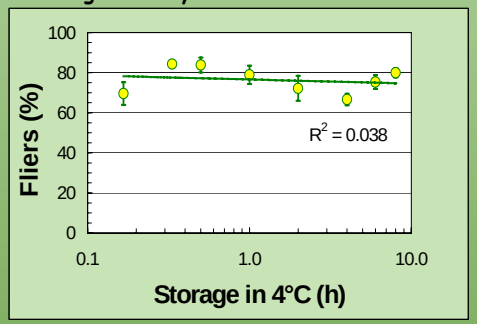
The test: would once a flier fly again?



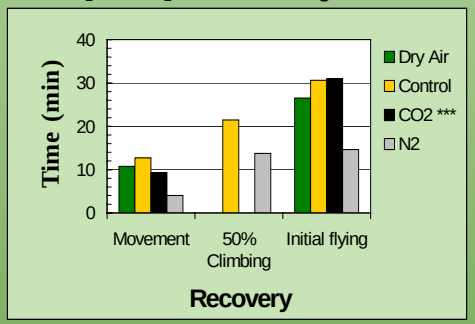
How long can Medfly take CO_2?



How long can they take 4°C?

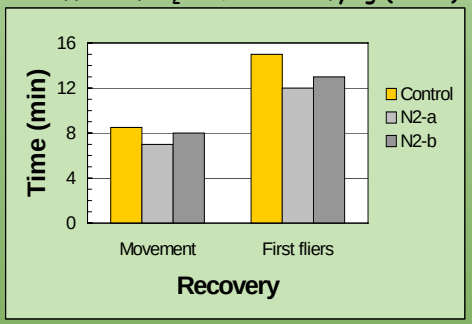


How CO_2 and N_2 affect awaking



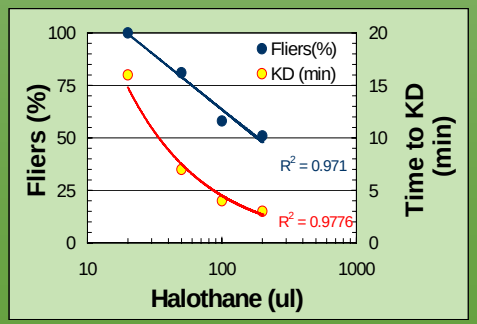
*** A few seconds in CO_2

The effect of N_2 in mass inertifying (AMEP)

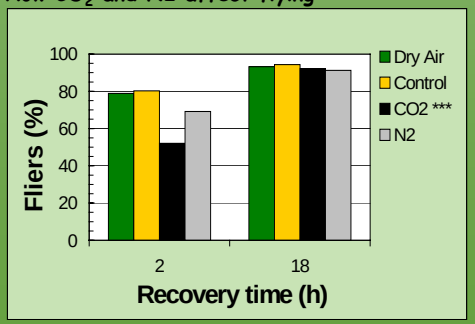


The additional effect of N_2 or CO_2 does not justify a switch to Nitrogen inertifying

Could Medflies be "anesthetized"?



How CO_2 and N2 affect flying



*** A few seconds in CO_2

EU-STREP–506495 CLEANFRUIT

Improving the quality of European Citrus & Fruit by developing Medfly (*Ceratitis capitata*) SIT technology so it can be widely applied in Europe and the Mediterranean

CleanFruit project has received funding from the European Community's Sixth Framework Programme.

PARAMETERS TO CONSIDER FOR THE SELECTION OF A LOCATION FOR AN INSECT MASS REARING FACILITY

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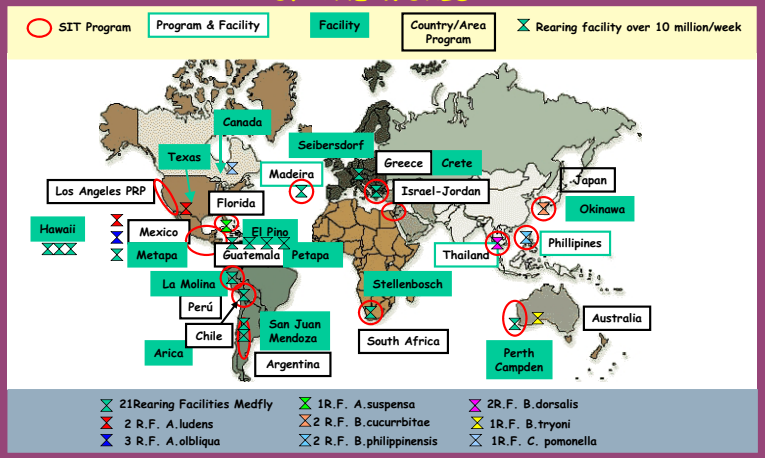
[(1)]ISCAMEN: Institute of Sanity and Agricultural Quality of Mendoza Argentina

MORE THAN THIRTY FOUR FACILITIES OF DIFFERENT SPECIES HAVE BEEN DISTRIBUTED AROUND THE WORLD.

MASS REARING FACILITIES AND SIT PROGRAMS OF THE WORLD



The facilities were located on places offered by the states. Some times some facilities were installed on isolated area or / and industrial areas. Considering absence of specific regulation and a null environmental impact of this kind of facilities, most of them were placed considering factors related with services possibilities, energy, petrol, water, etc.

The activities carry out on the insect mass rearing facilities have not critical activities that it will be able affect the environment however according the increment of factories related with the chemistry industry and secondary petrol products the Mass rearing facilities activities were affected by this activities. In other hand the posibility for the workers to work on a healthy facility as insect mass rearing facility create a new employment source.

In despite of a great advantages of the mass rearing activities, this healthy activity get other problems as follows:

*Population grew up very quickly and broken up a normal expansion planned.
*Increasing of the poverty in the people building very primitive houses close to the facilities.
*Environmental Impact laws were created withou any kind of specifications.
*Natural resistance of the people to Cobalt or Caesium.
*New technologies to control the pest without extra information in order to give to the people a great understanding of the new technologies.

New facilities are being affected seriously in the location, according the expansion of SIT and the ignorance of the people.

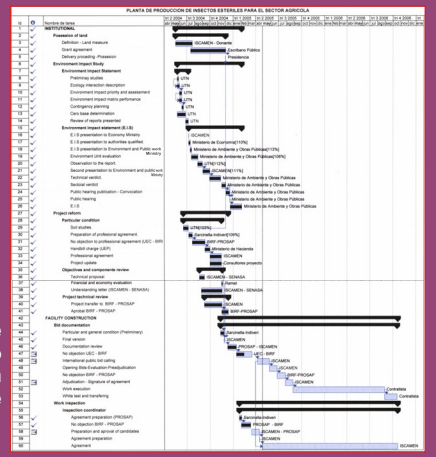
*Today a package of different topics must be considered to create a good location linked with the society, working with them and giving to the society a choice to participate in the discussion of this kind of activity. Differnt topics can be considered on the location.

ISCAMEN considered two groups of factors related with the location, dominants and non-dominants factors. In the dominants factor considered those factors indispensable to carry out the mass rearing activities. Non dominants factors were those factors that affect the production cost. The last factors were qualified giving a mark to the place selected:

Dominant factors:

*Distance to the town.
*Distance to the host.
*Access.
*Energy Service.
*Water posibility.
*Social receptivity.
*Land available.
*Negative environment influence on the biological activity.

The next map show the polygonal drawed to consider the place posibility, in order the dominants factor mentioned below.



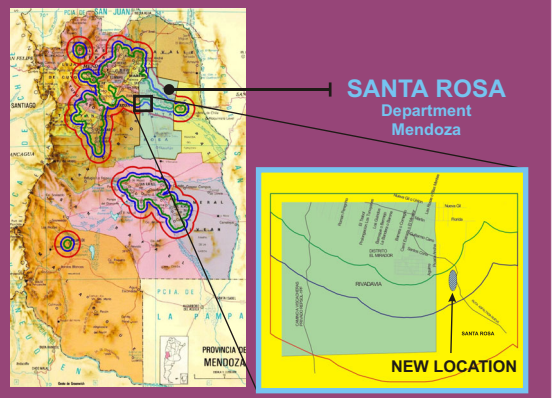
The red polygonal show the limit out of 20 Km to the last host, the blue line is 10 km to the last host and the green polygonal is 5km to the last host. The location selected is placed between 5 and 10km to the last host on desert area with the proximal population more than 10 km.

Non dominants factors:

*Gas service.
*Sewer Service.
*Drinkable water.
*Land tenure.
*Impuestos - Tasas y Servicios.
*Workforce.
*Transportation media.
*Municipal interes.
*Phone Service and cell phone signal.
*Health service and medical assistance.
*Supply sources posibilities.
*Weather influence.

Non dominants factors were qualified with a marck giving ponderate weight accoding the influence on the location. Once the location was selected cionsidering all factors, the environment studies were carried out and presented to the authorities qualified.

The next chronogram project are been executed in order to start the mass rearing of multipropose facility at the end of the next year.



The location selected is placed on Santa Rosa Department - Mendoza - Argentina. The new facility will be located in a land with more than 30 Has and it will have a cover surface at the first phase of the project of 12500m^2.

The objective of the facility will be provide sterile medfly males to Argentina Project and sterile codling moth to the region where the SIT will be applied (Valle de Uco region).

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Tel./fax:(0261) 4299015 / 4299013 / 4295450 / 4258741 / 4297315. E-mail:iscamen@iscamen.com.ar Http://www.iscamen.com.ar

AUTOMATED EGG COLLECTING SYSTEM AND PUPAE SEPARATOR FOR MEDFLY REARING

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CONTINUAL EGG COLLECTING SYSTEM

MERITS OF DEVICE
- Reduced demand for work
- Easy maintenance
- Optimal environment for eggs
- Exact age of collected eggs

COLLECTING CHANNELS

WATER PUMP

WATER INLET

EGG COLLECTING POT

PUPAE SEPARATING DEVICE

MERITS OF DEVICE
- Reduced demand for work
- Easy maintenance
- No damage of pupae
- No dust from pupation medium

EXHAUSTING DEVICE

ENGINE

STOCKER

ENDLESS MESH BELT

New larval diet for laboratory rearing of Ceratitis capitata (Diptera: Tephritidae)

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www.cleanfruitsit.org

Ingredients	Type of Diet/Amount (g)			
	Bran Diet	Cellulose Diet	Starch Diet	New Lab Diet
Agar	150	0	0	8
Wheat germs	0	0	0	40
Yeast	38	7.6	7.6	8
Sugar	70	14	14	16
Casein	0	0	0	18
Potato starch	0	0	51.5	0
Cellulose	0	30.2	0	0
Sodium benzoate	1.5	0.3	0.3	0
Methyl parabene	0	0	0	0.5
Sorbic acid	0	0	0	1
Formaldehyd 3.7%	0	0	0	5 ml
HCl	8	0	0	0
Water	230 ml	51 ml	30 ml	0

The ingredient composition of tested medfly larval diet

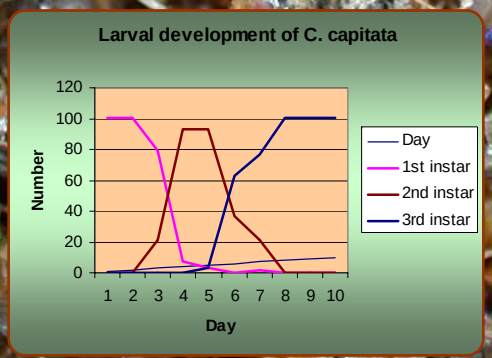
AIMS OF WORK:
- Development of reliable medfly larval diet for lab rearing
- Identification of developmental curve of all instars reared on new lab diet
- Identification of key morphological characters for distinguishing of larval instars

New larval diet for medfly lab rearing

Bran larval diet

Ingredients	Amount (g)	Ingredients	Amount (g)
Agar	8	Thiamin B_1	0.0025
Wheat germs	40	Riboflavin B_2	0.006
Casein	18	Pyridoxin B_6	0.0025
Yeast	8	Folic acid	0.0025
Sugar	16	Ascorbic acid	0.0025
Wesson salts	5	Streptomycin	0.05
Cholesterol	1.7	Kanamycin	0.05
Sorbic acid	1	Linseed oil	0.002
Methyl parabene	0.5	Formaldehyd 3.7%	5
Nicotin acid, PP	0.11	Water	500

The ingredient composition of complex pink bollworm (PB) larval diet which was used as initial diet for development of new medfly larval diet



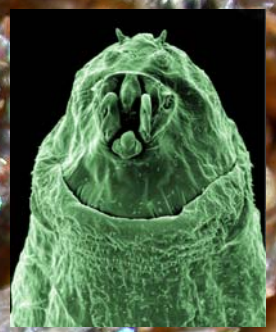
The developmental curve of all larval instars reared on new lab diet at 24±1°C

Type of Diet	Rearing Efficacy	Pupal Weight
Cellulose diet	≤ 5 %	≤ 7.5 g
Starch diet	≤ 5 %	≤ 7.5 g
PB lab diet	70.13 ± 4.59 %	9.53±0.213 mg
Bran diet	58.93 ± 2.22 %	9.29 ± 0.05 mg
New medfly lab diet	66.20 ± 1.33 %	8.68 ± 0.06 mg

Production parameters of diets tested in laboratory conditions

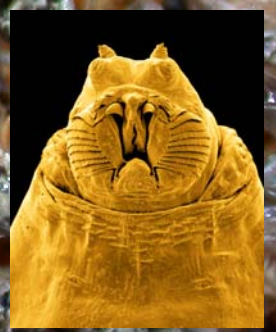
CONCLUSIONS:
- Well-balanced larval development
- Easy observation of all larval instars
- Lower cost

1st Instar Larva:
- Mandibles with two teeth
- Anterior spiracles absent
- Oral ridges absent



2nd Instar Larva:
- Mandibles with two teeth
- Anterior spiracles present
- Oral ridges present

3rd Instar Larva:
- Mandibles with one teeth
- Anterior spiracle present
- Oral ridges present



Morphological characters for identification of larval instars

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Effect of Irradiated Food on the Development of Egg Parasitoid, *Trichogramma chilonis* Ishii

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INTRODUCTION

- Trichogrammatids, are amenable to mass production & are frequently released enemies in the world.
- In many management programs, particularly after SIT release, it fits as a key component as they are found in a wide climatic ranges from temperate to tropical regions.
- over 18 million-hectare area is annually being treated with *Trichogramma* species in 16 different countries.
- About 18 species are being used to suppress pests on maize, sugarcane, rice, sugar beet, pine and vegetables.
- Out of many parasitoid species, *Trichogramma chilonis* Ishii is one of the most potent wasps and is extensively used in mass releases against a number of lepidopterous pests in Asia.

Continued .....

- The irradiation of this parasitoid and its adult food with low doses of gamma radiation may induce useful characters/biological attributes *viz.,* parasitization, fecundity, emergence, sex ratio, *etc.,* which might be exploited for improving the efficiency of the parasitoid.
- Though *T. chilonis* is one of the most extensively used egg parasitoid in IPM all over the world, no practical information is available on the effect of low doses of gamma radiation and irradiated adult food for incorporation of better biological attributes.
- In view of this, the present study was designed to investigate the effect of irradiated adult food on *T. chilonis,* which may lead to a better understanding of the role of irradiated food in the life of the parasitoid.

MATERIALS & METHODS

- Adult parasitoids were fed daily (treatment wise) on the fine streaks of 50% honey; 20% of fructose/sucrose/glucose/yeast hydrolysate and also on grape juice or distilled water, drawn on the inside wall of the tube with a fine needle.
- A group of parasites were also kept unfed during the course of the experiment. Proper care was taken to make streaks of the right thickness so that the parasitoids could be fed on them without getting stuck.
- For more critical and precise studies, the presence or absence of the food was observed at 10 hours interval. This experiment was continued up to 5 successive generations.
- In each generation freshly irradiated food was provided to the parasitoids.
- In all these cases including control (unfed), observations were recorded on fecundity, % adult emergence, % females, adult longevity, and on the total developmental period.

EFFECT OF IRRADIATED FOOD ON THE FECUNDITY OF *T. CHILONIS*

- Fecundity of the parasitoids increased significantly (at 5% level) at 2.5 and 5 Gy irradiated honey (50%) in all the generations as compared to the corresponding generations of the control.
- Similarly, in case of parasitoids maintained only on irradiated distilled water, the fecundity was found enhanced which was significantly superior over any of the generations of the control.

Table : Effect of irradiated food on the fecundity of *T. chilonis*

Food material	Generation	* Fecundity Con.	2.5 Gy	5 Gy	7.5 Gy	10 Gy	Stat. analysis	
Honey 50%)	F_1	84.9	96.1	93.8	89.5	88.3	S.Em ±	2.07
	F_2	87.2	93.5	95.9	92.6	86.2	C.D. 5%	5.74
	F_3	85.8	94.0	92.7	91.3	87.6	C.D. 1%	7.54
	F_4	84.1	95.7	94.8	89.4	85.2		
	F_5	86.5	94.6	93.0	92.1	86.7		
Fructose (20%)	F_1	84.9	85.7	84.6	86.1	85.6	S.Em ±	2.38
	F_2	86.3	83.2	82.9	83.6	82.5	C.D. 5%	6.60
	F_3	82.4	86.8	85.1	84.4	83.7	C.D. 1%	8.67
	F_4	85.2	84.6	83.2	85.0	82.1		
	F_5	84.3	83.9	84.1	82.7	85.2		
Glucose (20%)	F_1	83.9	83.1	82.5	83.3	82.8	S.Em ±	2.63
	F_2	81.5	80.9	83.7	80.2	83.2	C.D. 5%	7.29
	F_3	82.4	83.6	81.2	83.4	81.0	C.D. 1%	9.58
	F_4	80.6	82.7	83.9	81.5	80.4		
	F_5	82.7	81.8	82.0	82.8	81.3		
Sucrose (20%)	F_1	80.8	79.8	81.7	78.9	77.7	S.Em ±	2.56
	F_2	81.6	81.2	79.4	80.5	79.9	C.D. 5%	7.09
	F_3	78.2	80.4	77.9	81.0	80.6	C.D. 1%	9.32
	F_4	80.2	80.4	77.9	81.0	80.6		
	F_5	80.0	78.6	80.2	79.2	78.1		
Grape juice	F_1	79.3	80.1	78.7	80.9	78.5		
	F_2	72.8	83.5	84.6	85.7	76.9	S.Em ±	1.67
	F_3	70.6	82.8	85.8	83.4	74.8	C.D. 5%	4.63
	F_4	71.9	83.1	86.3	84.8	75.4	C.D. 1%	6.08
	F_5	72.1	81.4	84.4	83.3	72.3		
Yeast hydrolysate enzymatic (20 %)	F_1	70.4	82.9	85.1	84.1	73.2		
	F_2	55.6	52.8	53.0	51.6	49.2	S.Em ±	1.88
	F_3	52.2	54.3	52.6	52.9	46.4	C.D. 5%	5.21
	F_4	56.8	53.6	54.3	53.5	47.1	C.D. 1%	6.85
	F_5	53.7	54.1	53.9	51.1	48.6		
Distilled water	F_1	54.5	53.2	52.2	50.4	46.5		
	F_2	33.8	60.9	56.5	45.8	42.8	S.Em ±	1.83
	F_3	36.2	63.8	59.2	47.4	44.8	C.D. 5%	5.07
	F_4	31.4	62.1	57.0	44.6	40.5	C.D. 1%	6.66
	F_5	32.9	61.5	54.0	46.9	41.7		
Unfed	F_1	34.5	62.7	56.8	44.1	42.6		
	F_2	51.9						

EFFECT OF IRRADIATED FOOD ON THE ADULT EMERGENCE OF *T. CHILONIS*

- Adult emergence, on honey, initially in F1 and F2 generations was at par with the control
- Later in F3, F4 and F5 generations it was significantly superior.
- At 5, 7.5 and 10 Gy, however, the adult emergence was at par with the corresponding generations of the control except in few cases (*i.e.,* F2 of 5 and 7.5 Gy), where the emergence was superior over the respective generations of the control.
- In case of irradiated fructose, glucose, sucrose, grape juice and water, the adult emergence was lower than the corresponding generations of the control, at higher doses of 7.5 and 10 Gy.
- In case of 2.5 Gy irradiated distilled water, the adult emergence did not vary however, it was significantly superior at 5 Gy in all generations except in F4.
- Adult emergences in F2 and F5 generations of 7.5 Gy and F3 and F5 of 10 Gy treatments were found to be superior over the corresponding generations of the control.

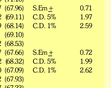

Table : Effect of irradiated food on adult emergence (%) of *T. chilonis*

Food material	Generation	* Adult emergence Control	2.5 Gy	5 Gy	7.5 Gy	10 Gy	Stat. analysis	
Honey (50%)	F_1	87.63 (69.46)	88.37 (70.11)	87.94 (69.73)	86.96 (68.87)	87.12 (69.02)	S.Em ±	0.65
	F_2	86.49 (68.48)	87.27 (69.17)	89.71 (71.34)	88.51 (70.23)	85.97 (68.04)	C.D. 5%	1.80
	F_3	86.89 (68.82)	89.29 (70.96)	87.22 (69.09)	89.11 (70.78)	86.46 (68.45)	C.D. 1%	2.37
	F_4	88.15 (69.92)	90.09 (71.71)	88.01 (69.78)	87.91 (69.70)	87.62 (69.42)		
	F_5	86.36 (68.36)	89.19 (70.83)	87.55 (69.37)	88.16 (69.92)	86.07 (68.12)		
Fructose (20%)	F_1	87.21 (69.10)	87.56 (69.39)	88.23 (69.97)	86.98 (68.89)	87.99 (69.78)	S.Em ±	0.68
	F_2	86.73 (68.68)	86.25 (68.28)	87.31 (69.19)	87.68 (69.51)	86.93 (68.86)	C.D. 5%	1.88
	F_3	87.87 (69.68)	90.01 (71.61)	86.68 (68.68)	86.79 (68.71)	87.12 (69.01)	C.D. 1%	2.47
	F_4	87.19 (69.08)	87.97 (69.77)	87.09 (69.98)	88.19 (69.94)	88.07 (69.84)		
	F_5	87.19 (69.08)	88.45 (70.18)	89.38 (71.03)	87.06 (68.97)	89.46 (71.10)		
Glucose (20%)	F_1	87.47 (69.33)	87.17 (69.06)	86.84 (68.79)	88.14 (69.97)	85.87 (67.96)	S.Em ±	0.71
	F_2	86.46 (68.48)	85.94 (68.02)	88.22 (70.00)	86.97 (68.90)	87.22 (69.11)	C.D. 5%	1.97
	F_3	86.93 (68.86)	88.05 (69.83)	85.89 (67.97)	87.62 (69.45)	86.09 (68.14)	C.D. 1%	2.59
	F_4	87.31 (69.19)	86.38 (68.37)	87.33 (69.21)	85.50 (67.66)	87.2 (69.10)		
	F_5	88.18 (69.94)	87.74 (69.55)	86.24 (68.26)	87.00 (68.91)	86.52 (68.53)		
Sucrose (20%)	F_1	86.33 (68.35)	85.69 (67.82)	87.26 (69.13)	86.20 (68.24)	85.47 (67.66)	S.Em ±	0.72
	F_2	85.99 (68.06)	87.12 (69.04)	85.27 (67.47)	84.99 (67.23)	86.32 (68.32)	C.D. 5%	1.99
	F_3	86.49 (68.48)	85.72 (68.68)	86.07 (68.12)	87.35 (69.18)	84.80 (67.09)	C.D. 1%	2.62
	F_4	87.03 (68.92)	85.10 (67.35)	87.14 (69.03)	85.57 (67.73)	85.82 (67.93)		
	F_5	85.66 (67.78)	86.26 (68.29)	85.79 (67.89)	87.01 (69.16)	84.97 (67.23)		

EFFECT OF IRRADIATED FOOD ON FEMALE EMERGENCE OF *T. CHILONIS*

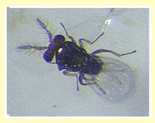

Table: Effect of irradiated food on emergence (%) of *T. chilonis* females

Food material	Generation	* % female emergence Control	2.5 Gy	5 Gy	7.5 Gy	10 Gy	Stat. analysis	
Honey (50%)	F_1	73.05 (58.74)	74.17 (59.46)	73.01 (58.72)	71.92 (58.01)	72.40 (58.32)	S.Em ±	0.44
	F_2	72.12 (58.15)	72.97 (58.68)	71.82 (57.95)	73.32 (58.95)	71.22 (57.58)	C.D. 5%	1.22
	F_3	72.75 (58.54)	73.44 (58.98)	72.98 (58.69)	72.58 (58.44)	73.48 (59.02)	C.D. 1%	1.60
	F_4	73.38 (58.95)	74.05 (59.38)	73.78 (59.22)	71.52 (57.77)	72.19 (58.20)		
	F_5	72.69 (58.33)	73.90 (59.29)	72.79 (58.43)	73.09 (58.76)	72.91 (58.64)		
Fructose (20%)	F_1	71.57 (57.79)	72.05 (58.12)	71.97 (58.06)	73.03 (58.73)	72.39 (58.32)	S.Em ±	0.60
	F_2	72.25 (58.24)	73.16 (58.81)	70.46 (57.10)	71.70 (57.89)	70.92 (57.40)	C.D. 5%	1.66
	F_3	72.47 (58.38)	72.48 (58.40)	73.26 (58.89)	72.82 (58.61)	73.18 (58.83)	C.D. 1%	2.18
	F_4	71.38 (57.68)	70.97 (57.56)	72.17 (58.18)	70.86 (57.35)	71.89 (57.99)		
	F_5	72.46 (58.37)	71.65 (57.86)	71.19 (57.58)	71.07 (57.48)			
Glucose (20%)	F_1	72.07 (58.12)	71.40 (57.68)	72.27 (58.24)	70.99 (57.43)	71.60 (57.82)	S.Em ±	0.48
	F_2	71.94 (58.02)	72.18 (58.18)	70.70 (57.24)	71.86 (57.98)	72.79 (58.57)	C.D. 5%	1.33
	F_3	72.14 (58.17)	70.86 (57.34)	71.11 (57.50)	72.67 (58.50)	69.93 (56.76)	C.D. 1%	1.75
	F_4	70.91 (57.37)	71.89 (58.02)	72.00 (58.07)	69.97 (56.78)	70.27 (56.97)		
	F_5	71.49 (57.76)	71.23 (57.60)	70.34 (57.01)	72.02 (58.08)	71.76 (57.91)		
Sucrose (20%)	F_1	71.38 (57.67)	70.15 (56.91)	72.93 (58.67)	70.40 (57.05)	71.12 (57.52)	S.Em ±	0.45
	F_2	72.19 (58.20)	72.14 (58.15)	70.85 (57.33)	71.70 (57.87)	70.57 (57.17)	C.D. 5%	1.25
	F_3	71.50 (57.54)	70.86 (57.35)	71.88 (57.99)	72.01 (58.08)	71.90 (58.01)	C.D. 1%	1.64
	F_4	71.17 (57.55)	71.22 (57.57)	71.10 (57.49)	69.88 (56.74)	69.32 (56.38)		
	F_5	71.82 (57.95)	71.78 (57.93)	72.32 (58.26)	71.13 (57.33)	70.25 (56.96)		

(Contd.)

Continued .....

Food material	Generation	* % female emergence Control	2.5 Gy	5 Gy	7.5 Gy	10 Gy	Stat. analysis	
Grape juice	F_1	71.68 (57.87)	72.17 (58.17)	73.42 (58.98)	72.11 (58.14)	70.88 (57.35)	S.Em ±	0.43
	F_2	71.14 (57.54)	70.36 (57.02)	72.25 (58.23)	70.77 (57.28)	71.31 (57.65)	C.D. 5%	1.19
	F_3	71.98 (58.06)	71.94 (58.02)	72.64 (58.48)	72.67 (58.49)	72.76 (58.55)	C.D. 1%	1.57
	F_4	70.66 (57.21)	70.66 (57.22)	73.01 (58.71)	71.84 (57.96)	70.55 (57.14)		
	F_5	71.83 (57.96)	71.76 (57.93)	71.31 (57.62)	70.34 (57.01)	71.06 (57.46)		
Yeast hydrolysate enzymatic (20 %)	F_1	70.52 (57.18)	71.42 (57.70)	69.46 (56.47)	70.34 (57.03)	70.09 (56.86)	S.Em ±	0.60
	F_2	69.58 (56.55)	69.10 (56.64)	71.58 (57.82)	71.13 (57.33)	68.90 (56.12)	C.D. 5%	1.66
	F_3	71.22 (57.56)	70.78 (57.31)	69.80 (56.70)	69.99 (56.75)	70.83 (57.33)	C.D. 1%	2.18
	F_4	70.06 (56.85)	68.88 (56.10)	70.49 (57.12)	68.40 (55.81)	69.78 (56.66)		
	F_5	70.76 (57.28)	67.94 (55.53)	69.72 (56.62)	69.18 (56.30)	69.10 (56.24)		
Distilled water	F_1	69.06 (56.23)	70.62 (57.19)	71.73 (57.89)	70.93 (57.39)	71.26 (57.59)	S.Em ±	0.58
	F_2	68.52 (55.91)	69.58 (56.55)	70.40 (57.05)	69.64 (56.58)	70.49 (57.10)	C.D. 5%	1.61
	F_3	68.72 (56.01)	70.14 (56.90)	68.87 (56.09)	71.27 (57.61)	69.37 (56.41)	C.D. 1%	2.11
	F_4	70.09 (56.86)	70.91 (57.37)	71.27 (57.59)	68.94 (56.14)	71.59 (57.80)		
	F_5	69.86 (56.72)	69.80 (56.67)	70.44 (57.07)	71.52 (57.77)	68.37 (55.79)		
Unfed	F_1	68.52 (55.91)						
	F_2	70.84 (57.34)						
	F_3	68.20 (55.72)						
	F_4	66.80 (54.92)						
	F_5	67.05 (55.28)						

*Mean of 10 replications — Data within parentheses indicate angular transformed values

Results on female emergence of *T. chilonis,* in relation to the feeding of different irradiated and non-irradiated food materials to the parasitoids indicate that there was no significant impact of radiation on female emergence in most of the treatments except in F_1 and F_4 generations of 5 Gy treated grape juice (>73.42 and 73.01%) and in in F_1 and F_2 of 5 Gy (>71.73 and 70.40 %) and in F_1 and F_3 of 7.5 Gy and F_1 and F_2 of 10 Gy treated distilled water.

EFFECT OF IRRADIATED FOOD ON MALE LONGEVITY OF *T. CHILONIS*

The male longevity, from F_1 - F_5, did not differ significantly in between and from any of the corresponding generations of the control, when the parasitoid was fed either on honey, or on all the three sugars, grape juice and yeast hydrolysate.

In case of distilled water, however, the normal male longevity of the parasitoids was reduced very significantly in all treatments including the control and the mean longevity varied from 1.0 to 1.4 days only.

Table: Effect of irradiated food on the male longevity of *T. chilonis*

Food material	Generation	* Male longevity (days) Cont.	2.5Gy	5Gy	7.5Gy	10 Gy	Statistical analysis	
Honey (50%)	F_1	3.6	3.9	3.7	3.8	3.6	S.Em.±	0.33
	F_2	3.8	3.7	3.6	3.9	3.5	C.D.5%	0.91
	F_3	3.9	3.5	3.8	3.7	3.6	C.D.1%	1.20
	F_4	3.7	3.6	3.9	3.5	3.4		
	F_5	3.8	3.8	3.6	3.7	3.6		
Fructose (20%)	F_1	3.6	3.6	3.5	3.7	3.4		
	F_2	3.5	3.4	3.2	3.5	3.7	S.Em.±	0.29
	F_3	3.6	3.2	3.4	3.6	3.3	C.D.5%	0.80
	F_4	3.4	3.5	3.6	3.2	3.5	C.D.1%	1.06
	F_5	3.3	3.7	3.3	3.3	3.6		
Glucose (20%)	F_1	3.4	3.3	3.4	3.2	3.5		
	F_2	3.2	3.5	3.2	3.3	3.1	S.Em.±	0.31
	F_3	3.3	3.0	3.3	3.0	3.3	C.D.5%	0.86
	F_4	3.5	3.2	3.4	3.4	3.2	C.D.1%	1.13
	F_5	3.4	3.4	3.1	3.2	3.0		
Sucrose (20%)	F_1	3.0	3.2	3.0	3.1	3.2		
	F_2	2.7	3.0	3.1	2.8	3.0	S.Em.±	0.30
	F_3	3.3	3.4	2.9	3.2	3.1	C.D.5%	0.83
	F_4	3.0	2.9	3.3	3.1	2.9	C.D.1%	1.09
	F_5	3.1	3.1	3.2	3.3	3.2		
Grape juice	F_1	2.0	2.2	2.6	2.4	2.1		
	F_2	2.1	2.0	2.3	2.5	2.2	S.Em.±	0.24
	F_3	2.3	2.3	2.5	2.2	2.0	C.D.5%	0.67
	F_4	1.9	2.2	2.4	2.2	2.1	C.D.1%	0.87
	F_5	2.1	2.1	2.3	2.2	2.0		
Yeast hydrolysate enzymatic (20%)	F_1	1.8	1.7	1.5	1.4	1.2		
	F_2	1.6	1.5	1.3	1.1	1.3	S.Em.±	0.16
	F_3	1.5	1.6	1.4	1.3	1.4	C.D.5%	0.46
	F_4	1.4	1.4	1.2	1.3	1.1	C.D.1%	0.58
	F_5	1.7	1.5	1.3	1.1	1.2		
Distilled water	F_1	1.2	1.3	1.1	1.2	1.0		
	F_2	1.0	1.2	1.3	1.1	1.2	S.Em.±	0.13
	F_3	1.2	1.4	1.2	1.1	1.1	C.D.5%	0.36
	F_4	1.3	1.1	1.3	1.2	1.4	C.D.1%	0.47
	F_5	1.1	1.3	1.2	1.4	1.2		
Unfed	F_1	1.1						
	F_2	1.3						
	F_3	1.2						
	F_4	1.1						
	F_5	1.2						

EFFECT OF IRRADIATED FOOD ON FEMALE LONGEVITY OF *T. CHILONIS*

Table : Effect of irradiated food on the female longevity of *T. chilonis*

Food material	Generation	* Female longevity (days) Cont.	2.5 Gy	5 Gy	7.5 Gy	10 Gy	Stat. analysis	
Honey (50%)	F_1	8.0	7.8	7.5	7.7	7.4		
	F_2	7.8	8.0	7.8	7.6	7.2	S.Em.±	0.38
	F_3	7.6	7.9	7.6	7.5	7.5	C.D. 5%	1.05
	F_4	7.9	7.6	7.5	7.8	7.3	C.D. 1%	1.38
	F_5	7.6	7.8	7.7	8.0	7.6		
Fructose (20%)	F_1	7.5	7.6	7.5	7.3	7.4		
	F_2	7.4	7.5	7.8	7.2	7.7	S.Em.±	0.42
	F_3	7.8	7.3	7.7	7.5	7.3	C.D. 5%	1.16
	F_4	7.6	7.7	7.4	7.4	7.5	C.D. 1%	1.53
	F_5	7.3	7.4	7.6	7.3	7.6		
Glucose (20%)	F_1	7.1	7.4	7.5	7.3	7.2		
	F_2	7.3	7.2	7.1	7.5	7.4	S.Em.±	0.48
	F_3	7.5	7.5	7.2	7.4	7.1	C.D. 5%	1.33
	F_4	7.1	7.0	7.3	7.2	7.3	C.D. 1%	1.75
	F_5	7.2	7.3	7.0	7.1	7.4		
Sucrose (20%)	F_1	7.1	7.1	6.9	7.2	7.0		
	F_2	6.9	7.4	7.2	7.3	7.3	S.Em.±	0.47
	F_3	7.3	7.2	7.4	7.0	7.1	C.D. 5%	1.30
	F_4	7.2	7.3	7.3	6.9	7.0	C.D. 1%	1.71
	F_5	7.0	7.0	7.1	7.2	7.2		
Grape juice	F_1	4.7	5.0	5.4	5.2	5.1		
	F_2	5.0	5.3	5.6	5.5	5.2	S.Em.±	0.28
	F_3	4.7	5.6	5.4	5.4	5.0	C.D. 5%	0.78
	F_4	4.9	5.4	5.5	5.4	5.3	C.D. 1%	1.02
	F_5	5.1	5.2	5.4	5.3	5.2		
Yeast hydrolysate enzymatic (20 %)	F_1	3.0	2.9	2.7	2.6	2.4		
	F_2	3.4	2.5	2.9	2.8	2.6	S.Em.±	0.27
	F_3	3.1	2.6	2.5	2.4	2.7	C.D. 5%	0.75
	F_4	3.3	2.7	2.8	2.7	2.5	C.D. 1%	0.98
	F_5	3.2	2.4	2.6	2.8	2.6		
Distilled water	F_1	1.4	1.7	1.9	1.8	1.6		
	F_2	1.6	2.0	1.8	1.9	1.7	S.Em.±	0.23
	F_3	1.5	1.8	1.6	1.7	1.6	C.D. 5%	0.64
	F_4	1.6	2.1	1.7	1.8	1.5	C.D. 1%	0.84
	F_5	1.5	1.9	1.6	1.7	1.4		
Unfed	F_1	1.7						
	F_2	1.9						
	F_3	1.6						
	F_4	1.7						
	F_5	1.8						

- The female longevity also did not differ, when fed on either irradiated honey or on the three sugars.
- In case of irradiated grape juice, a slight increase in the female longevity of the parasitoids was observed in all treatments as compared to the control.
- On the other hand, it was reduced both in irradiated and un-irradiated distilled water.
- It was, however, slightly higher in unfed condition, as compared to water.

EFFECT OF IRRADIATED FOOD ON THE DEVELOPMENT PERIOD OF *T. CHILONIS*

Studies on the effect of irradiated food on the development period of the parasitoid reveal that feeding on any of the test food had no effect, since developmental periods recorded in different generations of the treatments were on par with the corresponding generations of the control.

Table : Effect of irradiated food on the developmental **periods of *T. chilonis***

Food material	Generation	*Developmental period (days) Cont.	2.5 Gy	5 Gy	7.5 Gy	10 Gy	Stat. analysis	
Honey (50%)	F_1	9.1	9.5	9.3	9.2	9.4	S.Em.±	0.14
	F_2	9.0	9.2	9.4	9.0	9.5	C.D. 5%	0.40
	F_3	9.4	9.3	9.5	9.1	9.3	C.D. 1%	0.51
	F_4	9.2	9.1	9.3	9.2	9.1		
	F_5	9.3	9.2	9.1	9.3	9.2		
Fructose (20%)	F_1	9.5	9.4	9.3	9.5	9.4		
	F_2	9.3	9.1	9.5	9.2	9.1	S.Em.±	0.15
	F_3	9.5	9.2	9.4	9.3	9.3	C.D. 5%	0.42
	F_4	9.4	9.4	9.2	9.4	9.2	C.D. 1%	0.54
	F_5	9.2	9.3	9.3	9.2	9.5		
Glucose (20%)	F_1	9.0	9.1	9.3	9.2	9.4		
	F_2	9.2	9.2	9.5	9.4	9.1	S.Em.±	0.13
	F_3	9.3	9.4	9.2	9.3	9.3	C.D. 5%	0.36
	F_4	9.0	9.0	9.2	9.2	9.2	C.D. 1%	0.47
	F_5	9.1	9.3	9.1	9.3	9.0		
Sucrose (20%)	F_1	9.3	9.3	9.1	9.2	9.4		
	F_2	9.5	9.4	9.3	9.4	9.2	S.Em.±	0.13
	F_3	9.2	9.2	9.4	9.3	9.3	C.D. 5%	0.36
	F_4	9.1	9.0	9.2	9.1	9.0	C.D. 1%	0.47
	F_5	9.3	9.0	9.1	9.2	9.3		
Grape juice	F_1	9.5	9.3	9.1	9.4	9.2		
	F_2	9.3	9.5	9.3	9.2	9.4	S.Em.±	0.14
	F_3	9.2	9.4	9.2	9.1	9.3	C.D. 5%	0.39
	F_4	9.1	9.2	9.2	9.4	9.3	C.D. 1%	0.51
	F_5	9.4	9.3	9.4	9.3	9.3		
Yeast hydrolysate enzymatic (20 %)	F_1	9.3	9.2	9.3	9.2	9.4	S.Em.±	0.15
	F_2	9.1	9.5	9.2	9.4	9.3	C.D. 5%	0.42
	F_3	9.4	9.1	9.4	9.1	9.5	C.D. 1%	0.54
	F_4	9.2	9.4	9.1	9.3	9.3		
	F_5	9.3	9.3	9.2	9.4	9.2		
Distilled water	F_1	9.5	9.4	9.2	9.4	9.4	S.Em.±	0.09
	F_2	9.2	9.2	9.5	9.3	9.3	C.D. 5%	0.25
	F_3	9.4	9.4	9.2	9.3	9.2	C.D. 1%	0.33
	F_4	9.2	9.3	9.4	9.2	9.3		
	F_5	9.4	9.2	9.3	9.4	9.2		
Unfed	F_1	9.4						
	F_2	9.3						
	F_3	9.5						
	F_4	9.4						
	F_5	9.3						

* Mean of ten replications

EXPERIMENTAL FINDINGS

- Out of all irradiated food materials, honey (20%) at 2.5 and 5 Gy exhibited increase in mean fecundity of *T. chilonis* in all generations
- Adult emergence in F_3 – F_5, at 2.5 Gy, was observed to be significantly superior than the corresponding generation of the control
- Irradiated honey, at all test doses, did not improve the female emergence, adult longevity and the developmental period of the parasitoid
- Feeding parasitoid with irradiated fructose, sucrose and glucose (all 20%), at all test doses, did not improve any biological parameter of the parasitoid
- Increase in some biological attributes, in case of grape juice and water, is nullified since these increases were found to be always lowered than the 50% honey
- Irradiated yeast (20%) did not show any increase in biological attributes of the parasitoid.

Viability of Stored Polyhedrosis Virus of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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The redpalm weevil, *Rhynchophorus ferrugineus* (Olivier), is one of the most destructive pests attacking date palm trees, *Phoenix dactylifera* L. in the Middle East, North Africa, and Gulf States. The insect was first recorded in Egypt in 1992. Polyhedrosis virus was isolated for the first time in Egypt form dead larvae collected from destroyed palm trees. In fact, this Polyhedrosis virus was first recorded in India, 1990.

The laboratory bioassays revealed that the noctuid *Spodoptera littoralis* (Boisd.) was considerably susceptible to this polyhedrosis virus and suitable for the virus mass - production (1).

The aim of this work is to evaluate the efficacy of frozen stored *Rhynchophorus* polyhedrosis virus (FSRPV, stored for 18 months at - 4 °C) and the newly extracted *Rhynchophorus* polyhedrosis virus (NERPV) propagated in the laboratory on reared larvae.

Stock colonies were established from field-collected larvae, adults, and pupae. The larvae were easily reared on the carrot-sweet potato mash. Larvae were introduced individually into rearing cups. All rearing cups and jars were kept at 21.8 ± 0.2°C, 78.6 ±0.3% RH.

The FSRPV was bioassayed against larvae at different ages. the NERPV was isolated from natural diseased larvae of RPW collected from the field, propagated and kept as a suspension in the refrigerator. The polyhedral inclusion bodies (PIBs)of both suspensions were quantified by a haemacytometer.

Viral suspensions were introduced within the larval diet to three larval ages being 0-14 days old, 15- 45 days old and 46- 85 days old. Both LC_{50} and LT_{50} for the three concentrations (2.8×10^7, 4.2×10^7 and 5.6×10^7 PIBs/ 100g of diet) were calculated accordind to Finny.

The pathogenicity of both FSRPV and NERPV were established at different larval ages inoculated with median concentrations as shown in Figure 1. The influence of larval age of the RPW used in the bioassay was obvious (Table 1). The LC_{50} of the NERPV for 0-14 days old and 15 - 45 days old larvae were significantly lower than the 46 - 85 days old ones being 2.9, 2.6 and 4.0×10^7 PIBs/ 100g of diet, respectively.

In fact, no significant difference in LC_{50} occurred between FSRPV and NERPV treated larvae. The LC_{50} of FSRPV at 11, 12, 16 and 18 days post treament has no significant differences between young, middle and older larvae for concentrations of 3.5, 3.8, 3.4 and 3.3×10^7, respectively.

The time to attain 50% mortality was affected by both the age of tested larvae and the dose used, being from 2.4 to 24.3 days and form 3.2 to 25.9 days for NERPV and FSRPV, respectively. These results agree with the results of Alfazairy *et al.*, (2) who indicated that susceptibility level depends on larval age and virus concentrations.

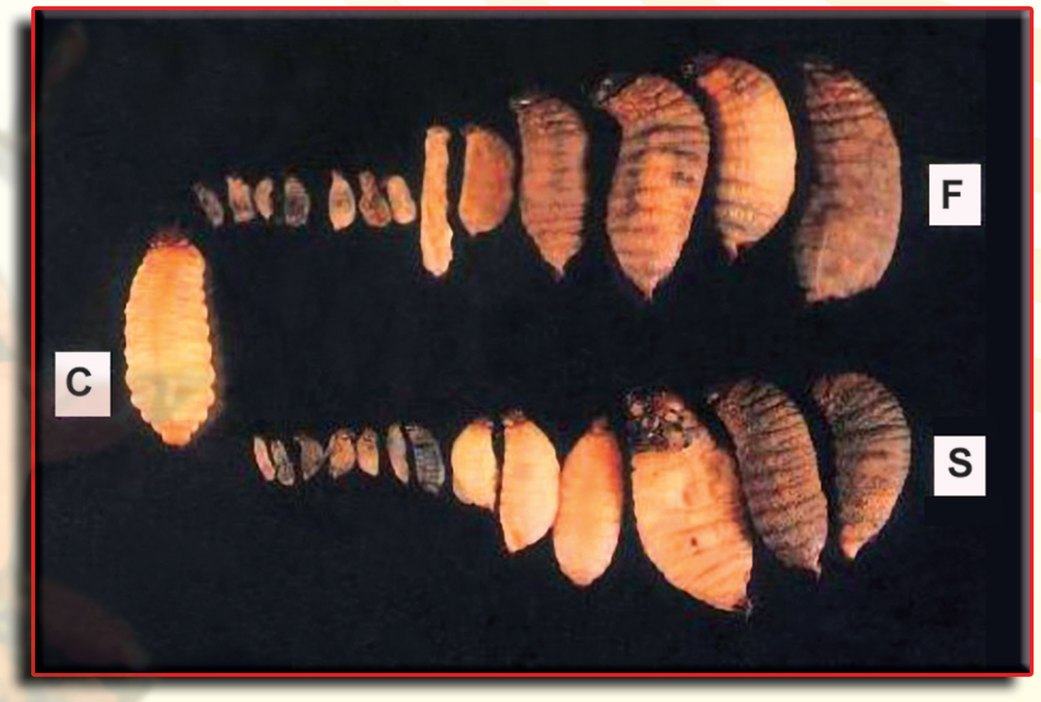

Figure 1 : Pathogenic symptoms of RPW infected larvae with the NERPV and FSRPV.
S : FSRPV F : NERPV C : Control

Table 1 : RPW larvae treated with different concentrations of both NERPV and FSRPV indicating first and last days of dead larvae after treatments.

Ages	Concentrations (PIBs/100 diet)	NERPV		FSRPV	
		First day of mortality	Last day of mortality	First day of mortality	Last day of mortality
0-14 days	Control	-	-	-	-
	2.8×10^7	5	18	4	21
	4.2×10^7	2	12	3	17
	5.6×10^7	1	4	3	6
15- 45 days	Control	-	-	-	-
	2.8×10^7	6	22	7	26
	4.2×10^7	4	15	5	21
	5.6×10^7	1	8	1	10
46- 85 days	Control	-	-	-	-
	2.8×10^7	8	31	9	33
	4.2×10^7	3	26	7	26
	5.6×10^7	2	18	2	18

[1] Alfazairy; A. A., A. M. El-Minshawy, H. H. Karam and R. A. Hendi. 2003b. The noctuid, *Spodoptera littorallis* (Boisd.) as an alternate host for propagation of a polyhedrosis virus of the curculionid *Rhynchophorus ferrugineus* (Olivier), and as an insect for bioassay *Bacillus thuringiensis* preparations The first Int. Egyptian-Romanian conf., Zagazig, Egypt, 191 - 194.

[2] Alfazairy; A. A., A. M. El-Minshawy, H. H. Karam and R. A. Hendi. 2003c. Naturally occurring viral and bacterial entomopathogens in the red palm weevil *Rhynchophorus ferrugineus* (Olivier), and their efficacy as microbial control agents for this curculionid pest. The first Int. Egyptian-Romanian conf., Zagazig, Egypt, 143 - 160.

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Control of CM (*Cydia pomonella*) using the area-wide approach in Chile

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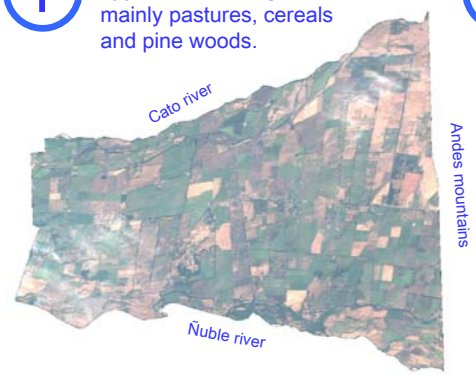
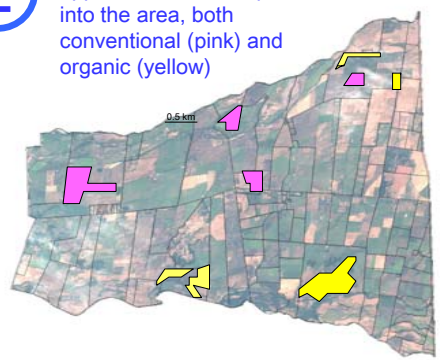
66P

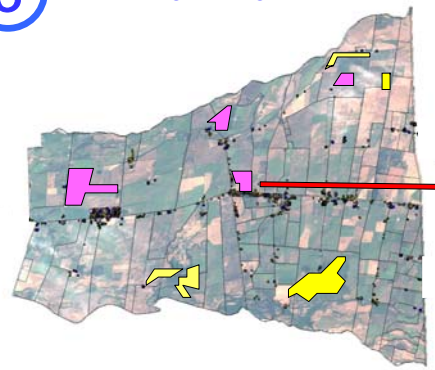
1 Area under area-wide approach covering 6000 ha, mainly pastures, cereals and pine woods.



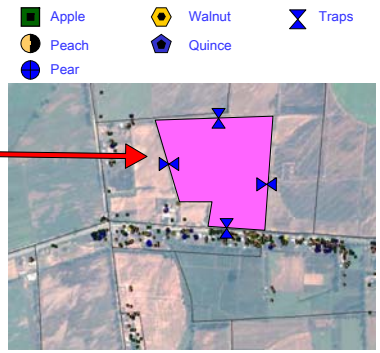
2 About 400 ha of commercial apple orchards are sparsed into the area, both conventional (pink) and organic (yellow)



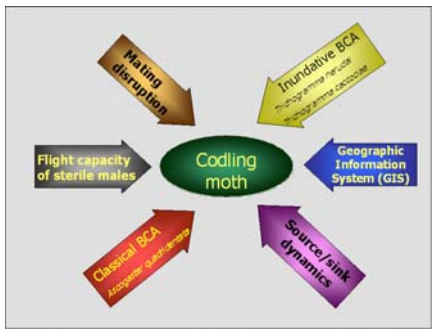
3 Many CM hosts grow in the area, acting as refugees



4 Trap pairs are put around every orchard and migrant moths are recorded through season.



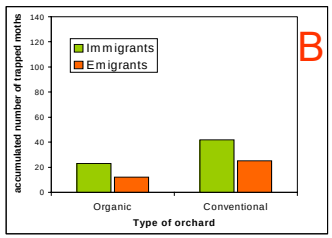
CM control is done integrating several techniques:

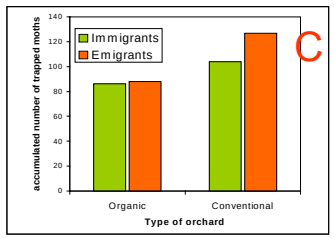


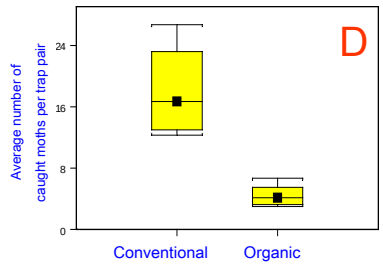
RESULTS

Population dynamics

CM dynamics between orchards and wild hosts were estimated using bidirectional traps (A). Inmigration is higher than emigration in the 1st half of season, October-December (B), but inmigration and emigration are equal in the 2nd half (C), January-March. More moths were caught in conventional than in organic orchards (D).



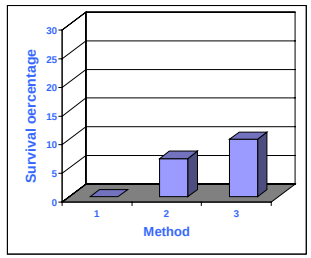




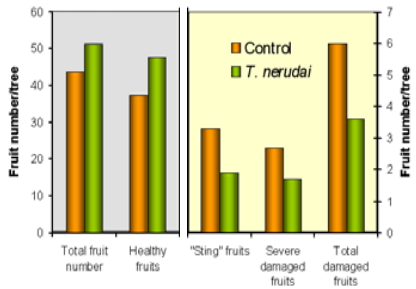
A. quadridentata release

Neonate CM parasitized by *A. quadridentata* were released on apples using a fine brush.

Three methods were compared: no injured apple (1); apple which a circle of skin was removed (2); and bagged apple (3).



Efficacy of Chilean trichogramma.



One sq inch/tree of the Chilean species *Trichogramma nerudai* was release weekly on the borders of an orchard under mating disruption (n=100).

Fruit dropping, light ("sting") and heavy damage were reduced about 13, 40 and 30%, respectively.

Effects of Host Density-age And Gamma Radiation on The Mass Production of *Nesolynx thymus* (Hymenoptera:Eulophidae), An Endoparasitoid of Uzifly *Exorista sorbillans* (Wied.)

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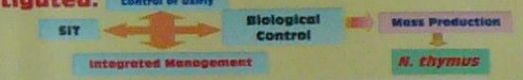
Introduction

- The Uzifly, ***Exorista sorbillans*** (Wiedmann) (Diptera:Tachinidae) is a serious endoparasitoid of the silkworm. It causes 20-40 % loss to silk industry.
- ***Nesolynx thymus*** (Girault) (Hymenoptera: Eulophidae) is a gregarious pupal parasitoid of Uzifly.
- ***N. thymus*** was found to have the best characteristics as potential control agents.
- ***N. thymus*** has a multiple host range. They attack a number of dipteran insect species including uzifly, blowfly, fleshfly and housefly.
- Effects of host age, density and gamma radiation (^{60}Co) on the mass-production of ***N. thymus*** were investigated.

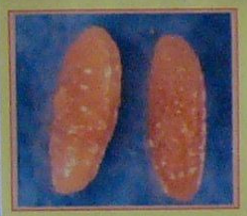


1

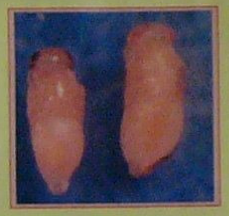
Results

- Host density and host-age significantly ($P<0.001$) influenced the sex-ratio of progeny in ***N. thymus***.
- Higher proportions of females were observed for all the host density levels and host-age groups.
- The proportion of females was gradually decreased as the density of host was increased for more or less all the age groups.
- Gamma irradiation significantly ($P<0.001$) increased the progeny production of ***N. thymus*** while reared either on early and late irradiated host puparia.
- The irradiated early host pupae were more suitable for mass production of ***N. thymus*** than the irradiated late pupae.

2

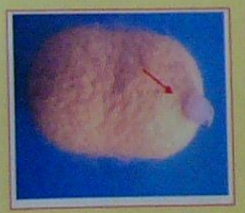


N. thymus larvae



N. thymus Pupae

N. thymus Adult

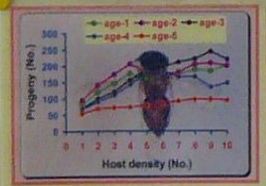


Uzi maggots perforating

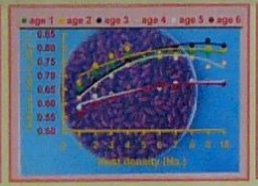
Perforated Uzi host pupae

Uzi adults

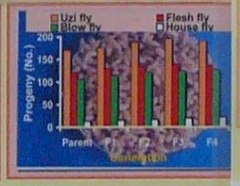
3



Effect of host density and age on the progeny production of ***N. thymus***

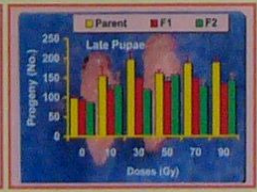


Intrinsic rate of increase for the progeny production of ***N. thymus***

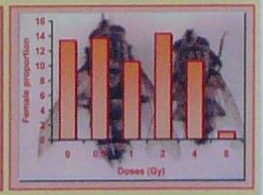


Host ranges for the progeny production of ***N. thymus***

Effect of gamma irradiation on the progeny production of ***N. thymus*** treated as early host pupae



Effect of gamma irradiation on the progeny production of ***N. thymus*** treated as late host pupae



Effect of gamma irradiation on the sex-ratios of ***N. thymus*** treated as early host pupae

4

Results

- All the parameters including host density, sex and their possible interactions showed significant ($P<0.001$) effect on the progeny production of ***N. thymus*** for all the host-age groups.
- Results revealed that there was a direct and progressive relationship between the progeny production and the increase in number of host puparia.
- There was a significant ($P<0.001$) decline in the number of progeny with the increase in host-age.
- The maximum progeny production can be obtained by maintaining the range between 1:5 to 1:8 parasitoid to host ratio for all the age groups.
- The 2-4 day-old host puparia were most suitable for obtaining the maximum progeny production of ***N. thymus*** for all the host-densities.
- The values for $r_m day^{-1}$, R_o and GRR increased with the increased host density

5

Results

- Female biased sex-ratios were observed for all the host-age groups. The sex-ratios were dose-dependent, *i.e.*, the number of females increased as the doses increased.
- A highly significant ($P<0.001$) difference was observed in the progeny production within the host range.
- The trend of progeny production was uzifly > fleshfly > blowfly > housefly.

conclusion

Ionizing radiation offers a reliable means to achieve developmental arrest of insect host for use in ***in vivo*** rearing prior to mass production of the parasitoid ***N. thymus***. These findings will be further tested in an area-wide demonstration site at sericultural practiced area, northern region of Bangladesh, where both ***N. thymus*** and sterile uzi flies will be released.

6 This project is funded by the IAEA [IAEA/BGD-10776]

Evaluation of Various Date Palm Cultivars for Red Date Palm Weevil *Rhynchophorus ferrugineus* (Oliv.) Rearing.

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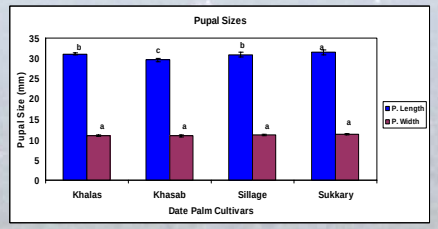
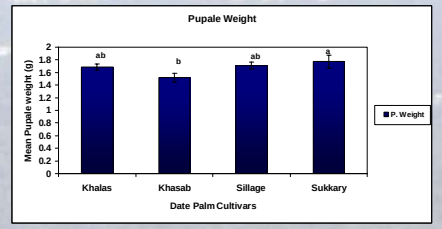
Introduction:

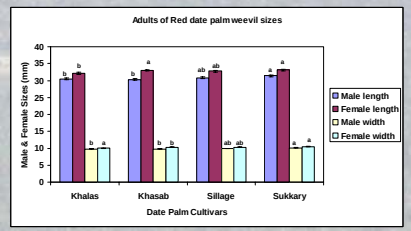
•Red date palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae) is the most destructive insect pest of date palm *Phoenix dactylifera* L on Saudi Arabia since 1986.
•The estimated area of date palm is 139,099 Hectares with approximately 817,887 tons of date production.
• Up to 10 tons/ha. reduction in yield due to Red date palm weevil infestation (Gush 1997).
• Saudi Arabia has approximately 400 date palm cultivars.
• Damage to date palm tree is mainly caused by larval stage of the insect that feeds on the trunk of the tree.

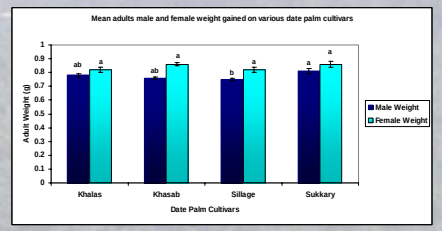
Objective:

Evaluation of various date palm cultivars on red date palm weevil *Rhynchophorus ferrugineus* Oliv. development.

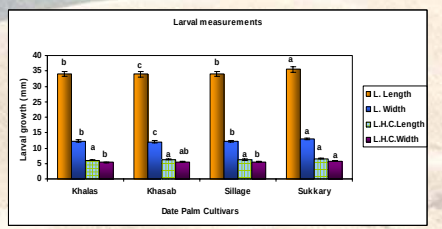


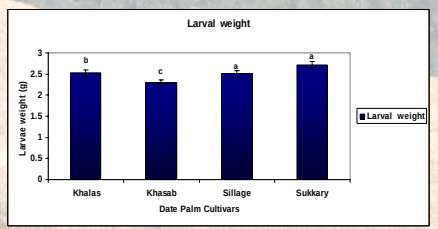






Date Palm Cultivars	Average females lifespan (Days)	Average number of eggs per female
Khalas	120.17 b	208.83 b
Khasab	186.17 a	229.50 b
Sillage	133.50 b	198.33 b
Sukkary	132.00 b	339.17 a





Conclusions:

1. Food quality differences have a significant impact on biological parameters.
2. Sukkary date palm cultivar proved to be the best basic food ingredient for insect growth.
3. Females fed on Khasab significantly have longer lifespan. However, more eggs were laid by females fed on Sukkary.

Acknowledgments:
I would like to thank Mr. Khawaja Gulam Rasool, Mr. Waleed S. Al Waneen and Mr. Waleed K. Al Abdulsalam for their technical assistance in the laboratory and field work,
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Activities of the Iraqi Project for Controlling OWSWF, *Chrysomya bezziana* and the Prospects for the Future

IAEA-CN-131/69P

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INTRODUCTION

The Old World Screwworm Fly, *Chrysomya bezziana*, is an obligate parasite of worm blooded animals, particularly cattle, sheep, goat--------etc. & human. The total cases reported by Iraqi Veterinarian Service from Sept. 1996 till December, 2004 was 119243, in addition to 23 cases in human being. Therefore, a project was initiated at IAEC/MoST since the identification of this pest in Iraq aiming to use SIT for eradicating it. Following some of the results obtained which appeared to be promising & encouraging.

RESULTS

First: Quality Control Parameters of the Colony of *Chrysomya bezziana* During the Last Twentieth Generations (Tables 1-5)

Table1. Some Biological Parameters of the Main Colony of *Chrysomya bezziana* Through Generations

Generation	No. of pupae produced	Total No. of adult emerged	Percent of emerged adult	No. of adult		Sex ratio
				Male	Female	
68	12325	11272	91.4	5878	5394	1.09:1
69	14125	11256	79.7	5034	6222	1:1.23
70	9248	8058	87.1	3699	4359	1:1.18
71	13877	13162	94.8	7049	6113	1.15:1
72	22100	14700	98.0	8016	66.84	1.19:1
73	12980	12187	93.9	6442	5745	1.21:1
74	25024	24054	96.1	13218	10836	1.22:1
75	21880	13794	63.0	6853	6841	1:1
76	11148	10946	98.2	7115	3831	1.86:1
77	25540	16677	65.3	8831	7846	1.13:1
78	13232	12047	91.0	7663	4384	1.75:1
79	10150	9403	92.6	5957	3446	1.73:1
80	16666	15582	93.5	8287	7295	1.41:1
81	10075	9410	93.4	5517	3894	1.42:1
82	12846	12240	95.3	6220	6020	1.03:1
84	14628	13808	94.4	6815	6993	1:1.03
86	16488	15663	95.0	7905	7758	1.02:1

*Average age of generation 23.9 ± 9.5 days (12-43 days)
**During some generations & as a result of failure in electricity low percent of adults emergence was observed.

Table 2. Sample of 500 Pupae of *Chrysomya bezziana* in Small Cage for Adult Emergence & % of Female Produced

Generation	Emergence (day)	No. of adult emerged		% female	Total No. of adult	% of emergence
		Male	Female			
60	1st	10	18	51.2	455(11)	91.0
	2nd	128	118			
	3th	84	97			
64	1st	10	10	42.3	378(12)	75.6
	2nd	183	143			
	3th	25	7			
66	1st	15	9	43.9	460(12)	92.0
	2nd	135	155			
	3th	108	38			
70	1st	8	14	47.1	433(13)	86.6
	2nd	128	109			
	3th	95	79			
77	1st	22	17	46.3	455(14)	89.0
	2nd	158	148			
	3th	59	41			
80	1st	18	16	48.7	495(17)	99.0
	2nd	168	154			
	3th	68	71			
84	1st	16	20	50.3	479(16)	95.8
	2nd	161	158			
	3th	61	63			

*Number between parenthesis represent the malformed adults.

Table 3. Flight Ability Index of *Chrysomya bezziana* Through Some Generations Under Laboratory and Field Conditions

Generation	F.A.I (L)	F.A.I(F)	Note
63	91.4	90.6	*Ten replicates / generation (Each replicate includes 100 pupa 4-5 days) **Economopoulos et. al. (1990) method was followed to calculate F.A.I ***F.A.I (L) & F.A.I. (F) means flight ability at laboratory & field conditions, respectively.
66	86.6	92.3	
71	92.2	90.8	
72	86.4	89.4	
75	90.6	88.6	
77	93.4	94.3	
79	94.3	95.7	
80	91.8	93.6	
82	89.7	92.8	
84	94.6	93.4	

Table 4. Survival of *Chrysomya bezziana* Adults with & without Food

Generation	Survival of adults (Days)		Note
	With food	Without food	
60	22.5	2.6	Ten replicates / generation (Each replicate includes 25 adults of 12-24 hrs old)
64	21.5	2.3	
66	22.4	2.7	
70	21.2	2.8	
77	21.9	3.2	
80	22.7	2.8	
84	21.9	2.6	

Table 5. Longevity of *Chrysomya bezziana* Males & Females Through Generations

Generation	Longevity (Days)		Note
	Male $\bar{X}$ ± S.D	Female $\bar{X}$ ± S.D	
60	22.7 ± 1.2	24.3 ± 0.6	Ten replicates / generation (Each replicate includes 25 adults of 12-24 hrs old)
64	22.3 ± 0.8	20.8 ± 0.8	
66	22.4 ± 1.3	22.4 ± 1.1	
70	20.9 ± 0.7	21.6 ± 0.6	
77	21.4 ± 0.6	22.4 ± 0.8	
80	21.8 ± 0.8	23.7 ± 0.8	
84	20.7 ± 0.9	23.2 ± 0.3	

Second/ Effect of Gamma Radiation on Some Biological Performance Parameters of *Chrysomya bezziana* (Tables 6-14)

Table 6. Effect of Different Doses of Gamma Rays on the Longevity of *Chrysomya bezziana* Males & Females Irradiated as Pupae at Different Ages

Age of irradiated Pupae (Day)	2		3		4		5	
Dose (Gy) / Sex	Male	Female	Male	Female	Male	Female	Male	Female
Control	21.7	22.5	23.0	22.5	23.0	22.5	22.7	24.2
15	7.2	7.7	10.9	9.0	13.7	13.8	22.4	24.0
30	4.8	5.0	8.7	9.0	12.0	11.8	20.7	22.6
45	4.0	4.0	8.7	8.6	11.5	11.2	19.9	20.8
60	4.0	3.7	7.0	8.0	10.2	10.7	20.5	19.4
75	3.0	3.5	6.7	8.0	10.2	9.7	19.9	18.8
90	2.0	3.0	6.2	7.0	8.2	9.0	17.4	18.6
LSD 0.05	3.0	2.9	2.8	3.0	1.6	1.8	N.S.	3.25

Table 7. Effect of Different Doses of Gamma Rays on the Percentage of *Chrysomya bezziana* Adult Emergence Irradiated as Pupae at Different Ages

Age of irradiated Pupae (Day)	2		3		4		5	
Dose (Gy) / Sex	Male	Female	Male	Female	Male	Female	Male	Female
15	10.0	11.7	38.3	26.7	48.3	45.0	43.5	47.5
30	8.3	13.3	36.7	26.7	45.0	40.0	40.7	39.2
45	8.3	11.7	30.0	31.7	41.7	40.0	39.2	42.2
60	5.0	6.4	31.7	26.7	31.7	40.0	41.3	37.7
75	5.0	7.3	18.7	28.3	25.0	41.7	41.5	37.7
90	3.3	8.3	16.7	20.0	31.6	41.7	30.3	36.7
Control	43.3	45.0	41.7	36.7	46.7	52.5	46.7	52.5
LSD 0.05	7.2	9.2	10.1	11.0	15.3	11.7	6.6	8.7

Table (8) Effect of Different Doses of Gamma Rays on the Sex Ratio of *Chrysomya bezziana* Irradiated as Pupae at Different Ages

Age of Irradiated Pupae (Day)	2		3		4		5	
Irradiated Dose (Gy) / Sex	Male	Female	Male	Female	Male	Female	Male	Female
15	1	1.17	1	0.69	1	0.93	1	1.09
30	1	1.60	1	0.73	1	0.88	1	0.96
45	1	1.40	1	1.00	1	0.96	1	1.08
60	1	1.30	1	0.84	1	1.26	1	0.91
75	1	1.66	1	1.51	1	1.76	1	0.90
90	1	2.51	1	0.75	1	1.32	1	1.21
Control	1	1	1	0.88	1	1.12	1	1.12

Table (9) Effect of Different Doses of Gamma Rays on the Flight Ability Index of *Chrysomya bezziana* Under Lab. & Field Conditions*

Irradiated Dose (Gy)	Flight Ability Index		
	Lab. Condition	Field Condition	Mean**
0.0 Control	90.1 a	84.7 a	87.4 a
30	84.0 a	81.0 b	82.5 ab
45	83.3 a	78.6 b	81.0 ab
60	82.4 a	73.0 c	77.7 b
75	80.1 a	72.1 c	76.1 b
Mean**	84.0 a	77.9 b	

* Means within a column followed by the same letter are not significantly different at 5% according to DMRT.
** Vertical & Horizontal Means followed by the same letter are not significantly different at 5% according to DMRT

Table (10) Mating Competitiveness Value (C.V.) of *Chrysomya bezziana* Males Irradiated as 4-5 Days Old Pupae and Released with Unirradiated Males & Females*

Type of Mating NF:NM:SM	Average No. of Egg Laid	Percentage of Hatch Eggs	Percentage of Unhatch Eggs	Expected Percent of Unhatch Eggs	C. V.
Dose 30 Gy					
1:1:0	297 a	93.5a	6.5	-	-
1:0:1:	243 a	0 d	100	-	-
1:1:1	273 a	56.1 b	43.9	53.3	0.82
1:1:5	207 a	42.9 b	57.1	84.4	0.67
1:1:10	286 a	5.9 c	94.1	91.5	1.03
Dose 60 Gy					
1:1:0	340 a	91.2 a	8.8	-	-
1:0:1	251 a	0 c	100	-	-
1:1:1	215 a	69.3 ab	30.7	54.4	0.6
1:1:5	292 a	58.3 ab	41.7	84.8	0.5
1:1:10	361 a	43.3 b	56.7	91.7	0.6

SM: Sterile Male, NM: Normal Male & NF: Normal Female
* Means within a column followed by the same letter are not significantly different at 5% according to DMRT

Table (11) Mating Competitiveness Value (C.V.) of *Chrysomya bezziana* Females Irradiated as 4-5 Days Old Pupae & Released with Unirradiated Males & Females *

Type of Mating NM:NF:SF	Average No. of Egg Laid	Percentage of Hatch Eggs	Percentage of Unhatch Eggs	Expected Percent of Unhatch Eggs	C. V.
Dose 30 Gy					
1:1:0	291 ab	94.9 a	5.1	-	-
1:0:1	0 c	0 c	100	-	-
1:1:1	103 bc	71.4 a	28.6	52.6	0.5
1:1:5	196 b	74.0 a	26.0	84.2	0.3
1:1:10	463 a	35.4 b	64.6	91.4	0.7
Dose 60 Gy					
1:1:0	301 a	96.7 a	3.3	-	-
1:0:1	0 b	0 c	100	-	-
1:1:1	224 a	80.2 a	19.8	51.6	0.4
1:1:5	286 a	71.2 a	28.8	83.9	0.3
1:1:10	408 a	49.1 b	50.9	91.2	0.6

SF: Sterile Female, NF: Normal Female & NM: Normal Male
* Means within a column followed by the same letter are not significantly different at 5% according to DMRT

Table (12) Mating Competitiveness Value (C.V.) of *Chrysomya bezziana* Male & Females Irradiated as 4-5 Days Old Pupae & Released with Unirradiated Males & Females*

Type of Mating NM:NF:SM:SF	Average No. of Egg Laid	Percentage of Hatch Eggs	Percentage of Unhatch Eggs	Expected Percent of Unhatch Eggs	C. V.
Dose 30 Gy					
1:1:0:0	268 a	94.5 a	5.5	-	-
0:0:1:1	0b	0 c	100	-	-
1:1:1:1	338 a	22.8 b	77.2	52.8	1.46
1:1:5:5	327 a	0.2 b	99.8	84.4	1.18
1:1:10:10	200 a	0 c	100	91.4	1.09
Dose 60 Gy					
1:1:0:0	337 a	87.2 a	12.8	-	-
0:0:1:1	0 c	0 c	100	-	-
1:1:1:1	298 ab	64.4 a	35.6	56.4	0.63
1:1:5:5	302 ab	24.8 b	75.2	85.5	0.87
1:1:10:10	169 b	16.3 b	83.7	92.1	0.90

SF: Sterile Female, SM: Sterile Male, NF: Normal Female & NM: Normal Male
* Means within a column followed by the same letter are not significantly different at 5% according to DMRT

Table (13) Effect of Gamma Rays & Mating Types on the Mean Number of Eggs Laid by *Chrysomya bezziana* Females*

Irradiated Dose (Gy)	Type of Mating		
	Irradiated Male × Irradiated female	Irradiated female × Unirradiated male	Irradiated male × Unirradiated female
0.0 (control)	632 a	632 a	632 b
15	114b	108 b	538 c
30	0.0 c	0.0 c	398 d
45	0.0 c	0.0 c	615 bc
60	0.0 c	0.0 c	698 b
75	0.0 c	0.0 c	1083 a

* Means within a column followed by the same letter are not significantly different at 5% according to DMRT.

Table (14) Effect of Gamma Rays & Mating Types on the Percentage of hatch Eggs of *Chrysomya bezziana**

Irradiated Dose (Gy)	Type of Mating		
	Irradiated Male × Irradiated female	Irradiated female × Unirradiated male	Irradiated male × Unirradiated female
0.0 (control)	93.8 a	93.8 a	93.8 a
15	1.7 b	13.0 b	1.7 b
30	0.0 c	0.0 c	0.0 c
45	0.0 c	0.0 c	0.0 c
60	0.0 c	0.0 c	0.0 c
75	0.0 c	0.0 c	0.0 c

* Means within a column followed by the same letter are not significantly different at 5% according to DMRT.

CONCLUSION

1. Success in rearing OWSWF Chrysomya bezziana on diet composed of minced meat free of fat 40%, whole blood 15% ; D.water 44.7% & formalin 0.3% continually for 88 generations.

2. The results obtained on the effect of gamma rays on adult emergence when fly irradiated as pupae at different ages, longevity of produced males & females, sex ratio, flight ability index under lab & field conditions & mating competitiveness value were very encouraged and important for the project.

3. The results obtained on the effect of gamma ray & mating type on the number of eggs laid & its percentage of hatch were very important & showed that dose of 30 Gy or higher caused complete sterility for both sexes. This was confirmed cytologically by examining the ovary & the testis.

4. The future plans aim to eradicate OWSWF from Iraq & other middle east countries using SIT through an project supervised by AOAD, FAO & IAEA. Several studies such as socio-economic feasibility study, further biological & ecological studies using artificial diet in stead of the diet mentioned above (paragraph 1 of the conclusion) & feasibility of using GIS computer for monitoring OWSWF in Iraq & other middle east countries are needed.

Gamma Radiation: A new Technique for Area Wide Control of *Tetranychus urticae* in Vegetable Crops in Egypt

IAEA-CN-131/70 P

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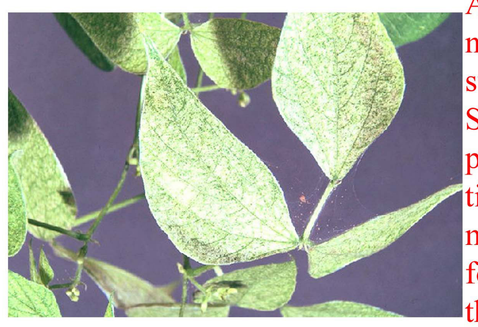
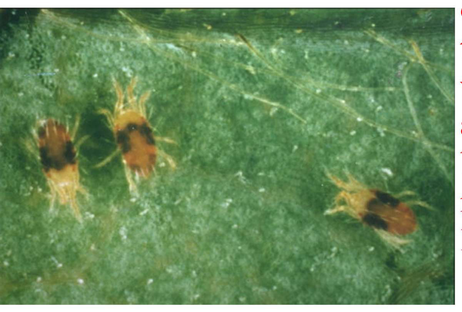
The twospotted spider mite, *Tetranychus urticae* Koch... An important pest in Eygpt

The twospotted spider mite is oval in shape, and may be brown or orange-red, but a green, greenish-yellow The adult is about 0.4 mm in length with an elliptical body that bears 12 pairs of dorsal setae. The body contents) large dark spots(are often visible through the transparent body wall. Since the spots are accumulation of body wastes, newly molted mites may lack the spots.



All mites have needle-like piercing-sucking mouthparts. Spider mites feed by penetrating the plant tissue with their mouthparts and are found primarily on the underside of the leaf.



The mites generally feed underneath the leaves and cause graying of the leaves due to mesophyll collapse and yellowing. Necrotic spots occur in the advanced stages of leaf damage.

The eggs are attached to fine silk webbing and hatch in approximately three days. The life cycle is composed of the egg, the larva, two nymphal stages) protonymph and deutonymph(and the adult. The length of time from egg to adult varies greatly depending on temperature.

A number of vegetable crops such as tomatoes, squash, eggplant, cucumber are also subject to twospotted spider mite infestations and damage.

The adult female lives two to four weeks and is capable of laying several hundred eggs during her life. The twospotted spider mite prefers the hot, dry weather of the summer and fall months, but may occur anytime during the year.

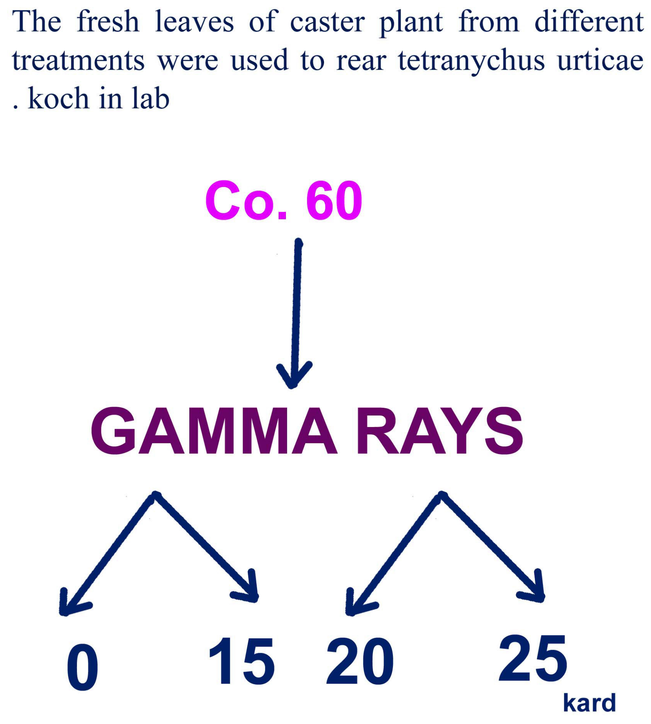
Fruit crops attacked include blackberries, blueberries and strawberries. The twospotted spider mite is also a serious pest in greenhouses

This study is aimed at gaining useful and enough disturbance in the biology of the two spotted spider mite

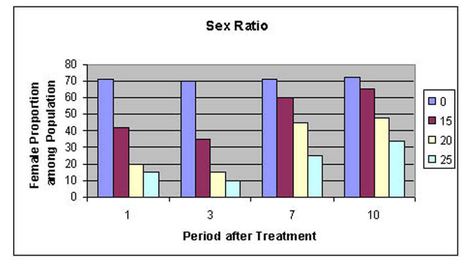
Castor oil plant is the most favorite host plant for the pest Tetranychus urticae koch by the begining of March spring season this host plant attracts the active generation of this pest from which it spreads to other vegetable crops Number of generation could reach 40-50/year All kinds of vegetable crops suffer from this pest attack

Equal size and mature seeds of caster oil plants were divided into 4 similar groups the first was kept as a control without any treatment, the other 3 groups were irradiated with 15, 20 and 25 krad of Gamma rays using Co 60 as a source of radiation

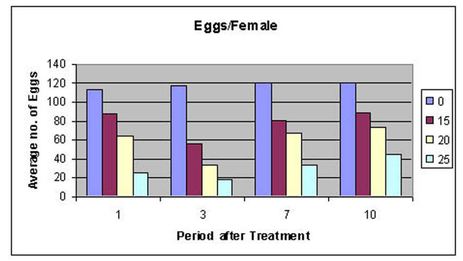
The fresh leaves of caster plant from different treatments were used to rear tetranychus urticae . koch in lab



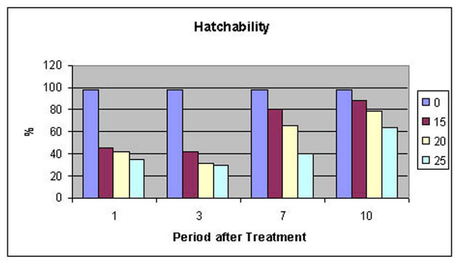
The biological aspects were directly affected by radiation the effects showed either linear positive or negative relationship with increasing the dose of Gamma radiation as follows:



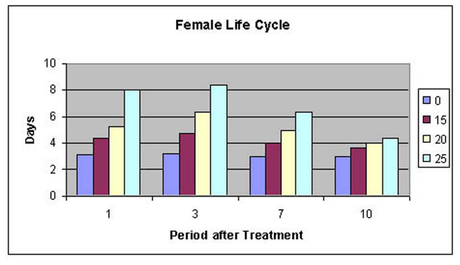
Female proportion among the whole population (Sex ratio) decreased to 29.6%



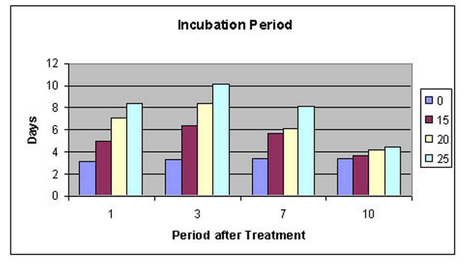
Number of Eggs/female decreased to 26%



Eggs hatchability decreased to 43%.



Female life cycle was prolonged and increased to 221% .



Incubation period of eggs was prolonged and increased to 236%. Compare with the control

- All mentioned observed aspects seem to be beneficial to control this pest .

- The severe effects of radiation were recorded at 3 weeks plant age, then the parameters tended to be similar to those of control treatment.

- **Suggested Plan of Action:**

Remove all caster plants scattered throughout the cultivated area . Arrange sowing the irradiated caster seeds in between the vegetable plantations before the peek time of the pest occurrence .

Management of melon fly infesting cucurbit growing areas of Bangladesh using Sterile Insect Technique

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Abstract

Protocols for the application of SIT have been developed to suppress the melon fly, *Bactrocera cucurbitae,* the key pests of cucurbit growing areas in the country. A collaborative approach have been initiated with the Entomology Division, Bangladesh Agricultural Research Institute (BARI) to transfer the SIT technology to the field levels. Insect Biotechnology Division of Institute of Food and Radiation Biology (IFRB) have developed mass rearing facility of fruit fly using artificial diet, protocols for SIT application i.e., sterility dose, mating competitiveness, quality control etc. Sterilizing dose for the melon fly was determined by exposing 72 h old pupae to gamma ray at various doses viz., 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy. A dose of 50 Gy was found to attain 100% sterility to melon fly. Mating competitiveness of the irradiated males and the virgin untreated females were conducted at ratios 1:1. Semi-field trials of SIT have been conducted in a netted cage placed in a field. A netted area of 3 x 1.5 x 2 meters (L x W x H) was used for the semi field trial experiments. The irradiated males (50 Gy) and unirradiated females were released at a ratio of 1:1 in the netted cages. Mating period was recorded between 5 p.m. and 9 p.m. A complete sterility was also recorded from the semi-field trial experiments. The mating of irradiated males with unirradiated females were recorded as 69.4 % ± 7.8.

Melon fly is the major pest of vegetables in Bangladesh. They cause serious damage in the field and are considered to be of great quarantine barrier for the free movement and trade of these commodities in the world market. Research programmes have been undertaken to introduce effective environment friendly approaches to control fruit fly. Nasiruddin *et al.* (2001) reported 50% reduction of fruit fly population in Jessore district area of Bangladesh by using bait traps of cuelure pheromone. Nasiruddin *et al.* (2001) also reported the higher capture of fruit fly using sex pheromone in combination with methyl eugenol in bait traps. Insect Biotechnology Division (IBD) of Bangladesh Atomic Energy Commission (BAEC) has been engaged to develop SIT of melon fly and to integrate the technique with the other eco-friendly approaches for effective and sustainable melon fly management in Bangladesh.

Results

Effect of irradiation on melon fly in laboratory condition

The 72 h old pupae of *B. cucurbitae* were subjected to gamma irradiation from 5 to 100 Gy. The results are presented in Figure 1. A complete sterility was achieved at 50 Gy gamma irradiation dose.

Mating competitiveness

The mating competitiveness of the laboratory reared irradiated males with unirradiated females were carried out in the netted field cage. The results are presented in figure 2. The 72 h old pupae were irradiated and the irradiated males were allowed to mate with the unirradiated virgin females at 1:1 ratio. The mating of 40 Gy treated males with the unirradiated females produced only 6% viable adults. But the application of gamma irradiation dose of 50 Gy to melon fly males and subsequent mating with the unirradiated females at 1:1 ratio did not produce any viable larvae, pupae and adult.

Optimization of cost-effective artificial diets for mass rearing of melon fly

The optimization of artificial diets for mass rearing of melon fly is one of the important aspects of our research for cost-effective quality mass production. Initially, three different compositions of wheat bran, soya bran and sweet gourd based larval diets have been tested (Table 1). The results are presented in Figures 3 and 4. Figure 3 shows the percent of egg hatch, pupation and subsequent adult emergence of melon fly reared in artificial diets. The results indicate that the percent adult emergence is about 85% when reared in diet C. On the other hand, slight reduction of adult emergence was recorded when the larvae of *B. cucurbitae* reared in diet B and A, respectively. Figure 4 shows the percent of mortality of the emerged adult in two weeks period reared in artificial diets. About 3 percent mortality were recorded in the fly population reared in artificial diet C. The mortality was recorded 13% in the fly population reared on artificial diet A.

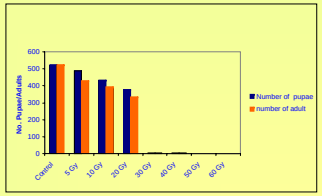


Figure 1. Number of pupae and adults from the mating of Irradiated males and unirradiated females

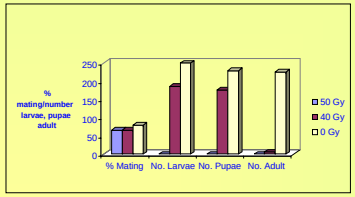


Figure 2. Mating competitiveness of irradiated males and unirradiated females

Ingredients	Diet A	Diet B	Diet C
Wheat bran	400 g	600 g	450 g
Soya bran	600 g	400 g	550 g
Sweet gourd	500 g	500 g	500 g
Sugar	200 g	200 g	200 g
$C_7H_5O_2Na$	10 g	10 g	10 g
HCl	3.75 ml	3.75 ml	3.75 ml
Vitamin C	25 g	25 g	25 g
Water	1275 ml	1275 ml	1275 ml

Table 1. Composition of soya and sweet gourd based artificial diets

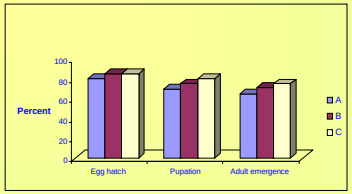


Figure 3. Hatching, pupation and adult emergence of melon fly reared in artificial diets

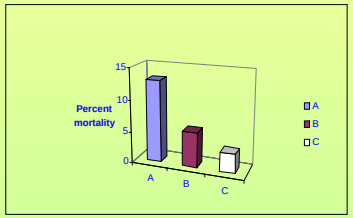


Figure 4. Mortality of adults reared on artificial diets in two weeks

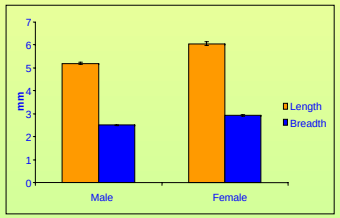


Figure 5. Length and breadth of male and female pupae of *B. cucurbitae*

Pupal size

The size of the pupae obtained from the larvae reared in artificial diet C were recorded and presented in figure 5. The size of the female pupae was found superior than the male pupae. Depending on the size of the pupae, the sexes were separated by mechanical sieving. About 70% separation could be achievable by this manual technique. The length of the male and female pupae was 5.19 and 6.05 mm respectively. The breadth of the male and female pupae was 2.5 and 2.94 mm respectively.

Conclusion

A considerable amount of cucurbit vegetables are grown in Bangladesh. The consequence of high population of fruit fly cause considerable damage to the cucurbit vegetables each year. Researchers of Bangladesh Agricultural Research Institute have been associated with considerable field research on pheromone baits for suppressing the wild population in cucurbit growing areas. Integrating the pheromone baits traps with the SIT programme would be very much desirable to minimize the SIT operational cost significantly. Considering the data obtained from irradiation studies and limited release of sterile flies in semi-field trials, we can conclude that some of the research areas needs further refinement before applying SIT in actual cucurbit growing areas. Vegetable farmers are continually increasing their support for the eco-friendly fruit fly control measures in Bangladesh.

References

Nasiruddin, M., Alam, S.N., Faruquzzaman, M., Khorsheduzzaman, Jasmine, H.S., Siddique Alam, Karim, A.N.M.R. and Rajotte, E. 2001. Management of cucurbit fruit fly, *Bactrocera cucurbitae,* in cucumber and sweet gourd by using pheromone and indigenous bait traps and its effect on year-round incidence of fruit fly. Abstract, Eight Annual Report of the Integrated Pest Management Collaborative Research Support Programme (IPM CRSP).

Nasiruddin, M., Alam, S.N., Zaman, M.F., Zaman, K., Jasmine, H.S., Siddique Alam, Talekar, N.S., Rajotte, E. and Luther, G.C. 2001. Comparative effectiveness of various sex pheromone dispensers and mashed sweet gourd bait traps for fruit fly control. Abstract, Eight Annual Report of the Integrated Pest Management Collaborative Research Support Programme (IPM CRSP).

Area-Wide Control of Fruit Fly Using Male Annihilation Technique in NWF Province of Pakistan

IAEA-CN-131/73 P

Sana Ullah Khan Khattak, Aman Ullah Khan, Abdus Sattar, Alamzeb, Abid Farid and Zahoor Salihah

Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan

Area wide control studies of fruit fly were carried out at 160 locations, using male annihilation technique. Lure baited traps of methyl eugenol, cue lure and trimed lure were used separately in various fruit orchards and vegetable fields including guava, peach, pear, plum, apricot, lychee, citrus, mango, persimmon, melons and cucurbits seasonally. The contents of the traps consisted of methyl eugenol, sugar and lure toxicant in the ratio of 85 : 10 : 5. The results showed that maximum number of 2268 flies/trap/day were recorded in guava at Kohat followed by 360 in lychee at Mardan and 327 flies in pear at Peshawar in June. Species recorded were *Bactrocera dorsalis* Hendel, *B. zonata* Saund and *B. cucurbitae* coq. No Med-fly was recorded in this region.

Mass trapping was done by distributing more than 15000 traps in about 45000 ha area. Annual population incidence studies were conducted at three different locations and fruit orchards. At Lala, peak population of 750 flies/trap/month (*B. dorsalis)* was recorded in September in pear using methyl eugenol. At Jhagra, using cue lure in persimmon, 50 flies (*B. cucurbitae*) were captured in August. At Tarnab, 92 flies (*B. zonata*) were recorded in peach in July.

Population suppression studies were conducted in pear and guava orchards at two different locations of Akbarpura and Kohat respectively. Methyl eugenol lure baited traps were installed in treated (@ 8 traps/acre) and untreated (1 trap/acre) orchards situated 15 km away. Using this technique, fly populations was suppressed by 60 and 87% (Figure) at these two locations respectively. Fly populations suppression was determined by using the following formula (Khattak *et al* 1988).

$$(A \times 100)/B = C \qquad 100 - C = D$$

A = Population infestation in treated orchard
B = Population infestation in untreated orchard
C = % population in treated orchard
D = % decrease in population in treated orchard

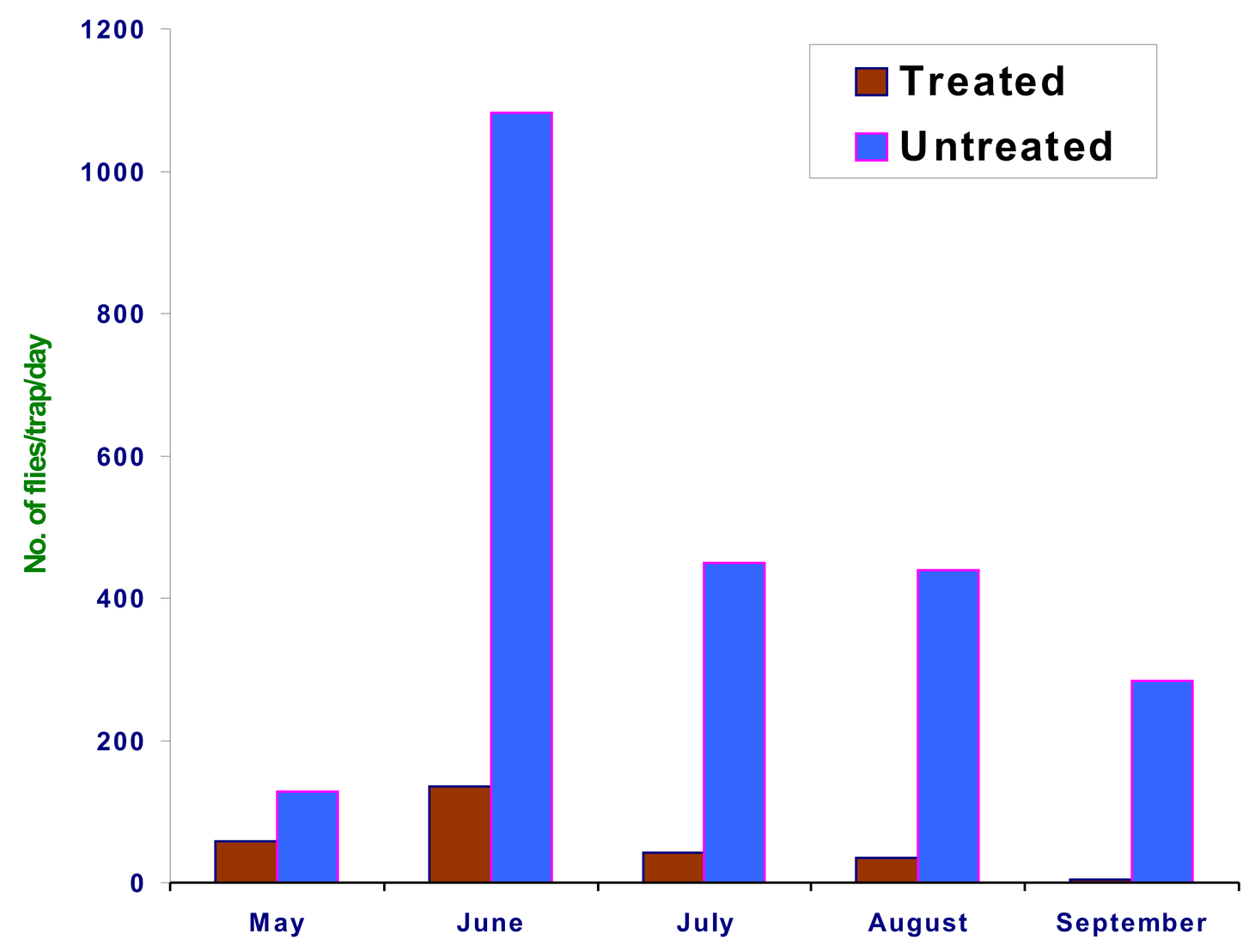


Figure: Population trend of *Bactrocera dorsalis* in treated and untreated guava orchards at Kohat.

CONTROL OF CABBAGE DIAMONDBACK MOTH BY USING STERILE INSECT TECHNIQUE AND PARASITOID IN MYANMAR

NILAR MAUNG AND HEATHER MORRIS

Plan Protection Division. Myanma Agriculture Service.

Ministry of Agriculture And Irrigation. Myanmar.

The cabbage Diamondback moth - DBM, is a serious pest of cruciferous crops through out the world. To control this pest, farmers use large quantities of insecticides. In the meantime, continuing use of insecticides resulted in DBM developing resistance to insecticides.

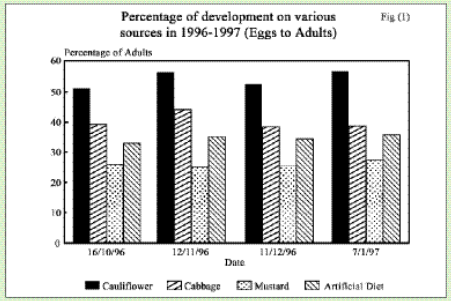
DBM-SIT in Myanmar Research contract No. MYA 7167/RB - IAEA / FAO from 1993 to 1998.

Objectives are to control the DBM pest pupulation, to reduce the use of chemical pesticides and to increase the population of natural enemies in cabbage growing area.

RESULTS

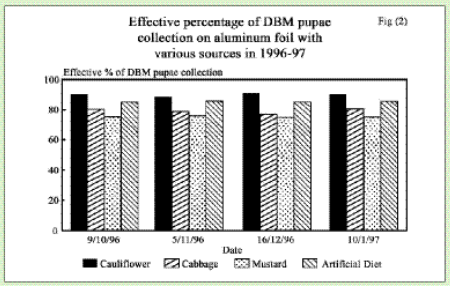
Mass-rearing of DBM on various foods

In figure (1), Effective percentage of mass - rearing of DBM were (51-56) % on cauliflower, (35-38) % on cabbage, (25-27) % on mustard and (33-35) % on artificial diet. Mass - rearing of DBM on cauliflower was best more than cabbage, mustard and artificial diet.



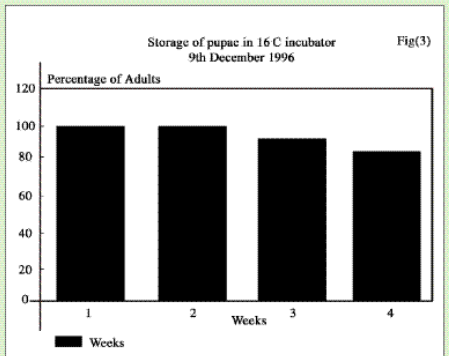
Pupae colloection on aluminum foil

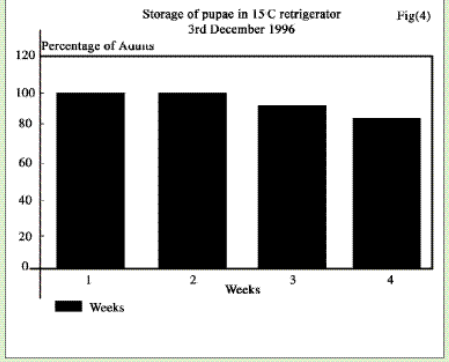
In figure (2), Effective percentage of DBM pupae collection on aluminum foil was (90-92) % on cauliflower, (79-82) % on cabbage, (76-77) % on mustard and (86-87) % on artificial diet. Pupae collection on cauliflower was best more than cabbage, mustard and artificial diet.

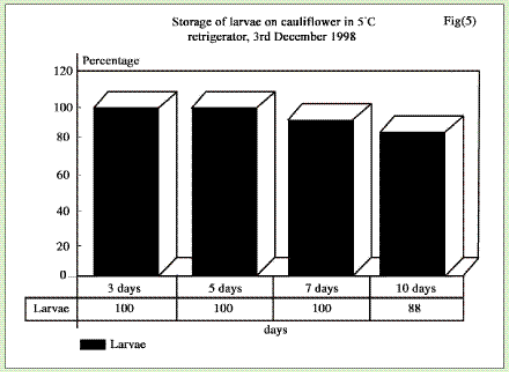


Storage of DBM pupae and Larvae

In figure (3) and (4) Pupae could be stored successfully for 2 weeks at 5-10˚C. Larvae reared on cauliflower could be stored for 7 days at 5-10˚C refrigerator. Larvae reared on cauliflower could be stored for 7 days at 5-10˚ C refrigerator in figure (5).







Tests of different dosage of gamma radiation

In Table (1), r-radiation rates of 10 Kr gave the best results than 15 Kr and 20 Kr.

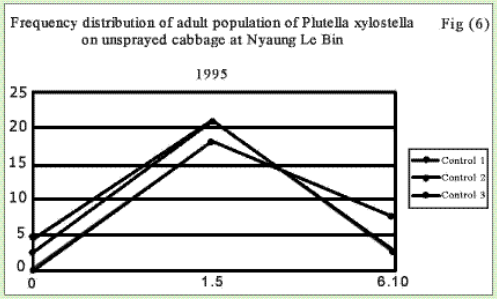
Table (1)

Fi studies on Diamond back Moth in 1997

10 Kr M(T) x F(N)			15 Kr M(T) x F(N)			20 Kr M(T) x F(N)		
Date	Test	No.	Date	Test	No.	Date	Test	No.
6.1.97	10 P		6.1.97	10 P		6.1.97	10 P	
7.1.97	10 x 10		7.1.97	10 x 10		7.1.97	10 x 10	
8.1.97	Egg	233	8.1.97	Egg	198	8.1.97	Egg	186
10.1.97	1st	152	10.1.97	1st	176	10.1.97	1st	169
17.1.97	4th	18	18.1.97	4th	16	18.1.97	4th	21
19.1.97	pupae	10	20.1.97	pupae	12	20.1.97	pupae	14
22.1.97	Adult	6	23.1.97	Adult	10	23.1.97	Adult	12
		3 x 3			5 x 5			6x6
22.1.97	3x3	F1	23.1.97	5x5	F1	23.1.97	6x6	F1
F1		F1						
23.1.97	Egg	24	24.1.97	Egg	45	24.1.97	Egg	51
25.1.97	1st	9	26.1.97	1st	25	26.1.97	1st	28
1.2.97	4th	4	4.2.97	4th	8	4.2.97	4th	15
3.2.97	pupae	2	7.2.97	pupae	4	7.2.97	pupae	6
6.2.97	adult	1	10.2.97	adult	4	10.2.97	adult	5

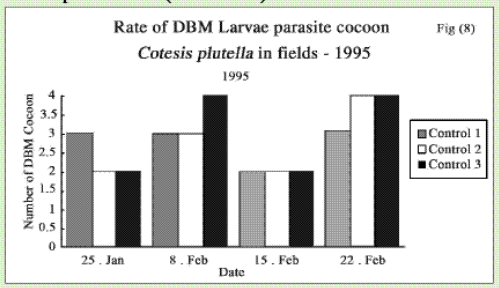
Frequency distribution of DBM population in field

Frequency distribution of plants containing different numbers of DBM adults was shown in figure (6). Most of the plants harboured 1-5 adults during the season. Weekly distribution of larvae population was shown in figure (7). Most of the plants harboured (2-25) larvae during the season.



Study the rate of DBM larvae parasitoids in fields

In figure (8), Weekly ten DBM larvae in each control area were collected. DBM larvae parasite. Cotesia plutellae or Apanteles plutellae were found and percentage of DBM larvae parasite was (30% - 40%).



Conclusions

Mass-rearing of DBM on various foods, pupae collection on aluminum foil, storage of DBM pupae and larvae, tests of different dosage of gamma radiation, frequency distrubution of DBM population in fields and Study the rate of DBM larvae parasitoids in lowlands area were important factors to control cabbage diamondback moth in cabbage growing area.

Large Scale Demonstration of IPM on Rainfed Chickpea

Chickpea
IAEA
CN
131/77P

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1. ectives of this P rogra e

i) o conserve bio-diversity
ii) o demonstrate the IPM module in a whole village involving maximum number of farm families
iii) o train youth and women in all aspects of IPM and to involve t em actually in eac o eration
iv) o popularise Indigenous crop protection practices
v) o convince and motivate farmers to the benefits of IPM
vi) o show the seriousness of pest in crop damage, loss of crop yield
vii) o convince the farmers about economic feasibility of IPM in rainfed chickpea
viii) o develop model IPM village

2. lage eason area of P

1. illages : Suregaon Ne asa . al: Ne asa Dist : .Nagar
2. Season : Ra i 2001
3. rea under IP :
   IP it NS 76 a 190 acres 75 farmers
   IP it out NS 66 a 165 acres 52 farmers
   otal 142 a 355 acres 127 farmers
4. arieties : i ay G-12

. P odule o erated

i rection of antennae : 12-25 a 10t to 24t Dec. 2001
ii se of eromone tra : 5 a 10t to 20t Dec. 2001
iii S raying of 5 NS : ne s ray 15t to 24t Dec. 2001
iv ollection of Larvae : 2 times 14t Dec. 2001 to *Helicoverpa)*

Av. yield and % increase in yield due to different treatments

Treatment	Av. yield q/ha	% increase over UT
IPM (with NSE) (Av. of 76 ha = 75 farmers)	15.2	50.04 %
IPM (without NSE) (Av. of 66 ha = 52 farmers)	12.4	22.41 %
Chemical control (Av. of 16 ha = 6 farmers)	12.5	23.40 %
Untreated (Av. of 166 ha = 70 farmers)	10.13	-

Economics of IPM plots :

Particulars	IPM (with NSE)	IPM (without NSE)	Chemical control	Untreated
Yield (q/ha)	15.20	12.40	12.50	10.13
Cost of plant prot. (Rs./ha)	670	320	600	-
Yield increase over UT (q/ha)	5.07	2.27	2.37	-
Net income over UT (Rs.)	8456	3766	3666	-

Total area (ha.)	**76**	**66**	**16**	**166**
Total increase in yield (q.)	**385**	**150**	**----**	**----**
Total Expt. on inputs from Agril. Deptt.	**24,320**	**+ 4,620**	**= 28,940**	
Total additional Income (Rs.)	**6,93000**	**+ 2,70,000**	**= 9,63,000**	

verall i act of P :

- ull confidence a out IP among c ic ea gro ers
- Lateral s read of tec nology from farmer to farmer and village to village
- ollection of neem seed from t eir o n villages and ado t ole IP tec nology in a ole village

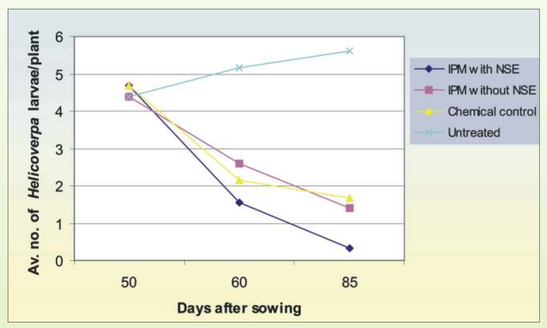
chieve ent :

- n ideal e am le for massive cam aign and to o ularise all as ects of IP in intensive c ic ea gro ing areas of t e state
- anagement of c ic ea est it out c emical esticides
- anaged eco-friendly environment it sustaina le c ic ea roduction
- Involved t e com ined artici ation of gril. niv. gril. De t. and all t e farmers of t e village
- armers from surrounding villages also decided to ado t IP tec nology on t eir o n accord

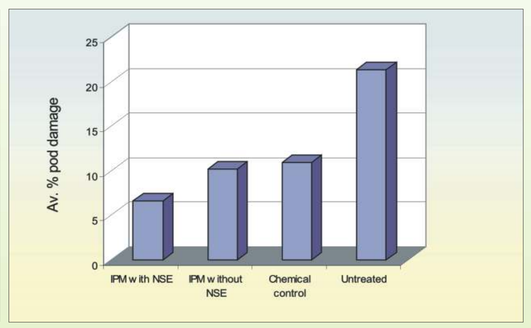
ocio econo ic i act:

- ormation of grou of IP farmers
- Develo ment of resource ersons for IP in t e village
- Reduction in cost of lant rotection
- areness a out t e ill-effects of c emical esticides on est out rea ealt environment
- isits of farmers from ot er villages to IP lots
- Im roved socio-economic status of IP farmers in t e village

e c er a larval o ulation



v. % od da age due to od orer



P eromone tra

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NS s raying demonstration

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P cro

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ollection of larvae

Future lan of action :

- Involvement of ole village covering 200-400 a of c ic ea under IP rogramme
- overing of ad oining villages in IP rogramme
- ollection of neem seed from t eir laces
- otivation of farmers for natural resources management i.e. Indigenous material and met ods
- Develo ment of self reliance and model village to ot er farmers t roug information tec nology and io-tec nology

Effectiveness of Synthetic Pheromone Traps for Monitoring of Important Polyphagous Field Crop Pests on Large Area

IAEA CN 131/78 P

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Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri- 413 722, Dist. Ahmednagar, Maharashtra (India)
e-mail : rvnakat@yahoo.com

E E

o evaluate t e erformance of different eromone lures against ma or ests *viz.* Gram od orer *Helicoverpa armigera* u . Diamond ac mot *Plutella xylostella* L. and ruit fly *Bactocera correcta* ezzi = *Daccus dersalis* in different commercially im ortant field cro s.

E E

e National emical La oratory Pune synt esized t ree eromones suc as -11- e adecenal -9- e adecenal 97:3 for *H. armigera* -11- e adecenal 11- e adecenyl acetate -11- e adecenol 10:10:0.1 for *P. xylostella* and t e su stitute derivatives 3 4- Dimet o y ro yl enzene for *B. correcta*

E

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Phero one -11- e adecenal -9- e adecenal 97:3
ro omato illi ic ea Pigeon ea.
ra distance 50 m
rea 0.40 a. 2-3 locations it 3 re lications
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Crop	Dose (mg/ trap)	Av. No. of moth catches / Trap
Tomato	1	0.5
	2	12
	5	57
Chilli	2.5	45.5
	5	62
Chickpea	2.5	152
	5	170

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Crop	Area	No. of traps	Av. moth catches	
			Mass confusion fields	Control fields
Chikpea	0.60 ha	60	6	89
Pigeonpea	0.40 ha.	30	0	18

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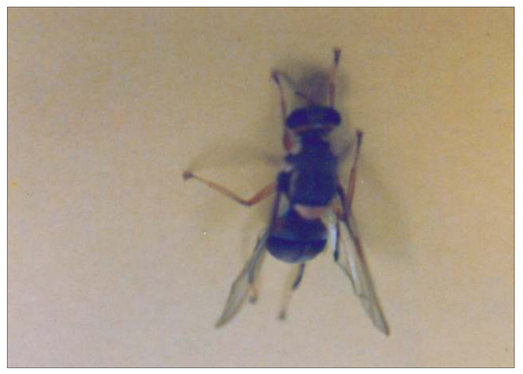

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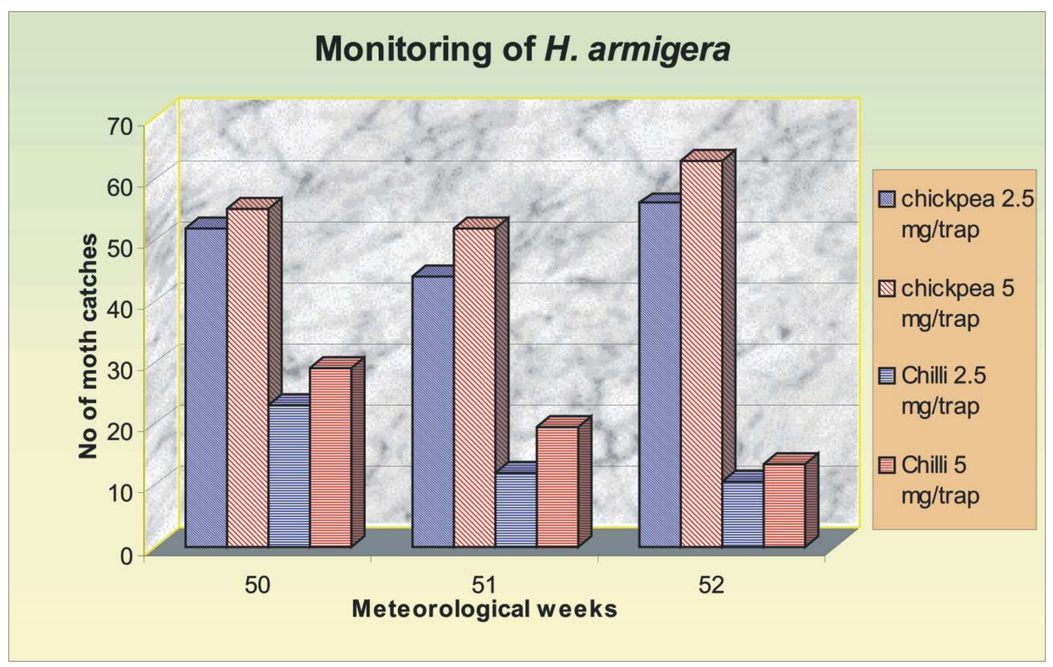

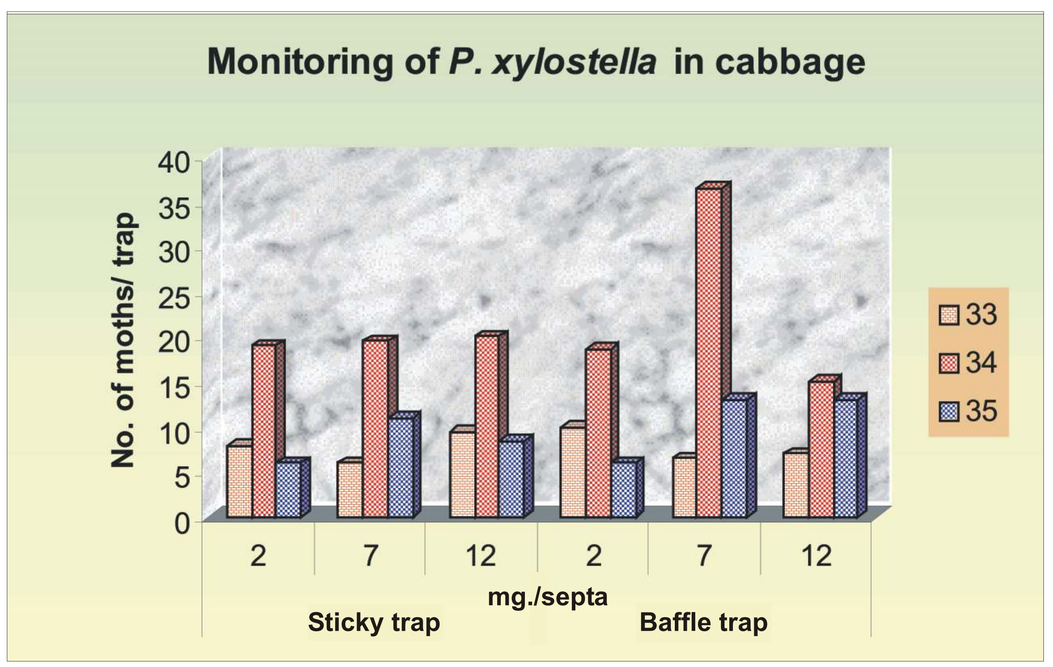

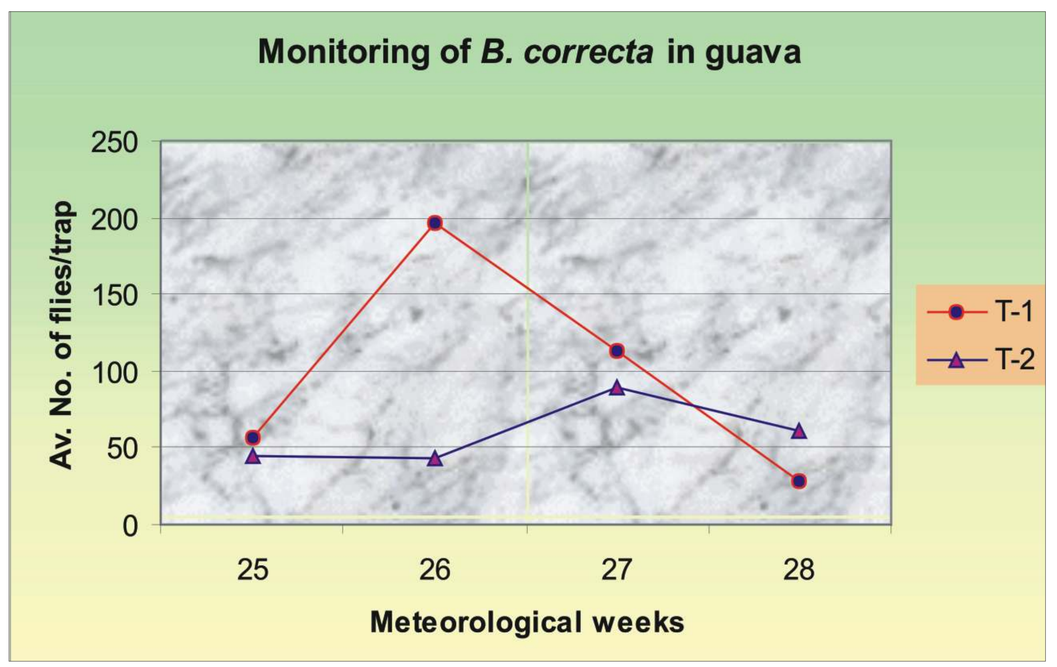

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- e ma imum mot catc es ere o tained it 5 mg eromone er se ta in all t e cro s.
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an s are due to De artment of iotec nology Govt.of India Ne Del i for roviding financial assistance for t is or and N L Pune for synt esis of eromones

D4-CN-131 / 80P

Management of sugarcane insect pests through environment friendly techniques in area wide approach in Tamil Nadu State, India

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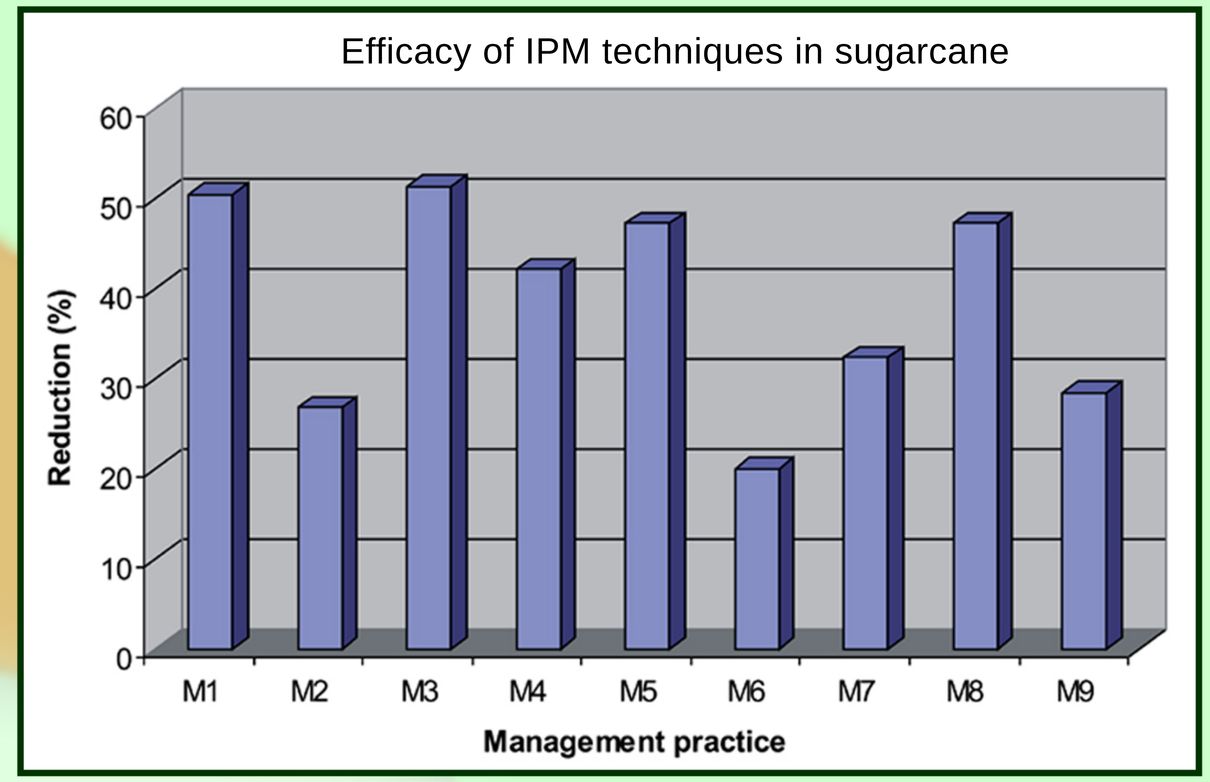
- Planting in Dec-Jan season escapes shoot borer incidence
- Trash mulching on I week reduces shoot borer up to 50%
- Higher sett rate of 30% at planting reduced shoot borer incidence even up to 43%.
- Intercropping on I week with pulses reduces shoot borer up to 50%
- Detrashing cane at 5th and 7th month of growth reduces sucking pests
- Release of *Trichogramma* parasitoid reduces internode borer incidence to 18%
- Naturally prevalent *Telenomus* parasitoid reduces internode borer incidence to 31%
- *Epiricania* parasitoid reduces Pyrilla pest up to 27-46%
- *Dipha*, *Micromus* predators reduce sugarcane woolly aphid up to 30%
- Balanced fertilizer, split application and judicious irrigation

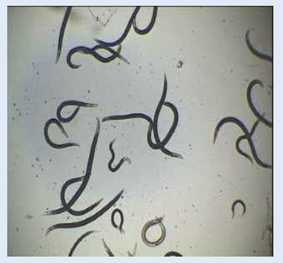


M1- Trash mulching on shoot borer
M2- Increased sett rate at planting on shoot borer
M3-Intercropping on shoot borer
M4- Detrashing on whitefly
M5- Detrashing on leafhopper
M6- Parasite *Trichogramma* on internode borer
M7- Parasite *Telenomus* on internode borer
M8- Parasite *Epiricania* as cocoons on leaf hopper
M9- Parasite *Epiricania* as eggs on leaf hopper

AREA WIDE APPROACH

- IPM demonstration trials carried out during the three seasons of 2002-2005.
- Incorporated the cultural components of higher sett rate at planting, trash mulching on ridges after planting, detrashing of older leaves on 5th and 7th months and biological component of release of egg parasite *Trichogramma chilonis* during vegetative phase of the crop.
- The incidence of shoot borer was reduced by 33.6 and 42.4%
- The internode borer incidence was reduced by 13.74 to 19.69%
- Realized increased cane yield of 23.3% over and above the plots where these practices are not followed up.
- Higher benefit ratio obtained.
- Area wide control advocated for Tamil Nadu state with sugarcane area of around 0.256 million ha

INFLUENCE OF HOST IRRADIATION ON THE BIO-INFECTIVITY OF *Steinernema glaseri* AS ENTOMOPATHOGENIC NEMATODES AND THEIR PERPETUATING PARASITIZATION POTENTIAL ON A SERIOUS TROPICAL LEPIDOPTERAN PEST, *Spodoptera litura*



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Department of Zoology, University of Delhi, Delhi-110 007, India

Biological and parabiological control measures [1] of the lepidopteran pest, *Spodoptera litura* seem to be eco-compatible pest suppression measures in view of increasing environmental hazards caused by the chemical pesticides and development of insecticide-resistance in this pest [2]. Among bio-control agents, the entomopathogenic nematodes (EPNs) appear to be promising due to their great ability for host searching and killing, high reproductive rate, and safety to the non target organisms [3].

The EPNs need to be transported within host-insect (*in vivo*) into the field for augmentative releases so as to have their proper viability. To avoid the risk of released host insect, if unparasitized, in building up of pest population in the field, radiation-sterilization of host was considered desirable. Therefore, an attempt was made to ascertain the bio-infectivity of EPNs, *Steinernema glaseri* vis-à-vis irradiated host, *Spodoptera litura,* and assess parasitization behaviour of the infective juveniles (IJs) derived from irradiated host, in order to establish this biocontrol strategy by using nuclear techniques. Radiation impact was ascertained on behaviour and reproduction of *S. litura,* in order to select the range of gamma doses for sterilizing the various insect stages. Radio-sensitivity decreased with age in this 'radio-resistant' pest, *S. litura.* Two gamma doses were selected (viz., 40Gy, 70Gy), for bio-evaluation of EPNs on treated host stages. 70Gy was determined as an overall sterilizing dose for late larval and prepupal stages. Although 40Gy induced about 88-90% sterility in insect host, but in view of reduced adult emergence (53.9%), marked degree of malformations in the emerged adults (61.2%) and reduced mating success(ca.40%), the effective sterility would be about 95% at 40Gy [4,5], hence 40Gy could also be feasibly used in an almost risk free mode (by avoiding potential pest release).

RESULTS & DISCUSSION

Influence of host-irradiation was ascertained on EPNs bioefficacy towards host reared on two different diets, i.e., castor (natural food) and semi-synthetic diet (used in mass rearing). Host-irradiation did not influence the parasitization behaviour of EPN and its proliferation on host insects reared on castor, as significantly as on semisynthetic diet.

The viability, assessed in terms of % survival of 7-10 days old IJs, was more than 90% in case of F_1 IJs and F_2 IJs, which was similar to control (92.3%). Also, the responsiveness of F_1 IJs and F_2 IJs, determined in terms of undulations per min, was not statistically influenced as compared to control.

A small field simulated experiment (on cotton pots having 12 insects /plant sprayed with 6mL EPN suspension containing 2000 IJs/mL) was conducted to ascertain the parasitization efficacy of F_1 IJs derived from host treated with 40Gy/70Gy. About 80-91% mortality was exhibited by F_1 IJs as compared to control (98.6%). The invasive efficiency of F_1 IJs emerged from irradiated host (40Gy/70Gy), determined after 48h, was not significantly affected with respect to control.

Further detailed bioassay was exercised to ascertain the parasitization behaviour of IJs derived from irradiated host reared on natural food, up to its ensuing generations, and understand the perpetuating potential of these entomophilous parasitoids (Fig.1). In the first phase, the F_1 IJs harvested from parasitized irradiated host, were evaluated for their bio-infectivity on normal (untreated) host, and compared with normal IJs response on treated host and control (i.e., normal IJs versus untreated host). In the next phase, the efficacy of F_2 progeny (i.e., next generation of F_1 IJs parasitizing normal host) was ascertained on normal host. The time required for causing mortality was significantly more in case of F_1 IJs with respect to F_2 IJs, which on contrary behaved almost similar to control. The parasitization efficacy of normal IJs on irradiated host, and F_1 IJs on normal host, was evidently less than control; whereas the F_2 IJs parasitization response was almost equivalent to control. Similar pattern was observed with respect to EPNs harvesting behaviour exhibited by F_1 IJs and F_2 IJs. This indicated almost revival response elicited by F_2 IJs, being similar to control, at 40Gy and 70Gy, although the revival response by F_2 IJs was more apparent at 40Gy.

Host sterilization at the gamma dose of 70Gy might be used for inundative releases of EPN; whereas considering the reduced adult survival and mating success, a high degree of adult malformations, along with behavioural dynamics of F_1 and F_2 IJs at 40Gy, this dose could also be effectively and safely used for propagation of EPNs within sterilized host towards inundative and inoculative releases of this entomogenous agent. The performance of these EPNs on irradiated host and behaviour of IJs harvested from irradiated host indicated the feasibility of using entomogenous *S. glaseri* as an ecologically sound biocontrol component for management of *S. litura*.

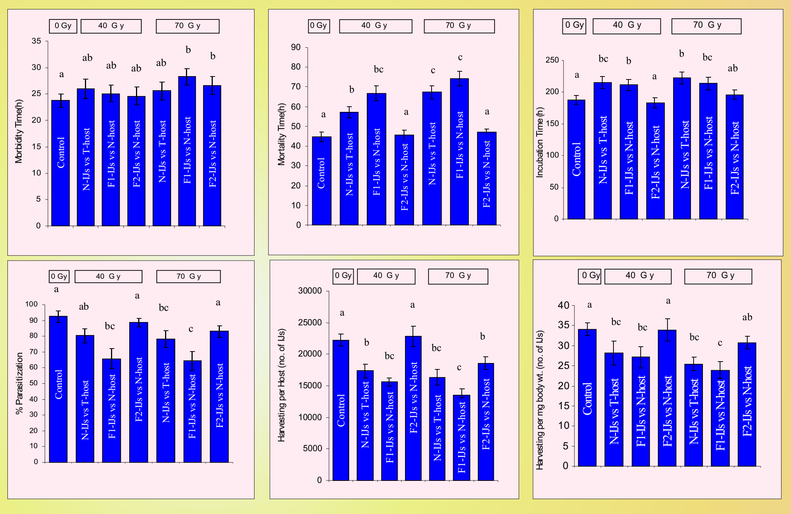
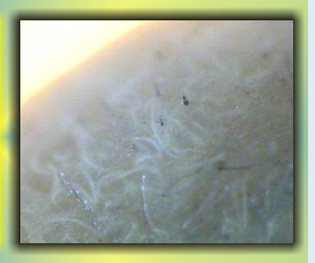


Fig.1 Perpetuating bio-infective potential of EPNs derived from host, *Spodoptera litura* irradiated with 40Gy / 70Gy up to its ensuing generations, in terms of time required for host morbidity and mortality, incubation time, per cent parasitization of host, and harvesting of IJs/host and IJs/per mg host-body weight

Control : Normal(N) IJs parasitizing Normal (unirradiated) host; N-IJs vs T-host : N-IJs parasitizing treated-host; F_1-IJs vs N-host : F_1-IJs parasitizing N-host ; F_2-IJs vs N-host: F_2-IJs parasitizing N-host; F_1-IJs: derived from N-IJs vs T-host; F_2-IJs: derived from F_1-IJs vs N-host ; Means ± SE denoted by the same letter in each figure (for a specific parameter), are not significantly different at $P \leq 0.05$ level (calculated using ANOVA followed by LSD post-test); Percentage parasitization data were transformed (arcsine) before ANOVA, but data in figure are back transformations; n = 12

- EPN killed L6 larva of *Spodoptera litura* with proliferating and developing EPN inside host cadaver

- Magnified view of parasitized host cadaver with EPN wriggling beneath the host-skin (just before IJs emergence)



- Harvesting Trap for EPN

SUMMARY AND CONCLUSION

- Host irradiation was considered for safe transport of EPN (*in vivo*)
- Interaction of host diet and host irradiation was assessed on bioinfectivity of EPN.
- Natural diet was slightly better than semisynthetic diet w.r.t. parasitization behaviour of EPN.
- Survival rate & responsiveness of F_1 and F_2 IJs were determined to be quite comparable to control.
- Viability (parasitization & invasive efficiency) of IJs derived from irradiated host, was tested by a preliminary field simulated experiment.
- Detailed bioassay was exercised to study the bioinfective behaviour of EPN derived from irradiated host up to its ensuing generations.
- A reasonable degree of bioinfectivity was retained by F_1 and F_2 IJs.
- Almost revival response was elicited by F_2 IJs, parallel to control; Better performance at 40Gy than 70Gy.
- Host irradiation at 40-70Gy might be recommended for inundative relaeses of EPN *in vivo*.
- 40Gy as host irradiation dose could also be effectively used for inoculative releases of EPNs *in-vivo*, due to its debilitating effects on adult behaviour and potential bioinfectivity up to F_2 IJs.

ACKNOWLEDGEMENTS

Financial assistance by the International Atomic Energy Agency, Vienna is gratefully acknowledged for supporting this research work under Research Contract No. IAEA/IND-10847/R0/RB in a Coordinated Research Project. Thanks are due to technical support provided by Ms. V Baweja and Mr. Manas K. Dhal.

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Assessing genetic variation in natural populations of the New Word screwworm fly, *Cochliomyia hominivorax*.

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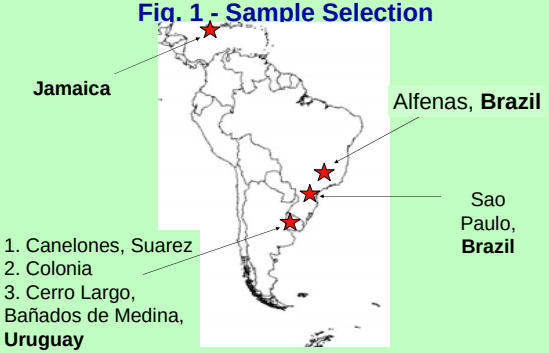
Abstract: Through participation in the IAEA/FAO programme '*Enabling Technologies for the Expansion of SIT for Old and New World Screwworm Fly*', we have been able to analyse samples of New World screwworm fly from across the range of this insect pest. This poster will bring together data from a range of genetic markers which have been used to assess levels of genetic variation within populations of the New World screwworm fly, *Cochliomyia hominivorax*. The markers used include: rRNA gene sequences, together with sequence information from protein coding genes, and microsatellites, allowing variation to be assessed at a range of levels from species to population.

Introduction:

- The new world screw worm (NSW), *Cochliomyia hominivorax*, is a major parasitic pest of Caribbean and South America.
- Eradication from N. America and much of Central America by sterile insect release techniques (SIT) mostly successful.
- Interbreeding between remaining isolated populations possibility-ability to affect ongoing eradication programmes.
- This study aims to characterise the genetic variability present in these populations and assess the gene flow between them.

This will be achieved through the use of molecular markers, designed to assess variability at differing resolutions; microsatellite markers will assess the population level differences, while sequence data from the Elongation Factor 1α (EF1α) will provide information about sub-species level variations.

Fig. 1 - Sample Selection



Microsatellite analysis:

A suite of 5 new microsatellite primers was developed to support those already in use (Torres *et al*, 2004). These were found to only amplify the Brazilian samples. From other arthropod based studies (Moulton, 2000 & 2003, Rubinoff & Sperling, 2002 and Zakharov *et al*, 2004) it is clear that EF1α is a rapidly evolving gene, capable of highlighting variation within populations of single species, hence it was decided to concentrate our efforts on the gene sequencing data.

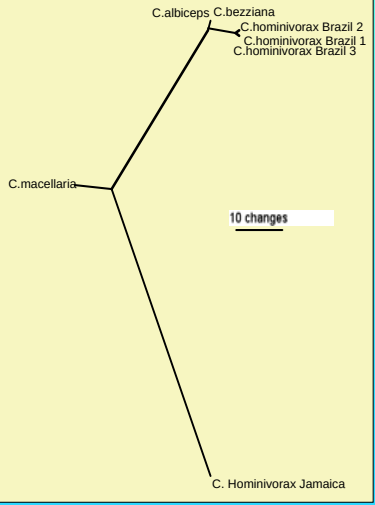
EF1α Gene Sequencing:

- Partial gene sequence was obtained from 3 separate *C. hominivorax* from Brazil, *C. hominivorax* from Jamaica, *Chrysomyia bezziana, Chrysomyia albiceps* and *Cochliomyia macellaria*.
- Amplification but not sequence was achieved for three samples of *C. hominivorax* from Uruguay.
- *C. macellaria* produced only a short sequence (130 bp) when compared to the other samples (400-600bp)

Phylogeny Production

- The partial EF1α sequence data was aligned and used to generate phylograms illustrating the relationships between the samples.
- The complete range of successful sequences is shown in Fig. 2, including only the shorter section of sequence shared by all samples.
- Fig 3 shows a phylogram generated using the full length sequences but excluding the *C. macellaria* sample, minimising any impact this shortened sequence would have on the phylogram.

Fig. 2 Phylogram of the shared section EF1α sequences

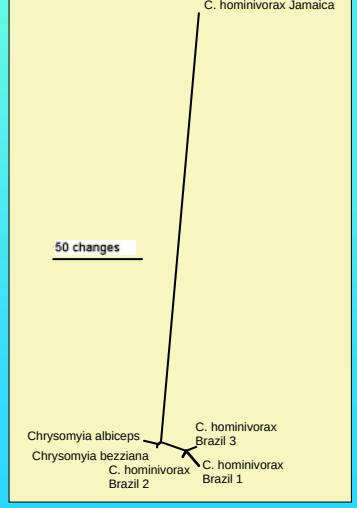


Discussion:

- Both phylograms clearly show same pattern ∴ *C. macellaria* sample is not affecting phylogeny.
- Brazilian *C. hominivorax* cluster very closely, not distinct at this gene level.
- Brazilian NSW clusters closley with related species, *Chrysomyia bezziana* and *Chrysomyia albiceps,* but is distinguished from them.
- Jamaican NSW is very different from both other NSW and the related species at this gene. Reasons for this are not clear, but geographical isolation is most likely.
- Further work, including extending the sample range and markers used, needed to explore this fully.

Cochliomyia samples wanted: please contact l.m.evans@ex.ac.uk

Fig. 3 Phylogram of long EF1α Sequences with C. macellaria sample removed



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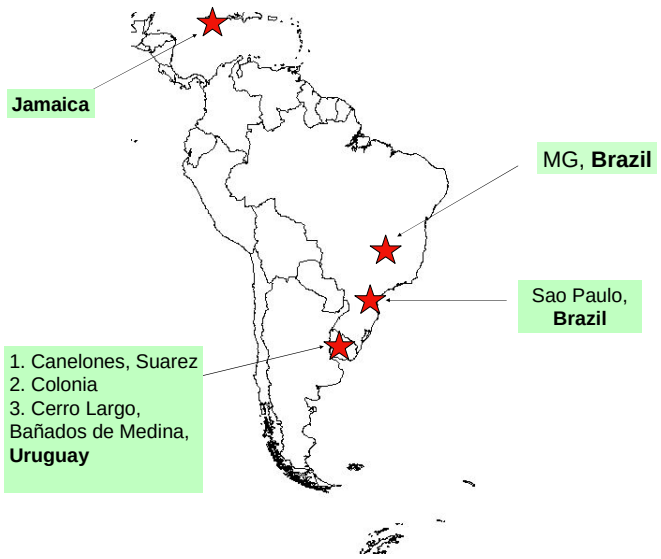
Acknowledgements: Many thanks to Kevin Moulton for his invaluable help with the the EF1α primers.


Jamaica
MG, **Brazil**
Sao Paulo, **Brazil**
1. Canelones, Suarez
2. Colonia
3. Cerro Largo, Bañados de Medina, **Uruguay**

ATTRACTION OF LEPIDOPTERAN MOTHS BY SEX PHEROMONES IN THE FRUIT ORCHARDS IN THE MEKONG DELTA OF VIETNAM

IAEA-CN 131/84P

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[2] Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan

MATERIAL & METHODS

Seven monoenyl acetates (Z5-10:OAc, Z5-12:OAc, Z7-12:OAc, Z9-12:OAc, Z9-14:OAc, Z11-14:OAc, and E11-14:OAc), three monoenyl alcohols (Z7-12:OH, Z11-14:OH, and E11-14:OH), and two dienyl acetates (Z9,E11-14:OAc and Z9,E12-14:OAc) with >97% purity were supplied by Shin-etsu Chem. Co., Ltd. (Tokyo, Japan). Monoenyl aldehyde (Z11-14:Ald) was prepared by PCC oxidation of Z11-14:OH. Four trienes (Z3,Z6,Z9-18:H, Z3,Z6,Z9-19:H, Z3,Z6,Z9-20:H, and Z3,Z6,Z9-21:H) and twelve epoxydienes (racemic mixtures of epo3,Z6,Z9-18:H, Z3,epo6,Z9-18:H, Z3,Z6,epo9-18:H, and their C19-C21 homologs) with > 95 % purity were synthesized from linolenic acid (Ando et al., 1993).

INTRODUCTION

Random screening tests of synthetic sex pheromones in order to gain information on the mating communication systems of lepidopteran species were conducted in the fruit orchardsof Chinese apple, guava, plum and logan in the Mekong Delta of Viet Nam over an approximately two year period starting from December 1998.

Family Species	Attractant	Main season of flight and (total number of captured males) [a]
Tortricidae		
Adoxophyes privatana Walker [b]	Z11-14:OAc + Z9-14:OAc (9:1)	March-April (435)*, June (69)*, Nov. (26)*
Archips atrolucens Diakonoff [b]	Z11-14:OAc + E11-14:OAc (5:5)	Dec.-March (21)*, May-June (13)*
Meridemis furtiva Diakonoff [b]	Z11-14:OAc	March-June (223)*
Cosmopterigidae		
Gen. et sp.	Z11-14:OAc	March-June (354)*
Gelechiidae		
Gen. et sp.	Z9-14:OAc	Jan.-March (234)*
Batrachedridae		
Batrachedra sp.	Z7-12:OAc + Z9-14:OAc (9:1)	Jan.-April (145)*, June (18)*, Aug.-Sept. (40)*
Noctuidae		
Argyrogramma signata F. [c]	Z5-10:OAc	Jan.-March (64)**, June-July (21)**, Oct. (25)**
Chrysodeixis eriosoma Doubleday [c]	Z7-12:OAc Z7-12:OAc + Z9-14:OAc (9:1) Z7-12:OAc + Z9-12:OAc (9:1)	Sept.-Nov. (23)* Dec.-Jan.(8)* March (14)**, Oct.-Nov. (70)**
Ctenoplusia agnata Staudinger [c]	Z7-12:OAc	Jan.-Apr. (251)**, Aug.-Dec. (424)**
Ctenoplusia albostriata Bremer & Grey [c]	Z7-12:OAc + Z5-10:OAc (9:1)	Jan.-March (152)**, Oct.-Nov. (16)**
Zonoplusia ochreata Walker [c]	Z7-12:OAc + Z5-12:OAc (5:5)	Feb.-May (73)**, Aug.-Dec. (97)**
Spodoptera pectinicornis Hampson[d]	Z7-12:OAc	June-Oct. (51)*
Hypena sp.[e]	Z3,Z6,epo9-21:H	Jan.-Feb. (28)**
Luceria sp.[f]	Z3,Z6,epo9-18:H	Dec.-Feb. (140)*, July-Sept. (58)*
Zanceognatha sp.[g]	Z3,epo6,Z9-19:H	Dec.-Jan. (29)*, Aug.-Sept. (38)*
Arctiidae		
Cyana sp. 1 [h]	Z3,Z6,epo9-18:H	Aug. (31)*
Schistophleps sp. 1 [h]	Z3,Z6,epo9-19:H	Dec.-March (106)*, June-July (14)*
Schistophleps sp. 2 [h]	Z3,Z6,Z9-19:H	Dec.-April (254)*, Aug.-Nov. (65)*
Schistophleps sp. 3 [h]	Z3,Z6,Z9-21:H	Feb.-Sept. (242)**

[a] Captured males in Test 1 from December 1998 to November 1999 (*), and in Test 2 from January to December 2000 (**).
[b] Tortricinae, [c] Plusiinae, [d] Amphipyrinae, [e] Hypeninae, [f] Ophiderinae, [g] Herminiinae, [h] Lithosiinae.

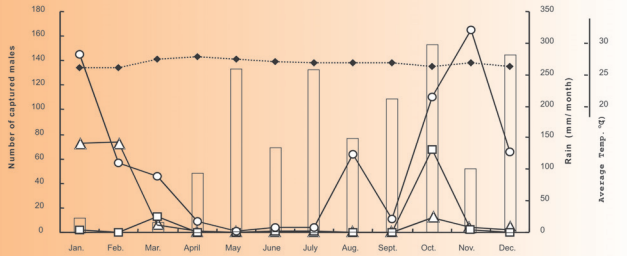

Fig. 1 Monitoring of threePlusiinaespecies by pheromone traps in orchards in the Mekong Delta (Cantho city) in 2000. *Chrysodeixis eriosoma* (□), *Acanthoplusia agnata* (○), and *Ctenoplusia albostriata* (△)

Trap in Chinese apple orchard

Moths captured by sticky pheromone trap

RESULTS & DISCUSSION

Table shows the attractants, the main seasons of flight, and the total number of captured moths. Field tests of synthetic pheromones at orchards in the Mekong Delta successfully revealed the species-specific male attraction of 19 lepidopteran species. Monoenyl acetates with a C_{10}-C_{14} chain newly attracted six species distributed mainly in Southeast Asia: *Adoxophyes privatana*, *Archips atrolucens*, and *Meridemis furtiva* in the Tortricidae family and *Argyrogramma signata*, *Spodoptera pectinicornis*, and *Zonoplusia ochreata* in the Noctuidae family. Furthermore, male moths of three species belonging to the Cosmopterigidae, Gelechiidae, or Batrachedridae family were also caught by traps baited with acetates. On the other hand, trienes with a C_{18}-C_{21} chain and their monoepoxides, which are stereotypes of pheromones secreted by females in the Geometridae family, did not show activity against any geometrid males but attracted three Noctuidae species and four Arctiidae species. Although only genera were defined for their taxonomic classification, the attraction is noteworthy considering that sex pheromones and attractants have not been reported for species in the same genera.

Management of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) in the Gharb area in Morocco

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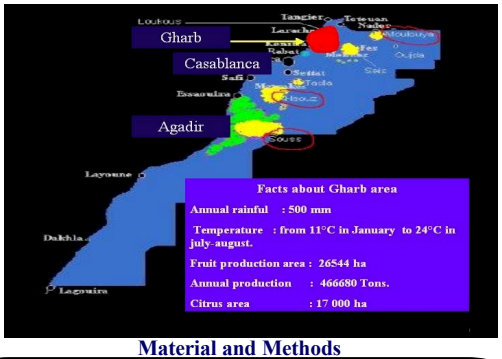
Introduction

The Mediterranean fruit fly (medfly), *Ceratitis capitata* Wiedmann is a major pest for fruit production, especially C*itrus* in Morocco (AIEA, 1995).
The Plant Protection Directorate (DPVCTRF) in collaboration with GTZ (German Co-operation) have developed a management program for medfly, based on spot treatment (Papacek, 1997). The present paper reports on an experiment conducted during the 2001 growing season in an effort to adapt the spot treatment method to the Moroccan context. To achieve this objective, we studied the efficiency of the spot treatment method compared to conventional one (Driba, 1998).

Map of Morocco showing the location of Gharb area and main fruit production areas



Material and Methods

Monitoring
Male flight was monitored weekly using MaghrebMed traps, Traps were placed at a density of 1 trap per experimental plot

Fruit sampling
100 fruits were collected from 20 randomly selected trees. The percentage of fruits with punctures was then estimated. A fruit with a minimum of one puncture was evaluated as an infested fruit.

Fruit harvest
The fruit harvest was carried out on Navel variety as scheduled : 15 Oct, 20 Oct, 24 Oct, 30 Oct and 15 Nov. The fruits were collected from 20 trees randomly and kept in 25°c chamber in order to accelerate fly punctures. After 10 days, actual damage was evaluated by slicing open and check of fruit fly larvae.

Economic evaluation
Economic evaluation was conducted by comparing the cost of conventional method and spot treatment method.

Presentation of conventional and spot treatment method for control of medfly

	Conventional method		Spot treatment method	
Period of monitoring	Starting From 1st September for Navel and 15 November for Maroc late		Starting From 1st September for Navel and 15 November for Maroc late	
Decision for spraying	Trap monitoring		Monitoring and Fruit sampling	
Treatment threshold and treatment method	*If 21 flies/trap/week , then localised spraying with attractant and insecticide of 1 row every 4 at 250 l of mixture /ha.		* If 21 flies/trap/week and/or 1 puncture /fruit, then spot spraying of 1 side of the tree at 16 l of mixture/ha	
	* If > 21 flies/trap/week, then generalised spraying with insecticide only at 2000 l of mixture /ha.		* If > 21 flies/trap/week, then spot spraying of both sides of the tree .at 32l of mixture /ha.	
Number of applications	Navel: 5 localised + 3 generalised	Maroc late: 0	Navel: 19	Maroc late: 30
Quantity of insecticide applied / cycle of prod.	3,625 l/ha	_	704 cc/ha	960 cc/ha
Volume of the mixture	2000 l/ha	-	16 l/ha	16 l/ha

Evaluation of fly population and apparent damaged fruits in the field (2000/2001)

Navel fruits apparently damaged reached a maximum of 2% and 3% respectively in spot treatment and conventional method (fig.1). On Maroc late rates of fruit damaged had a maximum of 1% and 16% respectively on spot treatment and control area (fig.3).

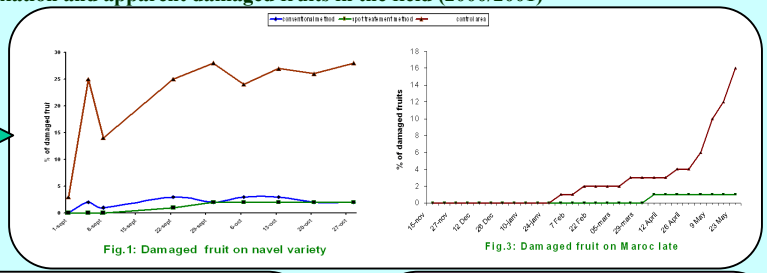


Fig.1: Damaged fruit on navel variety

Fig.3: Damaged fruit on Maroc late

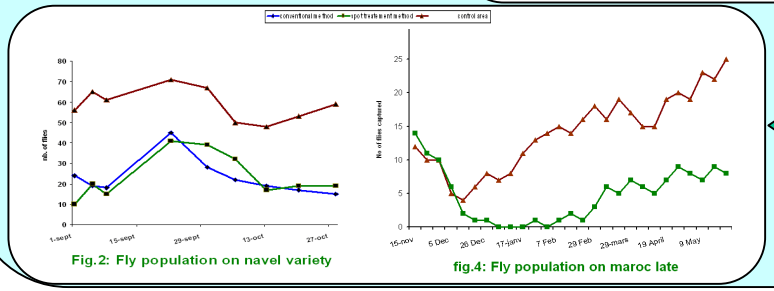


Fig.2: Fly population on navel variety

fig.4: Fly population on maroc late

A higher population was observed on Navel (till 70 flies / trap / week) (fig.2) compared with Maroc late variety where the maximum was 25 flies/ trap/ week (fig.4). Traps showed a permanent presence of medfly on control areas. In moroccan conditions medfly is present all the year (Smaili et al., 1999).
Irrespective to control method used , The number of flies captured was not significantly different
On Maroc late, the threshold (21 flies/trap/week) have not been reached; thus conventional method control was not adopted. As the threshold is commonly not reached on this variety, growers does not spray and supports damages

Cost comparison of spot treatment and conventional control method

Control method	Type of application	No. of application	Cost of one application ($/ha)			Total cost / application ($/ha)	Total cost /cycle of production ($/ha)
			Attractant + insecticide	Oil	Labour		
Conventional treatment	Localised spraying	5	12,37	0,42	0,3	13,09	65,45
	Generalised spraying	3	49,5	1,26	0,3	51,06	153,18
							218,63
Spot treatment	Spot	19	0,41		1	1,41	26,79

On Navel, the direct benefice using spot treatment method was evaluated to 200 $/ha.and the cost of the operation was only 26.79 $/ha instead of 218.6 $/ha using conventional method. on Maroc late, spot treatment method has allowed an increase of 500 $/ha of direct benefice compared with control area, with an average cost of only 42.3 $/ha .

Evaluation of damaged fruit from sampling after harvesting in order to confirm punctures with larvae and therefore real rates of damaged fruit on Navel variety

Dates on 2000/2001	Nbre of fruit sampled	Nbre, of fruit with apparent			Rate of fruits with apparent damages			Nbre, of fruit with larvae			Rate of fruits with larvae (%)		
		CA	CTM	STM	CA	CTM	STM	CA	CTM	STM	CA	CTM	STM
15-oct	200	49	5	4	24.5	2.5	2	41	3	2	20.5	105	1
20-oct	500	136	14	12	27.2	2.8	2.5	125	11	6	25	202	102
24-oct	600	159	18	13	26.5	3	2.16	141	9	7	2305	105	1.16
30-oct	800	204	20	18	25.5	2.5	2.25	191	11	8	2308	104	1
15-nov	1000	234	25	23	23.4	2.5	2.3	204	15	11	20.4	1.7	1.1
Average rate of infested fruit					**25.4**	**2.66**	**2.24**				**22.6**	**1.66**	**1.1**

At harvest, average proportions of fruits with apparent damages were evaluated at 2.24% and 2.66%respectively for conventional method and spot treatment method. Examination of the punctured fruits in the laboratory confirmed those results.

CA : control area
CTM : conventional treatment method
STM : spot treatment method

Conclusion

Spot treatment method was not only efficient but showed an extremely economic interest as the cost was reduced to 85% of conventional method cost; this method was approved by other authors (Vincenot and Quilici, 1995; Affellah and al, 2000) . Climatic conditions of Gharb area characterised by cold winter in December January contribute to the decrease of population especially on late varieties, additionally the use of spot treatment as an efficient, economic and friendly mean for control of medfly can replace the conventional method .This method could be successfully integrated in a future SIT area wide management of *Ceratitis capitata*.

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The unfaithful medfly females: impact on SIT?

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In the field, medfly females can copulate more than once, behavior that may be critical for the application of SIT against its natural populations. In the frequency of remating varies dependent on both ecological conditions and population density is under investigation. Moreover, the observation that in wild populations, remating is accompanied by a strong paternity skew, led to the formulation of hypothesis on the mechanisms that regulate the use of sperm from different males. The elucidation of these mechanisms has been undertaken in the laboratory, using fly strains with different internal molecular markers, and it will allow the description of the most significant medfly sexual/population behaviors to consider for SIT planning.

In the field

Two Mediterranean localities:

Chios (Greece)	Rehovot (Israel)
Summer '00	Sept. '01 April-May '02
36 wild caught females	20 fem. in '01; 30 in '02
26 producing offspring	12 with offspring
SSRs based paternity analysis	
18 Fem.>12 progeny	8 fem. > 12 progeny
681 inds. screened	422 inds. screened
1 remating (5.5%)	4 remating (50%)
P_2=0.48-0.95	P_2=0.37-0.99

Simulation of paternity assignment of two fathers by GERUD (Jones, 2001)

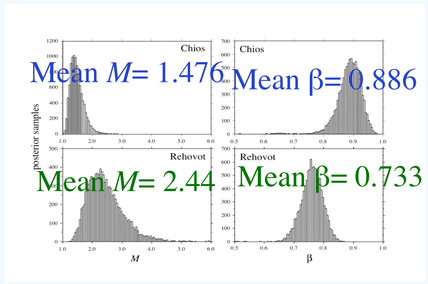
Simulation conditions		Correct detection of 2 fathers % over 10000 iterations	
N. Progeny	P1:P2	Chios	Rehovot
34	5:5	63.62	92.02
34	4:6	62.29	92.09
34	3:7	62.34	91.50

Mean *M*= 1.088

Mean *M*= 1.54

Goodness of Fit test (G test): comparison of the freq. remating between Chios and Rehovot: OBSERVED: G=5.88, 1 df: ESTIMATED: G=5.53, 1df. Both tests significant at p<0.025

Simultaneous estimation of the number of matings/female, *M*, and the proportion of progeny sired by the last male, β (SCARE program, Jones & Clark, 2003)

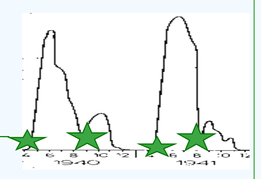


Medfly seasonal distribution

Chios (Katsoyannos *et al.*, 1998)

Israel (Rivany, 1941 Israeli *et. al.*, 2005)

Our collection times: population just appearing and/or recovering from the hot summer bottle-neck



- Large proportion of young flies
- Female receptivity negatively correlate with its age
- When the population size small, there may be a physiological change in the normally choosy females: their male receptivity increases…..maybe **MORE REMATINGS**

In the laboratory

- Three fly lines, which are genetically differentiable (different SSRs), were derived from the "South Africa" lab strain
- Two/three-day old virgin females were made mating for the first time- TOTAL 1050 MATINGS
- either ONE or THREE days after the first mating, the once mated females were re-exposed each to three males
- Every double mated female was allowed to oviposite for 5/8 days after the second mating or were dissected to count sperm-TOTAL of 147 doubly mated Females
- Reciprocal crosses were performed for the two different lines of fathers.- ANY GENOME EFFECT?

- REMATING FREQUENCY: 14-19%

- SECOND MATING
- Always shorter than the first one
- Its duration is not influenced by the inter-mating period

- FECUNDITY IN THE INTER-MATING PERIOD
- It does not influence the propensity to remate

CONCLUSIONS

From the field:

- The data from both Chios and Rehovot provide definitive evidence that medfly females remate in the wild and show that the level of remating may be influenced by local seasonal conditions.
- The strong second male sperm precedence detected in the wild enlights the importance of male sexual competitiveness- ANY CORRELATION BETWEEN THE FREQUENCY OF REMATING AND THE ALLOCATION OF SPERM?

From the laboratory experiments

- The frequency of remating is independent both of the inter-mating period (when it is between 1 and 3 days after the first mating) and the fecundity in the inter-mating period
- The precedence of the second mating male in siring the progeny is conserved in all our crosses-SPERM COMPETITION?
- RELEVANCE OF "MALE VIGOR"

QUANTITY OF SPERM FROM THE FIRST AND THE SECOND FATHER

- The quantity of sperm transferred by the second male is higher when the second mating takes place 3 days after the first mating than when it takes place after one day.
- Fathers from line B transfer more sperm than fathers from line C, both when used as first and second father-GENOME EFFECT?!

FOR THE REMATED FEMALES, DISTRIBUTION OF THE PROGENY BETWEEN THE FIRST AND THE SECOND FATHER

The second male sperm precedence ranges between 0.72 ±0.31 to 0.91±0.09; it is higher when the second father is from line B and tends to increase when the second mating is performed three days after the first one.

AREA-WIDE FRUIT FLY CONTROL IN MAURITIUS

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Introduction

In Mauritius, there exists several species of fruit flies namely the peach fruit fly *Bactrocera zonata* (Saunders), the Natal fly *Ceratitis rosa* (Karsch) and the Medfly *C. capitata* (Wiedemann) which attack fleshy fruits such as mango, peach, guava, papaya among others. The melon fly *B. cucurbitae* (Coquillet) attacks cucurbits only.

An area-wide National Fruit Fly Control Programme (NFFCP) was initiated in 1994. It was funded by the European Union until 1999 and is now fully financed by the Government of Mauritius.

Methodology

The NFFCP targets some 75,000 backyard fruit-tree owners mainly.

Use of bait application and male annihilation techniques.

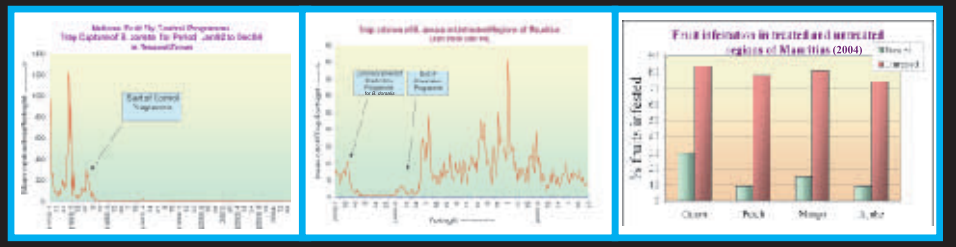
Fruit fly monitoring through traps and fruit collection.

The bait-insecticide mixture is being supplied free of charge to the public.

Results

Fruit fly populations in treated areas have been reduced.

The level of fruit fly damage to fruits has been reduced.



Future Strategy:

Integration of BAT/MAT with other control techniques (Use of female attractants and SIT)

Preliminary work on population genetics of *Glossina fuscipes fuscipes* in Uganda

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Introduction

Tsetse flies transmit several trypanosome species, including *Trypanosoma brucei brucei*, *T. congolense*, *T. simiae* and *T. vivax* that affect livestock. The fly also transmits *T. b. rhodesiense* and *T. b. gambiense* which cause Rhodesiense and Gambiense forms of human African Trypanosomosis (HAT) respectively. In Uganda, both forms of the disease exist with *Glossina fuscipes fuscipes* as the main vector (Okoth, 1999; Fevre, et. al., 2001).

Tsetse population in Uganda is estimated to infest approximately 2/3 of total land area. This population is fragmented into patches of sub-populations.

The aim of the present project is to investigate the genetic variation within and between different *G. f. fuscipes* sub-populations. Microsatellite markers are used to estimate genetic variation within and among tsetse populations (Solano et al., 1997).

Materials and Methods

Study Area

Samples were drawn from the districts of Soroti, Kamuli, Iganga, Tororo, and Lira

Genomic DNA Extraction

Extraction of genomic DNA was done using the salt-extraction procedure as described by Aljanabi and Martinez (1997).

Microsatellite DNA Amplification

Polymerase chain reactions (PCR) to amplify DNA fragments using microsatellite primers, were done.

A total of 13 microsatellite primer sets described by Luna et al. (2001) from *G. palpalis gambiense* were tested on *G. f. fuscipes.*

PCR was done and products were resolved on 12% polyacrylamide gels and visualized after ethidium bromide staining.

Results

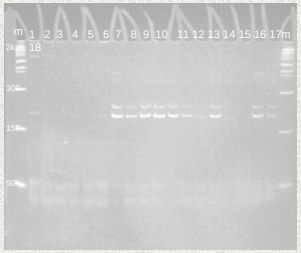
A total of 250 tsetse fly samples have been collected from the study districts. The details are in table 1 below.

A total of 13 Microsatellite primer sets (Pgp1, 8, 13, 17, 20, 22, 24, 28, 24, 29, 33,35 and 38) described in *G. palpalis gambiense* have been tested for population genetics studies on *G. f. fuscipes* field samples from Uganda.

Table 1:
G. f. fuscipes samples by district and trypanosome infection

	District					
Site of infection	Iganga	Kamuli	Lira	Soroti	Tororo	Total
Salivary gland	0	0	0	0	1	1
Mouth parts	4	0	5	0	2	11
Mid gut	7	4	3	0	6	20
Sample (n)	52	62	50	24	62	250

Figure 1:
Visualization of polymorphic microsatellite DNA amplified using primer set targeting the Pgp 13 loci in *G. f. fuscipes*



Conclusion

Primer sets described in *G. p. gambiense* are able to amplify genomic DNA from closely related *G. f. fuscipes*.

Future work

Microsatellite data will be analyzed for three major aspects; genetic diversity within populations, population differentiation, gene flow and phylogenetic relationships between populations.

FAO/IAEA International Conference on Area-wide icontrol of inscect pests: Intergrating the sterile insect and related Nuclearand other techniques, 9th to 13th May 2005, Vienna, Austria

Potential mobility differences between codling moths mass-reared using standard and diapause production protocols

STEPHANIE BLOEM, CBC at FAMU, Florida, USA
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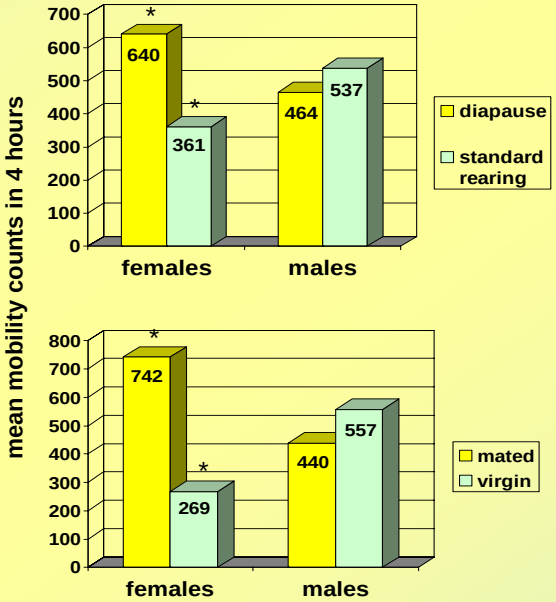
- Maintaining or improving the quality of mass-reared laboratory-adapted insects is essential to successful programs using the SIT.
- In codling moth (CM), *Cydia pomonella*, laboratory rearing at constant temperature (27°C) followed by irradiation results in CM that are less active in the spring when compared to wild CM that have overwintered in the orchard.
- To address this concern, a technique was developed to mass-rear CM through diapause. Subsequently, release-recapture rates of mass-reared diapaused CM were shown to be significantly higher than that of CM reared through standard protocols.

- Previously published studies on CM correlate high actograph mobility counts with increased dispersal under field conditions. Therefore, laboratory studies were conducted to ascertain whether the difference in release-recapture rates might be explained by differences in locomotor activity.
- We examined the effect of gender, mating status, overall temperature, dose of radiation and length of cold storage on the mobility of CM mass-reared through standard or diapaused protocols.
- This poster presents a subset of our results.

- CM were mass-reared in Osoyoos, British Columbia, Canada and experiments were conducted at the ETH laboratories in Zurich, Switzerland in 2005.
- We used 30 infra-red actographs housed in a climate controlled chamber (25ºC, 16L:08D, 60% relative humidity) to assess differences in locomotor activity.
- Each actograph has a transmitter that emits an infra-red beam that is captured by a receiver linked to a computer. CM in closed glass vials are positioned between the transmitter and the receiver. Any movement that intercepts the infra-red beam triggers a signal that is recorded by the computer.
- Tests lasted 4 hours and were initiated 1 hour before sunset. Light intensity was 3,750 lux at photophase (1 hour) and gradually diminished over the 2 hour “sunset” to reach 0.1 lux at scotophase (1 hour).



We found that :
- diapaused CM females were significantly more mobile than females reared through standard production protocols.
- mated CM females were significantly more mobile than virgin females; these data suggest that mated females become more active as they search for oviposition sites.
- no difference in locomotor activity due to rearing strategy was detected for males; these data suggest that the higher recapture rates for diapaused males obtained in previously published field studies resulted from an increased ability or higher propensity to respond to a pheromone signal and not from differences in dispersal ability.
- no difference in locomotor activity due to mating status was detected for males; these results suggest that released males will retain the same level of mobility even after they have mated in the field. In addition, because 12-15% of the mass-reared males have already mated before being released, our results suggest that their mobility is not compromised because of mating in the laboratory.

Comparison of pupal and other parameters in field and insectary-maintained *G. f. fuscipes* in Uganda

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- As part of quality control studies on tsetse fly materials to be availed for Sterile Insect Releases, co-ordinated research activities are ongoing to decide parameters to be included in criteria and protocols for tsetse rearing insectaries. Among conservative (non-changing) characters is size of pupa case. While pupal weight changes with age of pupae – becoming lighter as the larvae metamorphoses into a rather dry adult, the size of the pupal case is constant from day one. The size of pupa correlates with size of larvae that formed it and predicts the size and quality of the future adult to be of an acceptable size to successfully establish itself and compete with wild flies in the field, for niches and mates.
- Quality of tsetse fly product from mass rearing, is a pre-requisite for success of all SIT-based programs as it takes years before the product is available. The product should be un-changed in size, behaviour, etc., as to be able to successfully survive and compete with wild populations, whose phenotype/genotype is "regarded" the standard.
- Therefore setting up acceptable pupal size limits along with related pupal emergence periods will ensure that resulting progeny, with a acceptable mating propensity will be provided.
  Pupal size is determined from pupal diameter, a base for sorting pupal cases according to size. Pupal size is directly correlated to good nutritional status of mother tsetse fly, and assumes that the normal inter-larval period and consistently good insectary holding conditions and management regimen.

- <u>Comparison of field caught and insectary pupal weights for *G. f. fuscipes* in Uganda (study on-going)</u>

  Pupae collections from Buvuma Islands are foremost compared with insectary pupae of the same age. Age is correlated after emergence data of field collected pupae had been obtained.
  Weight status according to weeks since pupariation was recorded.
  There were no significant differences in weight between Buvuma and insectary pupae.

Class and weight parameters in pupae (Insectary and Buvuma)

COMPARISON

		Class A	Class B
		13.83%	86,16%
Average weight Vienna	2.6054g at 2 wks	20.5mg	26mg
Average weight Buvuma	2.7212g at 2 wks	21.5mg	25mg

- <u>Emergence trends of field (Buvuma) & insectary pupae</u>

  Pupae collected from Buvuma Islands were kept iindividually along with insectary-reared pupae in petridishes and emergence data recorded by day, sex and all un-emerged pupae dissected after day 40 in the lab.

  The data collection is still ongoing for batch 2-3. The study will be extended to mainland lake Victoria shoreline tsetse material.
  No conclusions will be drawn as study ongoing,
  but no significant differences between field collected and insectary material have been detected to-date

Viable flies		Dissected pupa status				
M	F	Watery	Red eye	Formed	Empty	Totals
Class B						
20	25	2	1	30	2	80
Class C						
43	26	2	3	25	1	100
Buvuma pupae – batch A						
32	26	-	7	9	18	92

- <u>Performance of Buvuma collected flies following establishment of make-shift membrane feeding on the field</u>

  Attempts to domesticate Buvuma strain of *Glossina f. fuscipes* have been ongoing with flies being transported to Tororo some 250km away without feeding. A dimension of feeding flies and only transporting flies that had fed and survived for 24-48 hours was pursued. Results showed that membrane feeding in the field greatly increased chances of fly survival and the flies larviposited in the insectary and fly survival of up to 2-3 moths was recorded. Improvements need to include ensuring survival and retaining males too for mating emergences.
  The study will be extended to the mainland.

Performance of Buvuma flies in insectary conditions

Dec 2003	Total females	Pupae dropped	Mortality	
Jan 13.04	80	59	5	Mating ? feeding ?
Jan 20.04	381	18	25	
Jan 27.04	377	12	22	By 29.2.04, 3 months later
Feb 01.04	50	1	16	
Feb 8.04	181	-	-	

- <u>Acknowledgements</u>
  Director and staff of Livestock Health Research Institute, Tororo, Uganda
  IAEA CRP N^{O}12543- ongoing

Village-level suppressive fruit fly management in India: Issues determining the optimum scale of cooperative control

IAEA-CN-131/92P

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Village Cooperative Control

There are often benefits to the coordinated, suppressive control of pests over an area larger than an individual farm, but smaller than hose used for high-tech applications such as SIT. This study evaluated the returns to fruit fly management at "farm" and "village" scales in India. Village-level application had double the effect of farm-level application. Interviews and discussions examined the social features making cooperative control sustainable at village level.

Five Keys to Sustainability

1 - ***Farm size*** Among large farms the number of farmers needed to obtain cooperative control is relatively small. Among very small farms only cooperative control may be effective, as immediate reinvasion from neighbouring plots undermines farm-level controls.

2 - ***Problem seriousness*** Sustainable cooperative control must overcome inertia, apathy and suspicion. The perception of the problem as serious is particularly important in overcoming this.

3 - ***Shared economy*** Sustainable cooperative control is enhanced when it can be "grafted" or "piggybacked" onto other cooperative activities - such as marketing or buying inputs – rather than starting from scratch.

4 - ***Social cohesion*** Some mutual trust is highly important. Farmers tend to trust recommendations from cooperatives for cultivation when these also buy their produce, the farmer can see a real interest by the cooperative in the success of production rather than the sale of the input.

5 – ***Tolerance of imperfection*** "Forgivingness" of incomplete application of area-wide controls, so their effect is not destroyed by a few isolated untreated areas, is important where there are truculent individuals who do not cooperate with a group effort. When cooperative control aims to be suppressive, rather than eradicative, private control by each individual can still obtain a return, regardless of the participation of neighbours, undermining the "free rider" strategy. This "forgivingness" is a function of the ecology of pests which are relatively "*K*-selected", such as fruit flies, rather than "*r*-selected" such as Hemiptera.

Farmers were interviewed singly and in groups (photo: HS Singh)

Cooperatives which buy produce are trusted to provide inputs (photo: J Stonehouse)

Cooperative pest management sits naturally alongside cooperative marketing (photos: J Thomas)

Acknowledgement: Work funded by the UK Department for International Development Crop Protection Programme, Project R8840, collaborating with the Indian Council for Agricultural Research

Indian fruit fly control and the S Asia Fruit Fly Network

IAEA-CN-131/94P

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Programme

The Indian Fruit Fly Research Project supports researchers at eight centres in India. The areas are representative of a range of horticultural systems within the country and includes work on the major Tephritid fruit flies affecting fruits and vegetables: *Bactrocera zonata*, *B. dorsalis*, *B. cucurbitae*. Participating centres:

- Kerala Agricultural University (KAU), Thrissur and Thiruvananthapuram
- Navsari Agricultural University (NAU), Gandevi, Gujarat
- Anand Agricultural University (AAU), Anand, Gujarat
- Sardarkrishinagar Dantiwada Agricultural University (SDAU), Palanpur, Gujarat
- Central Horticultural Experiment Station (CHES), Bhubaneswar, Orissa
- Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh
- Central Institute for Subtropical Horticulture (CISH), Lucknow, Uttar Pradesh

Monitoring cucurbit flies, damage in Kerala (photo: J Rajmohan)

Activities

Extensive trapping from 2003-2005 established seasonal patterns of fly abundance and damage. Cucurbits are worst affected in August, while tree fruit damage is spread throughout the year depending on the fruiting seasons (May for mango, July for sapota, December for guava).

Trials in 2003 and 2004 demonstrated that market quality fruit can be produced from area-wide male annihilation control. Further experiments in 2005 are testing this for vegetable fruit flies. Larger scale treatment, 1 km^2, gave double the effectiveness of male annihilation compared to farm-level treatment. Work with Mother Dairy Ltd has been examining how cooperative fruit fly control at village-level can be connected with other quality and value adding processes (such as grading and packing of produce locally) to increase small farm incomes and improve their position in the food supply chain.

A review of the Indian fruit fly literature has produced abstracts of over 300 reports and published papers in Indian journals going back to the 1930s on fruit flies in India. These will be made available to researchers through the web-based network.

Assessing damage to melons in Gujarat (photo: R K Patel)

Village male annihilation trial outside Thrissur, Kerala (photo: J Thomas)

S Asia Fruit Fly Network

The *South Asia Fruit Fly Network* (**www.southasiafruitfly.net**) will serve as a forum for fruit fly research. The Network's website will disseminate the research results and control recommendations arising from research in the region, and encourage the discussion of all aspects of fruit flies and their management in South Asia, through the on-line *SAFFN Newsletter,* the *Fruit Fly Forum* bulletin board, a list of *Connections and Contacts* to allow fruit fly workers to contact each other, and a page of announcements of upcoming events. The Network is hosted by Anand Agricultural University, Gujarat, and the site was officially opened on 11 January, 2005.

The human network at Delhi, Jan 2005 (photo: CABI India)

Acknowledgement: Work funded by the UK Department for International Development Crop Protection Programme, Project R8840, collaborating with the Indian Council for Agricultural Research

IAEA-CN-131/95P

Management of the rice stem borers in Yangtze Delta, China: an area-wide approach

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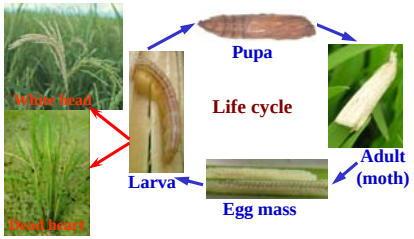
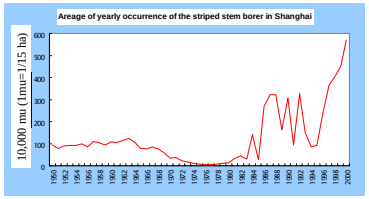
1.The problems of the rice stem borers in the Yangtze Delta, China

Yangtze Delta of China is one of the largest rice production region. It has been called as the rice big bowl for long time. The rice stem borers have been the most injurious insect pests with long history in this area. The rice striped stem borer (SSB) *Chilo suppressalis* (Walker), the yellow stem borer *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) and the pink stem borer (PSB) *Sesamia inferens* Walker (Lepidoptera: Noctuidae), are the main species.



Life cycle of the rice stem borers, as represented by the striped stem borer *Chilo suppressalis*

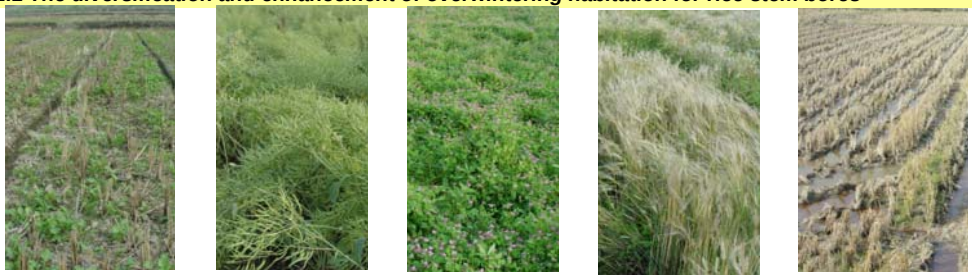
The field scale symptom of white heads



The pest status of the rice striped stem borer (SSB) has increased significantly in the last decade, as represented by the occurrence in Shanghai.

2. Main causes of the outbreak of the rice stem borers

2.1 The diversification and enhancement of overwintering habitation for rice stem bores



Directly-sowing of rape seeds or green manure crop in the 2nd-season rice field after harvest, overlap-growing of wheat or barley before rice harvest are helpful for overwintering for the rice stem borers

High-stubs remained by mechanical harvester favorite the survival of wintering larvae of stem borers.

2.2 The area-wide shift from pure double cropping system to single-double mixed cropping system

In such mixed system, rice sowing date varies from late-April to early-June, and the transplanting date varied from early-May to late-June. Thus almost all of the newly emerged adult moths in different time period from different overwintering sites can find the suitable rice plants of suitable growth stage for oviposition.

2.3 The increase in yield potential of new varieties

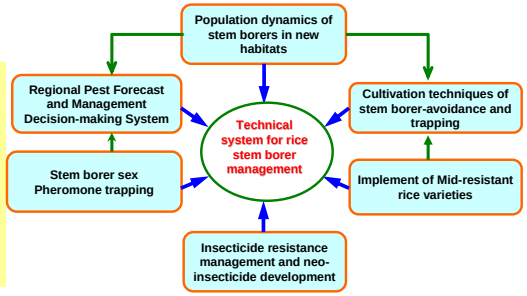
The *japonica* hybrid rice, the super-high yield hybrid and conventional *indica* rice, which are with features of relative higher plants, larger stem diameter and inner space, particularly with higher N/C ratio, are helpful for stem borers to borer, growth, survival, and reproduction.

2.4 The development of high resistant level in stem borers to main insecticides

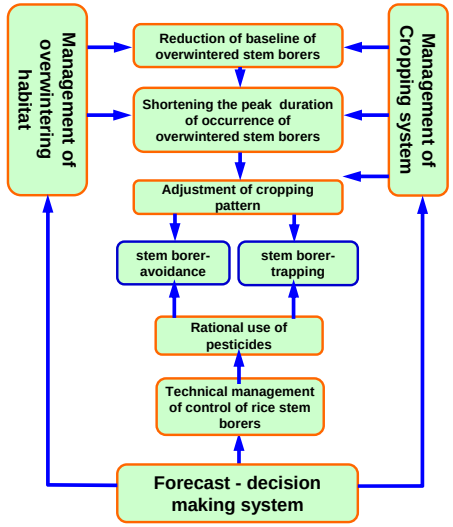
The resistance levels of YSB to nereistoxin insecticides monomehypo (monosultap) and dimehypo is as high as at 40-243 times. Resistance SSB to OP triazophos in central Yangtze Delta is still low, but in south fringe of the region it is as high as 40 times with a max. of 203 times.

3. Area-Wide management system

The indigenousness and characteristics with local dispersal of the rice stem borers among habitats implies that the area-wide approach is probably the best way to achieve the objectives of management of the rice stem borers to such a density under economic injury level, and to maintain rural environment and farmers healthy. The necessary and national policy of food-safety requires the area-wide approach in design and implement of rice pest management too.



Components of the area-wide management system of rice stem borers



Flow chart of techniques in the area-wide management system of rice stem borers


Flooding and mechanical plough during spring reduce the pupation and survival rates.

Collective raising of seedlings in a village for all farmers limits the amount of pesticide use and increase control efficacy during seedling nursery.

Light trap and sex pheromone trap are tools for forecast of population dynamics of rice stem boers. Sex pheromone traps are used in large scale for reduction of male density and therefore control of stem borers.

Postponing and synchronization of the seeding and transplanting dates of rice to reduce the oviposition opportunity of overwintered moths.

Carefully application of fertilizers and reasonable spray of insecticides according to the recommendations of local extension experts are effective ways to control the rice stem borers.

Acknowledgements

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EXPERIENCE IN THE MANAGEMENT OF AREA-WIDE CONTROL OF *GLOSSINA PALPALIS PALPALIS* IN GHANA USING BAIT TECHNOLOGY AND RELEASES OF STERILE MALES

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INTRODUCTION

Trypanosomosis causes a great deal of human suffering, agricultural productivity losses and hunger. The control or eradication of tsetse fly vectors is therefore critical. To permit the development of livestock industry and tourism in the onchocerciasis-freed zone the Northern sector of Ghana., BNARI in collaboration with TTCU embarked on a pilot project between 1995 and 1999 to develop the Sterile Insect Technique (SIT) to control tsetse flies, *Glossina palpalis palpalis* (Robineau Desvoidy) and *G. tachinoides* Westwood (Diptera: Glossinidae) which are responsible for cyclical transmission of trypanosomosis in riverine habitats across the country.

An integrated approach using a combination of bait technologies and sterile insect technique was the strategy adopted in the pilot study in the Savelugu-Nanton District in the Northern Region of Ghana (Map1).

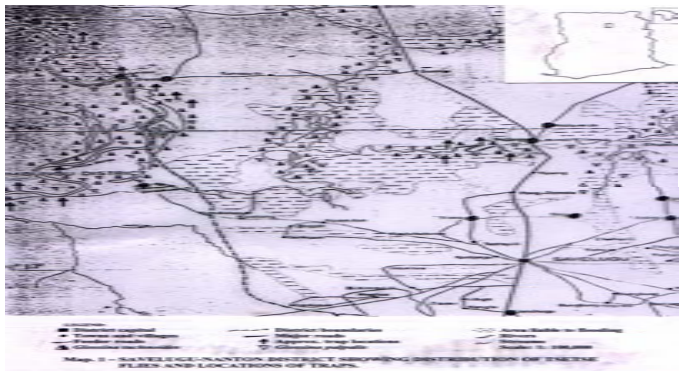
LABORATORY REARING OPERATIONS: The flies used have been maintained at BNARI since 1985. The rearing conditions, procedures and handling of puparia and adult flies were modifications of techniques described by Van der Vloedt (1982). The flies were fed mainly on mixed fresh frozen porcine and bovine blood (50:50 vol/vol) through silicone membrane. Male sterilization was by 120Gy gamma radiation from a cobalt-60 source.

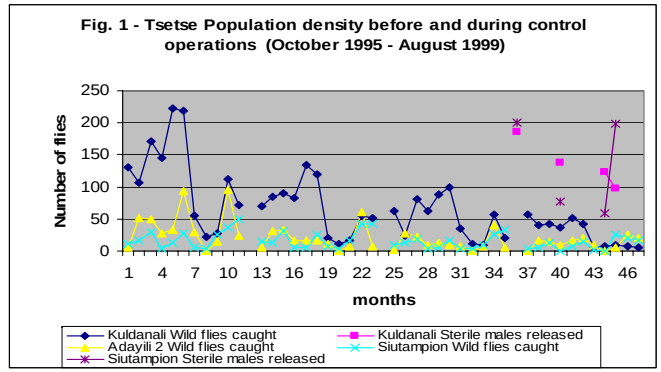
CONTROL OPERATIONS : To reduce wild population, 600 Unbaited blue biconical traps traps, 120 cattle treated with chemicals and 890 blue cloth screens impregnated with 200 mg deltamethrin were deployed over an area of 296km^2 with participation of 13 affected communities. Ten sites in three villages, Kuldanali (K), Adayili 2 and Siutampion (S) were used as fixed monitoring sites for tsetse population. Prevalence of trypanosomosis in cattle was evaluated.

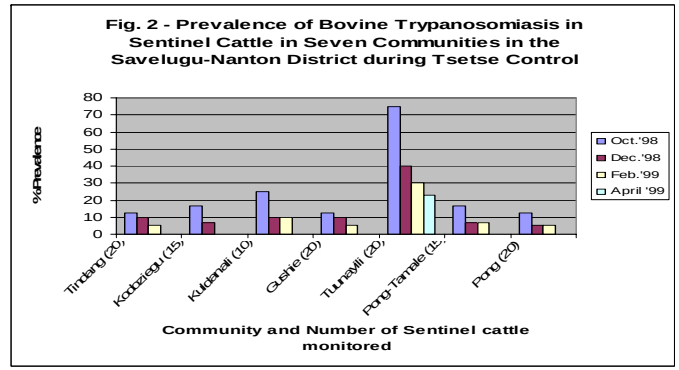
Sterile males were released at S and K from October 1998 to June 1999.

RESULTS AND DISCUSSIONS

- Map1 is the study area showing distribution of the two riverine tsetse flies species and locations of the monitoring traps.
- Fig. 1 and Fig. 2 show significant reduction in the tsetse population and prevalence of bovine trypanosomosis by integrating the use of traps, insecticide-treated screens and animals with release of sterile males. The reductions in population of *G. p. palpalis* and trypanosomosis were as around 98% and 80% respectively.
- The releases of sterile males were done at longer intervals to cut down on cost of operations, but the numbers of sterile males released were inadequate to establish the recommended ratio of 10 sterile to 1 wild fly. However, the reduced tsetse population indicated the additive effect of induced sterility. Therefore, the releases of sterile flies should be carried out either more frequently or the number of sterile flies be increased to ensure that sexually active sterile males are always present to mate with the native females.







CONCLUSIONS

❑ Tsetse eradication could not be achieved because the genetic load imposed by the limited number of sterile males released was inadequate. However, appreciable levels of suppression of tsetse population and reduction in trypanosomosis were achieved.

❑ Though we could not eradicate *G. p. palpalis* in this small-scale pilot trial, we have gained valuable practical experience in the integration of insecticide-impregnated screens and sterile male releases to control tsetse flies in the Savelugu-Nanton District in Northern Region of Ghana. The skills acquired will be used in the GHANA PATTEC for the nation-wide eradication of tsetse flies in the country.

❑ The results are in close conformity with those of Oladunmade et. al. (1990), Merot and Bauer (1990) and Vreysen et. al.(1999) in showing that integration of insecticide-impregnated targets and sterile males releases is effective in reduction in tsetse populations.

❑ This work has produced a base-line data of essential parameters required for planning and implementing an area-wide SIT programme With improved skills and facilities, SIT in combination with bait technologies could be an effective strategy for eradication of tsetse and trypanosmosis in Ghana.

ACKNOWLEDGEMENT

We acknowledge with thanks the technical support given in the form of equipment, training, expert services and supplies of puparia by the IAEA from 1985 to 1995.

FAO/IAEA International Conference on Area-wide Control of Insect Pests: Integrating the Sterile Insect and related Nuclear and other Techniques, Vienna, Austria, 9 – 13 May, 2005

Impact of Beta Cyfluthrin and Triflumuron on *Glossina fuscipes fuscipes* in Mageta Island, Kenya and the Implication for tsetse eradication in Lake Victoria.

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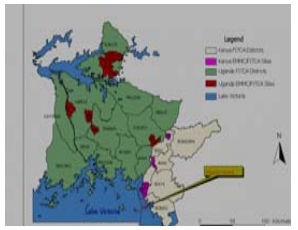
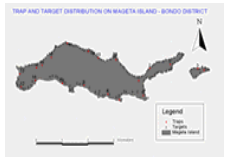
Mageta Island in Relation to Kenya and Ugandan Districts





The objectives of the study

- To prove that insecticide treated targets can control *G. f. fuscipes*
- To assess if Triflumuron can induce sterility in an island population
- To integrate the two techniques to eliminate *G. f. fuscipes* from Mageta island.

Treatment of blue-black targets with insecticide

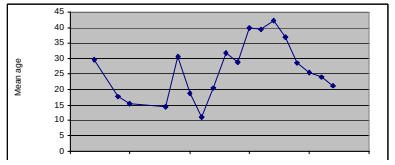
- Pre treatment monitoring - 3 mths
- Treatment - 0.6% ♌cyfluthrin (Bayer)
- Screens (1.0 x 1.5m) soaked and dried
- Set at 100m intervals (170 screens) in November 2002 to March 2003
- Targets set 25 cm
- Exposed for 5 months
- Monitor age categories apparent densities

Target

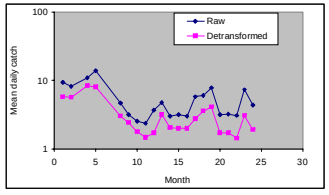
Treatment of blue-black targets with Triflumuron

- Applied immediately after removing insecticide treated targets.
- Treat with Triflumuron 3% (Bayer)
- Screens (1.0 x 1.50m) soaked and dried
- Set at 40m intervals (270 screens)
- Targets set 25 cm above the ground
- Exposed for 16 months
- Monitor age categories and apparent densities

Mean ages of two phases



Detransformed mean catches



CONCLUSIONS

- **Insecticide**
  - Kills flies on contact
  - High composition of young flies
  - % teneral flies increase post treatment
  - Higher rate of decline, growth rate –20.9%
  - The difference between the mean ages before and after treatment significant (P< 0.05)
  - remains persistent on fabric for long
  - Can effectively be used to suppress *G. f. fuscipes*
  - The fabric does not fade

CONCLUSIONS

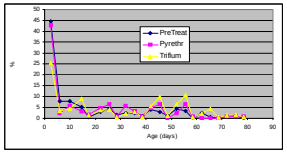
- **Triflumuron**
  - Disruption of ovulation- ovarian cycles not clear
  - High composition of young flies
  - The population did not show clear aging pattern as expected
  - Slow rate of decline (growth rate of 1.3%)
  - The difference between the mean ages before and after treatment not significant (p<0.05)
  - Persistence on fabric require investigation
  - Effectiveness may be compromised by other factors

Acknowledgements
- Bayer Animal Health, South Africa
- Vestergaard Frandsen, Nairobi, Kenya
- EU and AU/IBAR
- Veterinary Department Ministry of Livestock and Fisheries Development, Kenya
- Dr. Glyn Vale, Zimbabwe

Pooled data for all the treatment phases

Age range	All	Pretreat	Insecticide	Triflum
2.5 to 6	335	109	57	169
10 to 14.5	117	27	11	79
17.5 to 22	43	7	8	28
25.5 to 28	46	12	9	25
28.5 to 32	50	10	11	29
35.5 to 38.5	49	10	7	32
42 to 45.5	79	8	8	63
48.5 to 52	127	16	11	100
55.5 to 58.5	20	5	1	14
62 to 65.5	27	1	0	26
Above 68.5	26	4	3	19
Total	919	209	126	584

Effect of two treatments on age structure



Integration of Behavioural and Biological Control for the Management of Cotton Insect Pests: Significance and Cost Benefits

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OBJECTIVES

Development of effective, economical and environment friendly tactics for the management of cotton bollworms

COMPONENTS USED

Parasitoids and pheromones

ACHIEVEMENTS

- The infestation of pink bollworm (Table. 1) and the two *Earias* spp. (Table. 2) was least in blocks treated with pheromones PB/SB-ROPE in conjunction with egg parasitoids, *Trichogramma chilonis*.
- The infestation of cotton bollworms in blocks treated with pheromones and egg parasitoids separately was at par to insecticide treated blocks.
- Establishment of parasitoids was low during the hot cotton growing months and started increasing in the field as temperature and relative humidity became favourable (Table. 3).
- Peak parasitization in the field was recorded in the month of November, which may be due to the successive generations produced by the parasitoids at the end of the cotton season.

CONCLUSION

- On an aggregate basis, higher return was achieved with the integrated treatments of pheromones and the parasitoids.
- Separate treatment of pheromones or parasitoids, was less effective, requiring supplemental measures.

Table I.- Mean infestation percentage of pink bollworm in cotton treated with different environment friendly treatments.

Treatments	Flowers	Green bolls
Trichogramma chilonis + Pheromone (PB/SB-ROPE)	4.91[a]	4.05[a]
Pheromone (PB/SB-ROPE)	9.23[cb]	6.85[b]
Trichogramma chilonis	10.51[c]	8.57[c]
Insecticides (control)	6.79[b]	7.73[cb]

Means followed by similar letters are not significantly different (P≤0.05).

Table II.-Mean infestation percentage of *Earias* spp. in cotton treated with different environment friendly treatments.

Treatments	Flowers	Green bolls
Trichogramma chilonis + Pheromone (PB/SB-ROPE)	6.44[a]	5.29[a]

Table III. Mean percentage parasitization of Angoumois grain moth eggs exposed to *T. chilonis* in the field during different cotton growing months.

Months	Number of eggs exposed	Parasitism percentage	Mean Temp. (^{0}C)	Mean R.H. (%)
June	2000	0.25[e]	33.60	63.0
July	2000	0.26[e]	31.87	74.8
August	2000	1.57[d]	30.65	74.1
September	2000	10.89[c]	30.10	74.3
October	2000	23.50[b]	27.10	66.5
November	2000	38.51[a]	20.90	62.8

Means followed by similar letters are not significantly different (P≤0.05).

Table IV. Cost economics of different eco-friendly tactics for the management of cotton bollworms.

	Treatments			
	T. chilonis + Pheromones	Phero-mones	*T. chilonis*	Insecti-cides
Cost of treat./ha (Rs.)	4550	4000	550	2920
Appl.charges/ha (Rs.)	65	50	15	850
Total cost (Rs.)	4615	4050	565	3770
Seed cotton yield/ha (Kg)	2855	1842	1470	2485
Total income/ha* (Rs.)	66022	42596	33994	57466
Net income/ha (Rs.)	61407	38546	33429	53696
Benefit over insecticides (Rs.)	+ 7711	-15150	- 20267	--
Cost economic ratio over insecticides	1 : 1.14	1 :0.72	1 : 0.62	--

Rate of seed cotton Rs. 925 / 40 Kg.

USE OF GAMMA RADIATION FOR IMPROVEMENT IN MASS PRODUCTION OF BENEFICIAL INSECTS

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PARASITIZATION OF IRRADIATED HOST EGGS (*SITOTROGA CEREALELLA*) BY *TRICHOGRAMMA CHILONIS* ISHII

GAMMA RADIATION PROLONGED THE VIABILITY OF HOST EGGS FOR PARASITIZATION: FIG. 1

Un-treated eggs : Parasitization occurred only for 3 days.
Irradiated eggs (5 to 55 Gy) : Parasitization increased to 7 days. Parasitization increased with the increase in doses. Highly effective dose: 55 Gy (82% on 1st day, 45% on 7th day)

FEEDING EFFECTS OF GAMMA IRRADIATED EGGS ON *CHRYSOPERLA CARNEA* STEPHENS

IRRADIATED EGGS INCREASED LARVAL LIFE: FIG. 2

Control : Survival of larvae: 56%.
Irradiated eggs (5 to 45 Gy) : Survival enhanced with the increase in doses. Highly effective dose: 45 Gy (survival increased to 89% in P, followed by 87% in F-1, and 78% in F-2).

IRRADIATED EGGS INCREASED INSECT FECUNDITY: FIG. 3

Control : Fecundity remained 272 eggs/ female.
Irradiated eggs (5 to 45 Gy) : Fecundity directly correlated with doses. Highly effective dose: 45 Gy (444 eggs/ female in P, followed by 397 in F-1 and 311 eggs/ female in F-2)

IRRADIATED EGGS INCREASED FEMALE SEX RATIO: FIG. 4

Control : Male to female sex ratio (1.4: 1)
Irradiated eggs (5 to 45 Gy) : Low doses increased male to female ratio (at 5 Gy, 4:1 in P, 1.7: 1 in F-1, 2.3: 1 in F-2). High doses increased female ratio (at 45 Gy, 0.5: 1 in P, 0.2:1 in F-1 and 0.2:1 in F-2)

Fig.1 : Parasitization of irradiated host eggs.

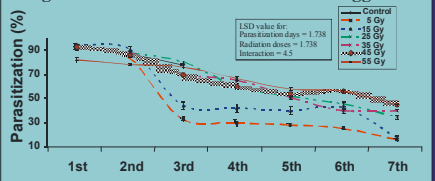


Fig. 2: Effect of irradiated host eggs on survival (%) of *C. carnea* larvae in successive generations

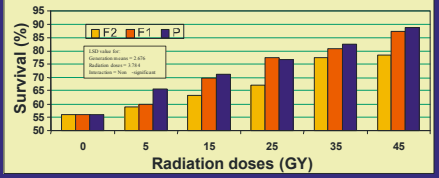


Fig. 3: Effect of irradiated host eggs on fecundity of *C. carnea* in generations

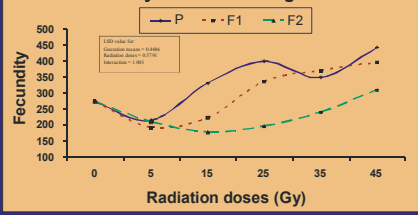
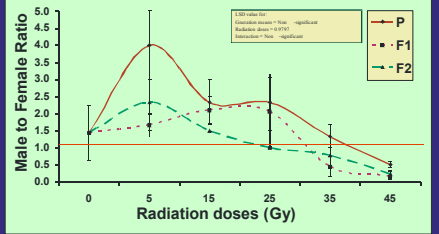


Fig. 4: Effect of irradiated host on sex ratio.



CONCLUSIONS:

- Gamma radiation prolonged the viability of host (*Sitotroga cerealella*) eggs for parasitization up to 7 days. High doses proved better than low doses.
- Parasitization of *Trichogramma chilonis* was not adversely affected by applying gamma radiation.
- Gamma radiation enabled to run the insectaries in remote areas by supplying irradiated host eggs with low rearing cost and to fulfill the requirement of area-wide releases of *T. chilonis*
- Feeding of gamma radiated host eggs increased percent larval survival and fecundity of *Chrysoperla carnea.*
- Female to male sex ratio of *C. carnea* was increased with the increase in gamma radiation doses to host and vice versa

IAEA-CN-131/102P

Sound analysis: a potential strategy of quality control for sterilized insect pests

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TARGETS:

The increasing threat caused by sleeping sickness urgently calls for intensifying the control of tsetse flies. The established method of releasing sterilized males requires expensive breeding technology and a reliable quality control of sterilized males.

An important step to improve implementation is to develop a simple and inexpensive quality control for the sterilized males.

POSSIBLE SOLUTIONS:

The required apparatus consists of a soundproof box with an inserted microphone (temp. 25°C, hum. 95-100%) and a standardized illumination.

The obtained results point to two possible solutions:

Determination of the "time balance" of the songs. Advantage: A small group of Glossinas can be kept in the box (max. 5 males). Disadvantage: Relatively time consuming, evaluation not possible on the individual level. Requirement: A computer with a simple sound analysis program (for example Avisoft, Raven res. Canary).

Determination of the "stamina power" of the sterilized male tsetse flies. This evaluation is based on how many males are still singing on the 4th day. Advantage: A bigger random sample is possible. Disadvantage: The "stamina power" rather than the "impetus" is tested. (Fig. 3,4 and 5)

FACTS, RESULTS:

1. The only acoustic signal of *Glossina pallidipes* (Fig. 6) that is reproducible under laboratory conditions is the "spontaneous sound" (which probably has a mating and assembling function).
2. Weakening males show no changes in frequency parameters (fundamental frequency, overtones, changes of frequency) of the "spontaneous sound".
3. The weakened male Glossinas show no changes in volume (sound pressure level).
4. The only change in weakened flies is an extreme decrease in the impetus to produce acoustic signals from the 4th day on.
5. The same results (points 1 – 4 above pertaining to "spontaneous sounds") apply to the "flight sounds". (Fig, 1 and 2)
6. Tsetse flies are not capable of simultaneously flying and singing because the flight apparatus and the sound - producing system are functionally coupled. (Fig. 1)
7. Singly kept males could not be motivated to sing.
8. The songs of the individuals differ only minimally therefore they could be distinguished by sonagrams.

METHODS:

In *Glossina pallidipes* we tested whether the acoustic parameters (frequency, volume, time) change distinct by enough in males that become weaker to easily define the change. For comparative values, the flies were weakened by starvation under identical conditions (temp. 25°C, humidity 95-100 %).

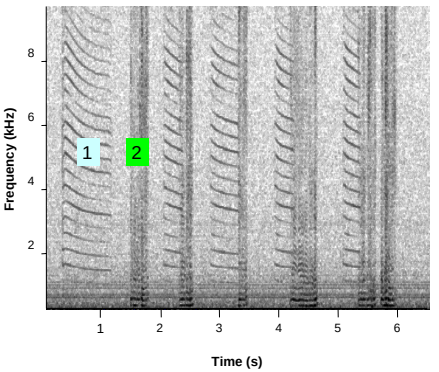

Fig. 1: *Glossina pallidipes* song (1) and flight sond (2)

PROBLEMS:

1. Neither the frequency parameters nor the volume could be used as representative values for quality or fitness.
2. As the singly kept male Glossinas could not be stimulated to produce acoustic signals and group - kept males could not be distinguished by the human ear, only a small number of flies can be acoustically distinguished by at once (by sonagram).(Fig 2)
3. Flight sounds also occur in singly kept males, but the acoustic signals are preferable because they are most probably correlated with mating success.

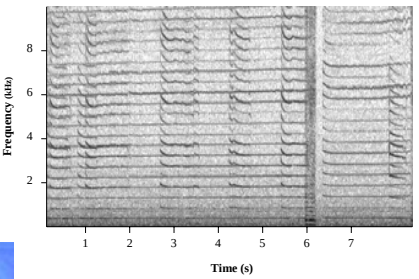

Fig. 2: *Glossina pallidipes* male chorus

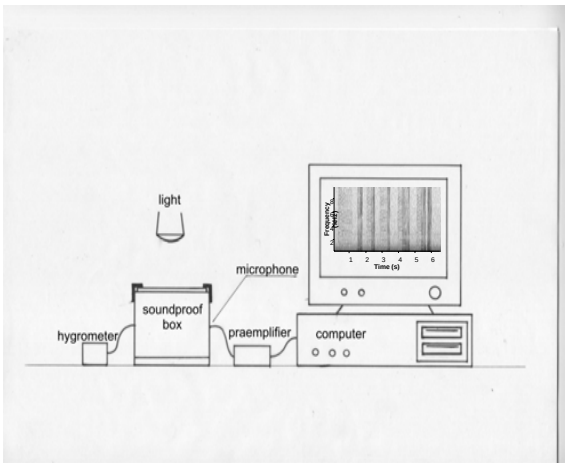

Fig. 3: Model of the system for acoustical quality control

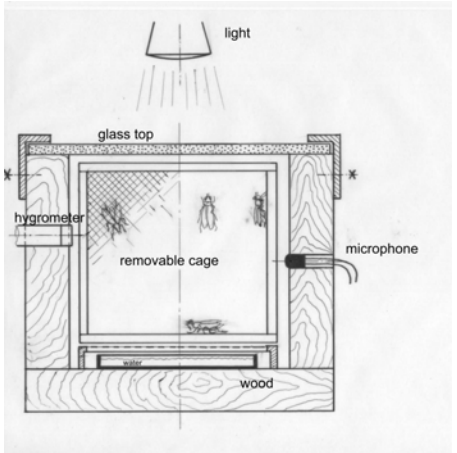

Fig. 4: soundproof box

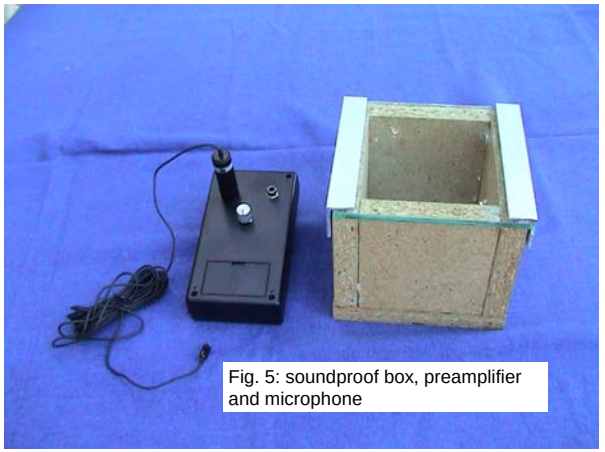

Fig. 5: soundproof box, preamplifier and microphone

Fig. 6: *Glossina pallidipes*

Monitoring of Some Major Pests of Palms in Nigeria - A Strategy towards an Area-wide insect control

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Synopsis

The major insect pests destructive to the oil, coconut and date palms in Nigeria were monitored. *Oryctes monoceros* Olivier (Coleoptera: Scarabaeidae) was found to be a major destructive pest of these three palms in NIFOR (Rain forest, oil palm belt), Badagry (coastal coconut belt) and Dutse (Sudan-savannah date palm growing belt) of Nigeria. *Latoia viridissima* (*Lepidoptera;Limacodidae*) was also found destructive to the oil palm.

The prospect of using sterile male *Oryctes monoceros* releases, combined pheromone based trapping and microbial infection (Trap-infect-and Release method) as inputs in the Integrated control of *Oryctes monoceros* is discussed. Also discussed is the prospect of using Gamma irradiation in reducing the rate of infestation of insect pests of date palm fruits (in bulk storage).

Introduction

Palms (oil, coconut,date and Raphia) are important food and cash crops in the economy of most countries in the humid tropics. Despite this fact, their entomological problems are often neglected. Among these major pests are Oryctes monoceros, Rhynchophorus phoenicis, Latoia viridissima, Sternocera klugii, Ornithacris turbida, Odontotermes sp. near pauperans, Macrotermes bellicosus, Oryzaephilus mercator and Plodia interpunctella (Aisagbonhi et al, 2004).

The process of monitoring insect pests in our plantations must therefore be regularly undertaken in order to determine the pest status of insect pests in the field and plan their integrated control measures. The methods of monitoring these major pest populations of palms and the prospects of integrating them with sterile insect and other control technologies in Area-wide insect control are therefore examined in this paper.

MATERIALS AND METHODS

Sampling Sites: The sampling sites of these studies were the Nigerian Institute For Oil Palm Research (NFOR) main station, Benin city, NIFOR Coconut substation, Badagry, Lagos state and NIFOR Date Palm substation, Dutse, Jigawa state, Nigeria.

RESULTS AND DISCUSSION

Census counts of *Latoia viridissima*:

Table 1 indicates the census counts of various larval instars and cocoons during the period of survey. It reflects the counts in August, 2003, before application of control measures (pruning); in November (4 weeks after pruning); in January and in July 2004. There was a mean number of 0.9 larvae and 6.9 cocoons per frond/palm sampled before pruning, which decreased to 0.8 larvae and 3.2 cocoons/frond after pruning. In late November,2003, insecticidal spraying of "Best Action" insecticide (25% Dimethoate + 30% cypermethrin) was undertaken.

The counts of *L.viridissima* per frond sampled in the same field in January, and July, 2004, are also indicated. Census count of the live stages of this insect undertaken in January, 2004, indicated an upsurge (mean of 10.1 larvae and 5.9 cocoons); and a significant crash in numbers (zero larvae and 4.3 cocoons) in July, 2004, 6 months later. (P= 0.005).This suggests it is better to resort to a long term and more sustainable use of natural enemies and weather factors in control of this Lepidopteran pest.

Handpicking Recoveries of *Oryctes monoceros*

Table 2 indicates the monthly handpicking recoveries from June to November 2003, from 79 randomly sampled coconut palms. 11 live *Oryctes monoceros* were handpicked and destroyed within the period.

Table 3 similarly indicates the handpicking recoveries from 103 coconut palms randomly sampled in Badagry from 1- 2 September, 2003. A Total of 13 live adult *Oryctes monoceros* were handpicked and destroyed at Badagry.

Fig. 1 indicates handpicking recoveries of *O. monoceros* on date palms at Dutse, from June to December, 1993. These results indicate that *O.monoceros* is a very destructive pest of coconut and date palms in these three ecological zones surveyed.

Pheromone Based Mass Trapping Recoveries:

Aisagbonhi and Oehlschlager (1999) found Ethyl – 4- methyloctanoate based mass traps very effective in monitoring *O.monoceros* on date palms at Dutse (Fig.2) and the corresponding pheromone extract of *Rhynchophorus phoenicis* very effective in monitoring *R.phoenicis* population on newly clear felled and replanted oil palm field at NIFOR, Benin city,(Fig.3).

The possibility of using pheromone based mass traps to trap *O.monoceros* , combined with appropriate microbial infection and releasing adults back into the palm ecosystem is an area that will be further explored. The present cost of these pheromone baits ($6 and $3 per unit of *O.monoceros* and *R.phoenicis* bait respectively) is however expensive to the resource-poor farmer and sourcing for the prepared baits from the manufacturers may also be presently difficult for poor farmers.

Philippe and Derry (2004) have found old fishing nets very useful in trapping O. monoceros in Ghana. This method may hold a better promise for resource-poor farmers, if adapted in Nigeria.

The possibility of capturing live adult males O. monoceros and *R.phoenicis* and exposing them to 10 krad of gamma irradiation from a caesium-137 source in a nitrogen atmosphere, and then releasing them back to the palm ecosystem is an area worth investigating. Such irradiated insects may lead to sterile generations, and reduction in numbers of these destructive insects.

Insect Infestation in Microscopically Examined Date Palm Fruit samples:

The following destructive insects were recovered from marketed date palm fruits examined under the microscope in results obtained by Aisagbonhi (1988):

Oryzaephilus mercator (Fauvel)(Coleoptera:Scolytidae)

Coccotrypes dactyliperda Fabricius (Coleoptera:Scolytidae)

Araecerus fasciculatus Degeer (Coleoptera: Anthribidae)

Plodia interpunctella Hubner (Lepidoptera:Pyralidae).

Al- Hakkak et al (1984) evaluated the safety of irradiated dates for human consumption in their wholesomeness studies with a full diet of irradiated dates on the insect *Ephestia cautella* (Walker). They used *Ephestia cautella* which was reared for 5 generations on a 100% diet of date fruits treated with 100 or 200 of gamma radiation. They checked each generation for (i) development from egg to adult stage (ii) female fecundity (iii) mating frequency and (iv) the percentage of egg hatchability as indicators for genetic effects. The joint FAO/IAEA/WHO Expert committeee in it's Geneva meeting in 1980 assessed the safety of irradiated date fruits. Accordingling, an unconditional acceptance was granted for date irradiation for the purpose of controlling infestation by stored product insects using an average dose of up to 100 krad (WHO, 1981).

Gamma irradiation of date fruits could similarly be investigated to improve on the local methods of packaging date fruits in the date growing regions of Nigeria.

Conclusion

The prospect of using sterile male *Oryctes monoceros* releases, combined pheromone based trapping and microbial infection (capture-infect-and Release Method) as inputs in the Integrated control of *Oryctes monoceros* and other major pests in Nigeria is an area worth investigating. Studies on using Gamma irradiation in reducing the rate of infestation of insect pests of date palm fruits (in bulk storage) also needs to be investigated, for Area-wide, insect control in Nigeria.

Table 1: Mean Numbers of *Latoia viridissima* per frond sampled in Field 34 in NIFOR, from 2003-2004 (10 palms sampled on each occasion)

Date of survey	Mean no. of larvae	Mean no. of cocoons	Remarks
Aug.2003	0.9	6.9	
Nov.2003	0.8	3.2	
Jan. 2004	10.1	6.0	
July 2004	0.0	4.3	Mainly mummified cocoons due to attack of microbial pathogens.

Table 2: *Oryctes monoceros* Handpicking Recoveries from Axils of coconut fronds in NIFOR field 63 from June to Nov. 2003.

Month	No. of coconut palms surveyed	No. of Live *O. monoceros* recovered
June	10	1
July	10	3
Aug	1	1
September	10	1
Oct	24	4
Nov	24	1
Total	79	11

Table 3: No. of Oryctes monoceros recovered per Row of coconut palms surveyed in Badagry from 1/9 – 1/9/2003

Rows	1	2	3	4	5	6	Total
No. of insects recovered	0	3	4	4	0	2	13
Total No. of palms	19	17	18	17	19	13	103

Plate 1: Ethyl Chrysanthemumate trap set up on date palm experimental site Dutse

ACKNOWLEDGEMENT

I am grateful to the IAEA conference organizing secretariat for the award of travel grant that enabled me attend the conference. I also thank the Executive Director, NIFOR, for Institutional support.

Plate 2: Typical destructive effect of *Oryctes monoceros* on Coconut palms in Nigeria

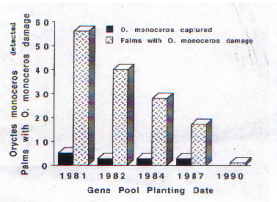

Fig.1: Histogram of *Oryctes monoceros* detected and date palms with signs of *O. monoceros* damage at Dutse from June to December, 1993.

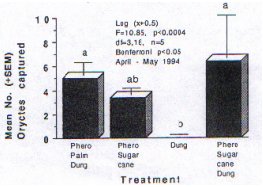

Fig.2: Oryctes catches at Dutse using 9mg/day pheromoe date palm frond pieces, sugarcane, cured cow dung impreganated with sevin 1gm/liter

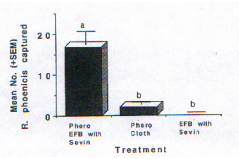

Fig.3: R. *phoenicis* catches using pheromone 3 mg/day with EFB or cloth impregnated with sevin 1g/L ANOVA F=20.68, p<0.0002;df=2,11 Bonferoni p<0.05 N=5 Feb-May 1994

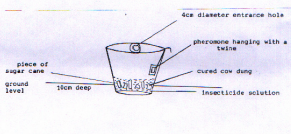

Fig.4: 4L white plastic bucket, containing pheromone and organic matter, immersed in insecticide solution, and partially buried in the ground

IAEA-CN-131 105P

Lufenuron in solid Baits as Chemosterilant traps against *Ceratitis capitata* (Wiedemann)

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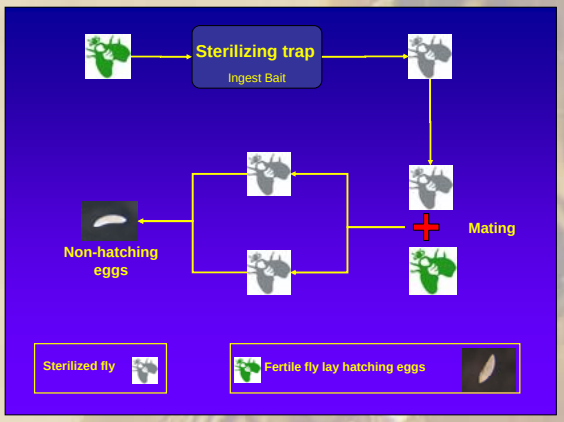
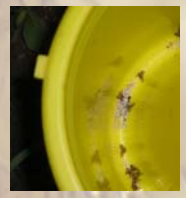
INTRODUCTION

Lufenuron prevents medfly egg hatching when females ingest a bait with 0.1% of lufenuron. Moreover, females that mated with lufenuron treated males (0,5% a.i. in diet) lay non-hatching eggs, in laboratory experiments.





MATERIALS AND METHODS

Sterilizing trap:

The gel is a protein bait that contains a 3% of lufenuron. The gel was introduced in a 9 cm diameter petri dish, with around 80 ml of gel per dish. The petri dishes with the gels were placed in delta traps, which were suspended on the south-east face of trees, 1.5 metres above ground (24 traps per Hectare). This bait remained in the field inside the trap during the whole season. Inside the trap we placed a trimedlure dispenser and a female "amine-lure" in order to attract flies from large distances.

Trial fields:

The check field was treated aerial with malathion+protein bait. Trials were carried out in:

1) A citrus orchard located in an isolated valley "Casella Valley" (Alzira, Valencia, Spain) with sweet oranges and mandarins cultivated on over 80 ha. This trial has run during 4 years.
2) Citrus, peaches and persimon are the most important grown on 3.600 ha in the south of Valencia at Carlet-Alcudia.

Evaluation:

The Medfly population was monitored with 80 McPhail plastic traps in the 80 Hectares treated with lufenuron and 11 in the check area (one trap per hectare trap grid). A Russell plug of trimedlure and a DDVP strip were placed inside the trap.

New sterilizing traps:

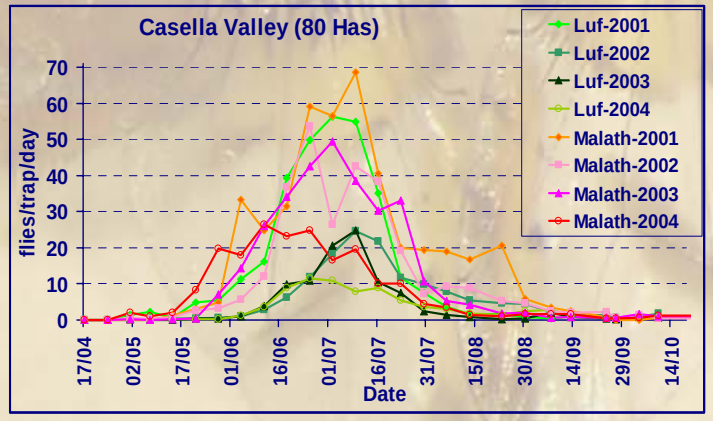
Using a new trap design in 2004, a cylindrical body covers the protein gel which contains the lufenuron. Attractants are enclosed in the cylinder that fix the dish with the cover.

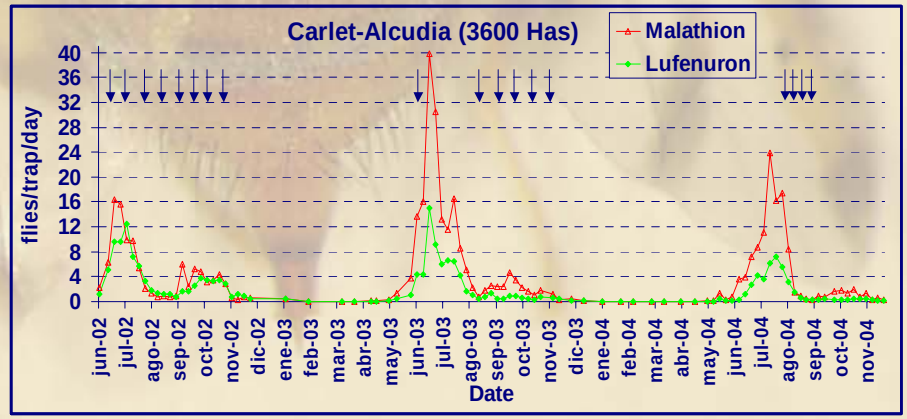
RESULTS

Casella Valley: Medfly population decreased year after year with lufenuron treatments. In four years the annual maximum population was reduced from 57 flies per trap and day in 2001 to 11 flies per trap and day in 2004. Medfly population in the check fields was not reduced in 3 years of aerial treatments. Second year population reduction was near 50% when compared with malathion aerial treatments. In the third year the medfly population was reduced by 60% when compared with malathion treatments.

Carlet-Alcudia: In the first year of lufenuron treatment no medfly population reduction was produced in comparison to the malathion treatment. In the second and third year the reduction was nearly 60% of the Medfly population. The Medfly population reduction was progressive and accumulative with the years.

In 2005 a 100 Has field will be treated with SIT and chemosterilization. In 2006 we will obtain the results of combine the two methods.





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The fruit fly exclusion programme in Chile

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INTRODUCTION

Chile is a fruit fly free country, no species of the genera *Anastrepha, Bactrocera, Ceratitis, Dacus, Toxotrypana*, etc., exist in the country. Natural Isolation given by desert, mountain ranges and ocean help to hamper pest entries. Nevertheless, the country faces a permanent biological pressure which results in Medfly entries almost every year.

Fig 1: Example of isolation Los Andes Ranges along the country.

II. Preventative measures

They are applied at three levels:

- Beyond the frontier, through binational agreements where joint work is carried out.
- At the border, through severe inspection of cargo and passengers
- Within the Chilean territory, with a national detection system, able to early alert any entry of the pest.

SIT for preventative purposes, applied in the province of Arica.

III. Detection system

A permanent trap network from regions I to XI, with 10,000 traps, baited with specific lures.

Target	Trap/attractant	Density
Medfly (males)	Jackson/ trimedlure	4 traps/km^2
Medfly (females)	Multilure/bioure	8 traps/km^2 *
Anastrepha	McPhail/protein hidrolisate	1 trap/km^2
Bactrocera dorsalis	Steiner or Jackson/Methyl eugenol	1 trap every10km^2
Bactrocera cucurbitae	Steiner or Jackson/cuelure	1 trap every 10km^2

*: currently in use only in SIT areas
Fruit sampling is conducted as a supplement.

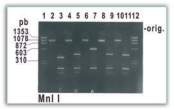
Fig 2: Upon detection the response plan is triggered.

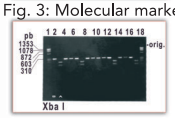
IV. Response Plan

- Single detection: (one male or unmated female): Intensive surveillance is undertaken 64 km^2 from the finding site, for two insect generations.
- Multiple catch: (two or more specimens, one mated female or immature stages): The area under surveillance is increased to 196 km^2, control is applied to all the developmental stages, quarantine is applied in regulated areas of 7.2 km radius. Eradication is declared after three cycles without detection.

V. DNA Analysis

To ascertain the most likely source and determine risk pathways, mDNA analysis is done to the specimens detected. The resulting haplotype helps with this analysis. Four molecular markers are used: Eco RV; XbaI; Mnl I and Hae III





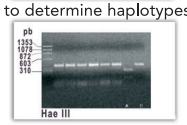


Fig. 3: Molecular markers to determine haplotypes.

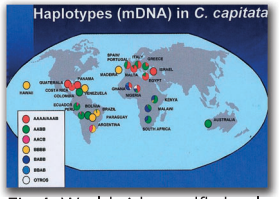


Fig 4: Worldwide medfly haplotypes distribution

VI Medfly situation, year 2005

The current year, three outbreaks have been detected in Chile, located in urban areas of Los Andes, Santiago and Rancagua. The response plan was triggered and to date, populations dropped to zero detection. All the three outbreaks belong to different haplotypes.

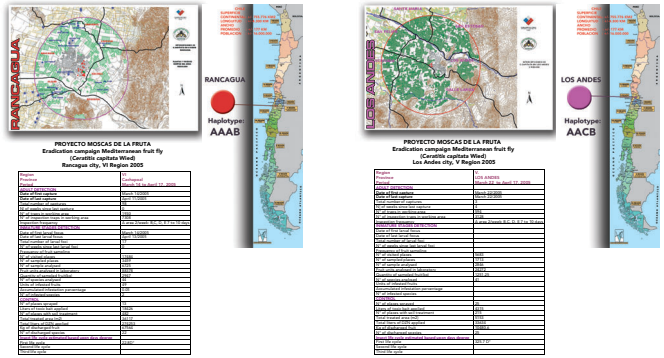


Fig 5: Medfly detection in Chile and regulated areas established, year 2005.

VII Fruit flies research

Through an agreement with a private foundation, applied research on fruit flies detection and control is being conducted since 2003.

Fig 6: Detection and control assays conducted in partnership with private sector.

VIII The use of SIT

As a PRP strategy, sterile medfly males are released twice a week in the province of Arica. After the eradication, achieved in November 2004, the SIT continued to be applied.

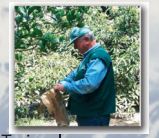


Fig VII: The use of SIT air releases.

Inhibition of responses to the pheromone of the European corn borer *Ostrinia nubilalis* as a prospective strategy in insect control

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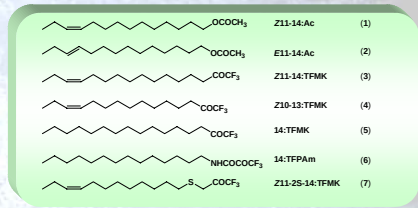
1. Introduction

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), is a major pest of maize and other crops, such as potato, green pepper, and winter wheat, in Europe, North America, North of Africa, the Philippines, and Japan. The species displays polymorphism in the pheromone communication system and, thus, the Z strain uses a blend of (*Z*)-11-tetradecenyl acetate (**1**) and (*E*)-11-tetradecenyl acetate (**2**) in 97:3 ratio, whereas the E strain utilizes the same compounds in blends from 1:99 to 4:96 ratios[1]. Trifluoromethyl ketones (TFMKs) are known to inhibit a number of enzymes, particularly, the antennal esterases of insect olfactory tissues[2]. We present herein the activity of compounds **3**, **4**, **5**, **6** and **7** as possible pheromone antagonists in wind tunnel bioassays, in the field and as esterase inhibitors of the Z strain of the insect[3].



2. Wind Tunnel Assays

Z11-14:TFMK exerted a significant antagonist effect when mixed with the pheromone in a 5:1 ratio, in which only 25% of the insects were able to contact with the lure (fig. 1). The antagonistic effect of Z11-14:TFMK became evident when a flight track of a moth flying to a source containing a 1:2 mixture of pheromone and antagonist was video recorded (fig. 2). Z10-13:TFMK needed a 10:1 mixture with the pheromone to reduce the number of contacts to only 7%.

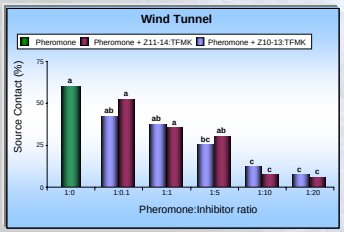


Figure 1. Plot of behavioral responses of *O. nubilalis* males flying towards a source baited with mixtures of pheromone and Z11-14:TFMK or Z10-13:TFMK in several ratios in a wind tunnel (N=40)

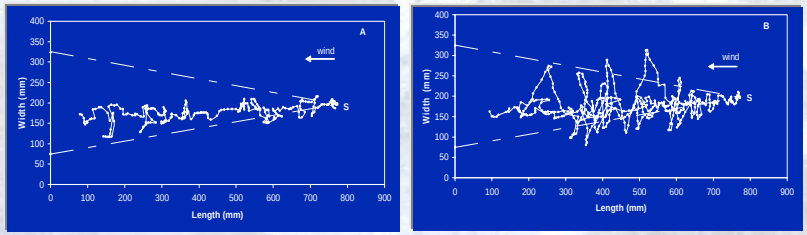


Figure 2. Representative flight track of *O. nubilalis* males flying towards a dispenser containing a 1:2 mixture of pheromone blend and Z11-14:TFMK (**B**) relative to control (**A**). White dots represent insect positions at 0.04s intervals

3. Field Tests

The activity of the antagonists in the field was evaluated by comparing the number of males caught with mixtures of the chemicals and the pheromone relative to those trapped with the pheromone alone. In tests carried out in 2001 and 2003, Z11-14:TFMK induced a significant reduction of catches when mixed with the pheromone in a 10:1 mixture. In a similar bait, Z10-13:TFMK also reduced the number of males caught but the effect was lower.

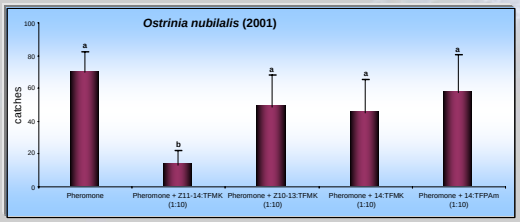


Figure 3. Number of catches of *O. nubilalis* males in traps baited with mixtures of compounds **3**, **4**, **5** and **6** and pheromone in a 10:1 ratio

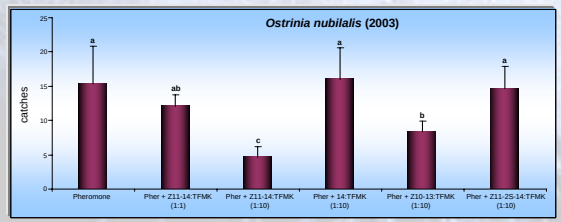
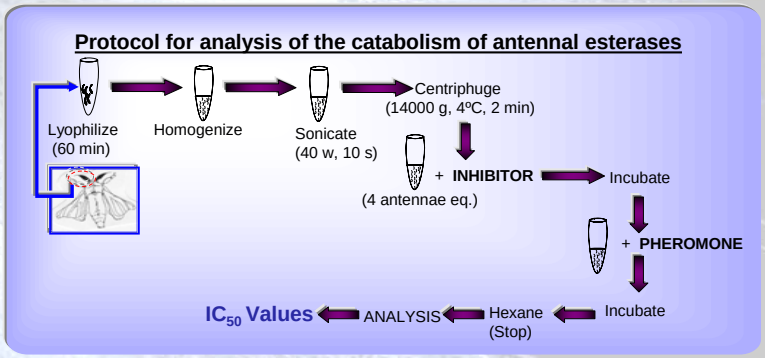


Figure 4. Number of catches of *O. nubilalis* males in traps baited with mixtures of compounds **3**, **4**, **5** and **7** and pheromone in a 1:1 and 10:1 ratio

4. Esterase Inhibition Assays

Z11-14:TFMK and Z10-13:TFMK were chosen as putative esterase inhibitors. The IC_{50} values displayed by both compounds were 0.28 μM for Z11-14:TFMK and 7.55 μM for Z10-13:TFMK. For Z11-14:TFMK, the most similar analogue of the major component of the pheromone, incubation of 1 ng on a 4-male antennae equivalents was sufficient to inhibit the total esterase activity of the extract by 40%.



5. Conclusions

Our results show that for the Z strain of the ECB only TFMKs closely related to the structure of the major component of the pheromone are effective antennal esterase inhibitors and good behavioral antagonists of the pheromone response in the laboratory and in the field. This paves the way to deeper disruptive studies of the chemical communication system of the insect to control the pest.

6. References

1. Peña, A.; Arn, H.; Buser, H.-R.; Rauscher, S.; Bigler, F.; Brunetti, R.; Maini, S. Tóth, M. *J. Chem. Ecol.* **1988**, *14*, 1359-1366.
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3. Riba, M.; Sans, A.; Solé, J.; Muñoz, L.; Bosch, M. P.; Rosell, G.; Guerrero, A. *J. Agric. Food Chem.* **2005**, *53*, 1158-1165.

ESTIMATION OF THE OLIVE FRUIT FLY – *BACTROCERA OLEAE* GMEL. (DIPTERA, TEPHRITIDAE) ATRACTANTS FOR AREA – WIDE MONITORING AND SUPPRESION PROGRAMS

IAEA-CN-131/108P

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MATERIALS AND METHODS

Experimental plots: The experimental plots were located along the area of Kaštela field near city of Split in central Dalmacija. The choosen plots are collection orchards of different olive cultivars, and same are possitioned in production area. The experimental plots are located 500 metars from the Adriatic sea, and 15-20 metars above sea, with slight southern exposition. The miksture of different domestic and introduced cultivars in orchards bring enough yeald, and create good conditions for olive fruit fly reproduction. For many years, there were no any control measures agains olive fruit fly.

Material: The tested atractants were: hidrolysed protein with trade name Buminal (Bayer, Hellas) in liquid form, which was used undiluted. The Buminal was applied through bait station made from 5 cm long, 1,5 cm diametar PVC tube with cotton wick inside in the ammount of 5 ml. The ammount of Buminal were refreshed once per month. Amonium salt – diamonium hydrogen phosphate (Pliva d.d. Croatia) was applied in solid form through the same bait station as described, and 2,5 grams were rolled in 7 cm diametar cotton patch. The complete part inside tube was refreshed once per mounth. Sexual pheromon (Isagro S.p.A. Italy) were applied in original ampules, which consist 1 ml of sinthetic pheromone. The old ampulew were replaced with new ones in monthly intervals.

The traps used were Populus sp. wooden boards, usualy used for apple boxes production. After cutting on 300 x 200 x 4 mm dimensions, the boards were kept on dry air over 60 days to lose fresh wood smell. The holes for bait stations were made in the middle of the traps proportionaly. Before aplication of the bait stations, the boards were treated with glue in sprey form (Soveurode Aerosol) on both sides. The glue were refreshed in monthly intervals and in few cases, when it was necesary, even before.

Methods: **Exp. 1:** PH : AM : HP; **Exp. 2:** HP: PH : HP+PH; **Exp. 3:** AM : PH : AM+PH; **Exp. 4:** AM+PH : HP+PH : AM+ HP; **Exp. 5:** PH+AM+HP : HP+PH : AM+PH : AM+HP. All experiments include five replications. Total capture number, number of males and number of females were counted. Estimation of single and join combinations of tested atractants were done through quantitative value analysis. Before analysis, the data were transformed with √x+0,5. The datas were analysed with Tukey's HSD and values were represented with nontransformed numbers. In the same time, the qualitative values were showed by graphicone of flight analysis, separately for males and females on every experimental plot.

Legend: PH-Pheromone; AM-Diamonium hydrogen phosphate; HP-Hidrolysed protein.

RESULTS:

Fig. 1. Captures of B. oleae on wooden boards, Kaštel Štaflić-Koludrovac, year 2000.

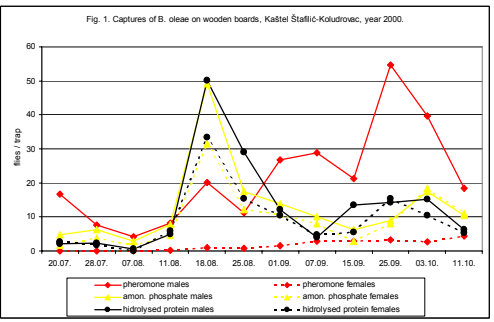


Table 1. Captures of the olive fruit fly – B. oleae between 20.07. and 11.10.2000. at Kaštel Štaflić - Koludrovac location.

	males	females	total	% females
PH	21,50 a	1,62 b	23,10 a	5,40 b
AM	13,40 b	9,88 a	22,50 a	39,30 a
HP	12,85 b	9,25 a	22,10 a	45,10 a
F	8,67	22,7	0,74	64,1
P	0	0	0,47	0

Fig.4. Captures of B.oleae on wooden boards, Kaštel Lukšić-Ostrog, year 2000.

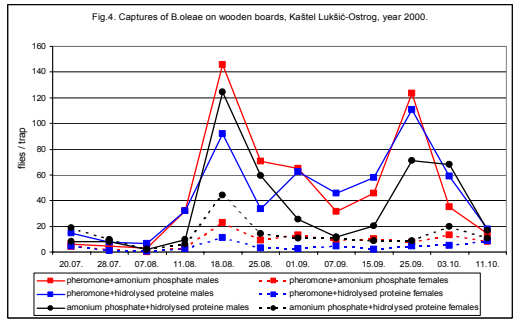


Table 4. Captures of the olive fruit fly – B.oleae between 20.07. and 11.10. 2000. at Kaštel Lukšić – Ostrog location

	males	females	total	% females
AM,PH	48,10 a	6,80 b	56,70 a	16,69 b
HP,PH	44,90 a	4,15 c	49,12 a	9,77 c
AM,HP	35,40 a	13,70 a	49,10 a	36,10 a
F	2,18	16,3	0,41	27,5
P	0,12	0	0,67	0

Fig. 2. Captures of B. oleae on wooden boards, Kaštel Štaflić-Rudine, year 2000.

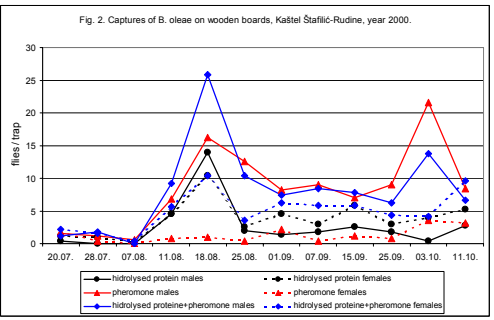


Table 2. Captures of the olive fruit fly – B. oleae between 20.07. and 11.10.2000. at Kaštel Štaflić - Rudine location.

	males	females	total	% females
HP	2,65 b	3,80 a	6,50 b	66,80 a
PH	8,50 a	1,32 b	9,80 a	14,90 c
HP,PH	8,22 a	4,98 a	13,20 a	42,90 b
F	17,8	21,7	8,3	42,6
P	0	0	0	0

Fig. 5. Captures of B.oleae on wooden boards, Kaštel Stari, year 2000.

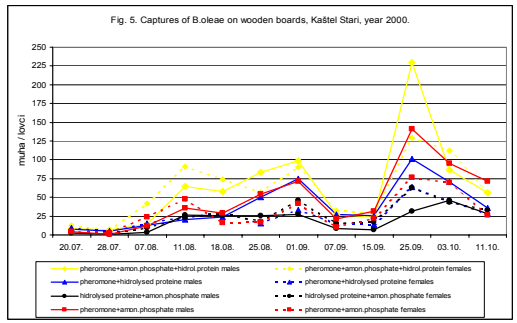


Table 5. Captures of the olive fruit fly – B.oleae between 20.07. and 11.10. 2000. at Kaštel Stari location.

	males	females	total	% females
PH,AM,HP	63,12 a	60,90 a	124,0 a	52,60 a
HP,PH	37,90 b	24,60 b	62,50 b	41,60 b
AM,HP	14,30 c	25,10 b	44,40 b	59,00 a
AM,PH	47,30 a	29,80 b	77,00 b	42,10 b
F	11,8	17,4	13,7	12,4
P	0	0	0	0

Fig. 3. Captures of B.oleae on wooden boards, Kaštel Stari-olive collection, year 2000.

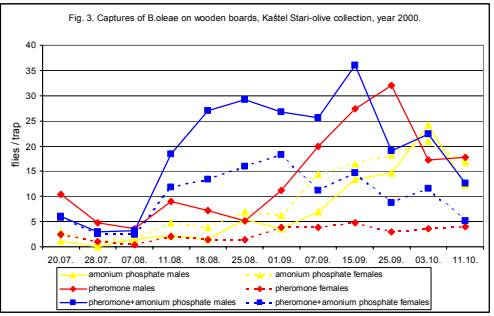
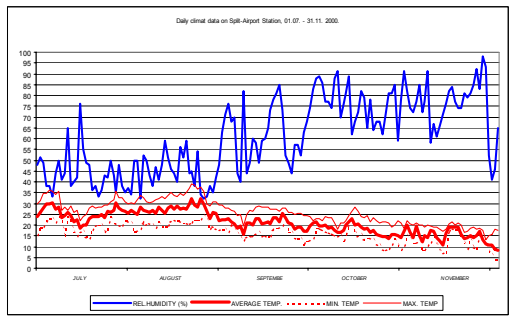


Table 3. Captures of the olive fruit fly – B. oleae between 20.07. and 11.10.2000. at Kaštel Stari – maslinarska stanica location.

	males	females	total	% females
AM	7,25 b	9,53 a	16,80 b	63,60 a
PH	13,80 a	2,63 b	16,45 b	16,80 c
AM,PH	19,10 a	10,13 a	29,20 a	36,00 b
F	25,9	37,8	14,1	12,7
P	0	0	0	0



CONCLUSIONS:

1.Atractant pheromone showes no difference from other two food atractatnts in values of total adult captures. Also pheromone showes significantly higher number of male capture, than other two atractants.

2.Atractant pheromone is most effective during the period of higher humidity conditions, specialy over 60 % rel. humidity, while under 30 % rel. humidity, it's efficasy decrise.

3.Join combinations of pheromone and diamonium hidrogen phosphate same as pheromone and hidrolysed protein, capture significantly higher total fly number than single values of each of tested atractants.

4.During the period of higher humidity conditions, join combination of pheromone and hidrolysed protein capture significantly more females than only pheromone.

5.During the period from end of August till third decade of September, when the capture on all tested combinations decriese, the join combination of pheromone and diamonium hydrogen phosphate showes highes capture efficasy.

6.The food atractants showes highest capture efficasy during the summer period of low humidity and high temperature conditions, specialy during August.

7.There is no significant difference between single values of diamonium hydrogen phosphate and hidrolysed protein and their join combination.

8.During September, join combination of two food atractants showes significantly lower capture, than join combination of pheromone with each two food atractants.

9.Join combination of three atractants, pheromone, diamonium hydrogen phosphate and hidrolysed protein, showes significantly higher captures than three join combinations of two atractantns.

10.The tested atractants showes single disadvantage in relations to climat changes during the season. Join action of each food atractant with pheromone fill up their disadvantages during the season.

11.Yoin combination of three atractants, showes the same qualitative values as yoin combinations of two atractants with great advantage of significantly higher quantitative values of three atractants combination.

12.Join combination of pheromone, diamonium hydrogen phosphate and hidrolysed protein was most effective in climat conditions of central Dalmacija regione and same could be used in monitoring and suppresion programmes.

Bicarbonate Production Inhibitors as a Novel Transmission-blocking Approach

IAEA-CN

131/109P

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Introduction

Plasmodium and *Leishmania* parasites propagate naturally in the midgut of female anopheline mosquitoes and sand flies in which the pH is slightly alkaline. It has been suggested that the enzyme responsible for generating the bicarbonate necessary to maintain this pH is carbonic anhydrase (CA). We have used molecular biology as well as pharmacology to study the role that CA plays in the development of *Plasmodium* and *Leishmania* parasites inside the female dipteran midgut with aims to interrupt their life cycle inside the vector.

We postulate that by inhibiting carbonic anhydrase activity in the midgut of these dipterans, we inhibit production and transport of bicarbonate, altering the pH maintenance mechanism in the midgut and depriving the parasites of this vital ion.

CA activity has been localized in the posterior midgut in Anopheline mosquitoes (where the blood meal is stored) using Hansson's histochemical method.

An. quadrimaculatus

An. albimanus

Sequence analysis of CA's from *An. gambiae* and *D. melanogaster* show high homology among dipterans as the sequence below exemplifies. Homology facilitates the design of probes and markers

```
D.MELANOGASTER   MERILRGVMGYRNTTREQMVKEFQKVRDNPEPEAVFFTCMDSRMIPTRYTDTHVGDMFVV
AN. GAMBIAE      MERILRGVMGYRHTTREQMVQEFRKVRDNPQPKAVFFTCMDERMIPTRFTETHVGDMFVV
                 ************.*******.**.*******.*****************.* *********

D.MELANOGASTER   RNAGNLIPRAQH---FQDEYFSCEPAALELGCVVNDIRHIIVCGHSDCKAMKLLYQLKDP
AN. GAMBIAE      RNAGNLVPHAEH---FQDEYFSCEPAALELGCVVNNIEHIIVCGHSDCKAMNLLYKLKDP
                 ******.***.*   ******************** * ******************.*.**

D.MELANOGASTER   EFASKLNKRLSPLRSWLCTKANTSLEKFQEWKDAGMEDFIFFSETPLRKFVAYIDSEKK
AN. GAMBIAE      EFASLDNKRISPLRAWLCEKANTSLAKFQNLKEIGLDKPLIFSSETPLRKFVAYIDPENN
                 ****  ***.****.*** ****** ***   *.  *. ********.****** *

D.MELANOGASTER   FAIEDKLSQINTLQQMENIASYGFLKARLESNDLRHIHALWFDIYTGDIYYFSRGAKQFIA
AN. GAMBIAE      FAIEDKLSQVNTLQQIENVASYGFLKRRLESNDLRHIHALWFDIYTGDIYFFSRNSKRFIA
                 *********.*****. *.******* ************************.***. * ***

D.MELANOGASTER   VDEDSVDRLGAEVRRFYS
AN. GAMBIAE      IDESSIDRLLDEVRRYYS
                 .** *.***  ****.**
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In situ hybridization with probes designed to localize this particular CA in *An. albimanus* and *L. longipalpis* adult female guts showed signal in the posterior midgut in both species.

An. albimanus adult female gut

L. longipalpis female adult gut

CA inhibitors such as methazolamide and acetazolamide have an effect on the pH inside the midgut in Anopheline mosquitoes

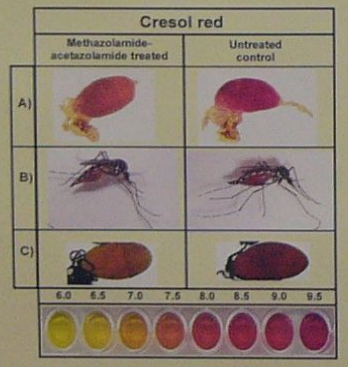
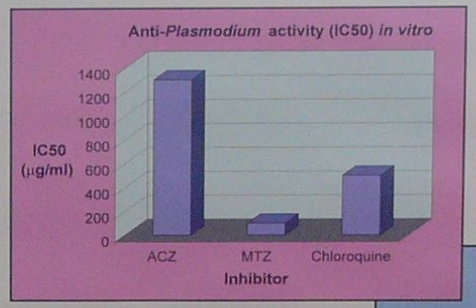
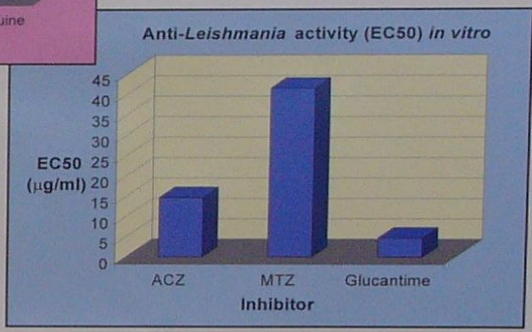


Figure by Maria Isabel Salazar, Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado,USA

Anopheline adult female mosquitoes develop fewer oocysts in the midgut in the presence of CA inhibitor.

Final MTZ Molarity (M)	Average oocysts	% decrease in number of oocysts developed with respect to the positive control	(P value) with reference to the FBS control	(P value) with reference to the positive control
10^{-4}	41.04	73.3	0.43*	3e-10
10^{-5}	18.71	87.8	0.04	0
10^{-6}	17	88.9	0.02	0
10^{-7}	15.12	90.16	6e-3	0
10^{-8}	44	71.4	0.3*	3e-9
10^{-10}	43.76	71.5	0.43*	1e-6
FBS control	32.96	78.6		
Positive control for infection	153.7	0.0		





Conclusions

CA is present in the posterior midgut of adult Anopheline mosquitoes and sandflies.

CA inhibitors have an effect on the pH maintenance inside the midgut, interrupt development of *Plasmodium* and *Leishmania* parasites *In vitro* and interrupt development of *Plasmodium* parasites inside the midgut of Anopheline adult mosquitoes.

By inhibiting CA activity in the female mosquito or sand fly the transmission of *Plasmodium* and *Leishmania* parasites can be blocked.

This strategy combined with existing methods such as the sterile insect technique, will in turn allow the design of integrated methods to control the spreading of malaria and leishmaniasis in area-wide programs by targetting both males and females.

By taking advantage of the similarities observed in the physiology of the midgut of the mosquito and sandfly vectors, efforts can be significantly reduced in terms of cost and time invested.

Acknowledgements

Our gratitude to Dr. William E. Collins (CDC), Dr. JoAnn Sullivan (CDC), Dr. Socrates Herrera and Dr. Felipe Zamora (Instituto de Inmunologia del Valle) for their help with *In vivo* studies and Lynn Milstead for her help with graphics.

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Codling Moth Trans-Hemispheric Compatibility Studies and Effect of Long Distance Air-Freighting on Adult Longevity and Mating

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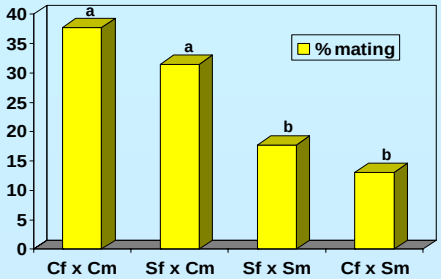
Stephanie Bloem, IAEA Consultant, USA
James E. Carpenter, USDA-ARS-CPMRU, USA

- The codling moth (CM), *Cydia pomonella* (L.), is the key pest in pome fruit orchards in South Africa.
- South Africa is interested in evaluating SIT for CM by conducting a season-long validation under local conditions.
- The time and expense of conducting this validation would be greatly reduced if CM produced by the SIR Program in Canada were found to be compatible with South African CM. The SIR Program produces 14-15 million moths/week and the mass-rearing facility is at full production for 6 months/year.
- Because the fruit growing seasons in South Africa and Canada occur at opposite times of the year, the SIR Program could provide CM to South Africa by maintaining year-round production. The SIR Program would benefit through more efficient use of their facility and from revenue from sales and South Africa would benefit by conducting the SIT validation before incurring the expense of building a mass-rearing facility and establishing a laboratory-adapted CM colony.
- We conducted codling moth compatibility studies in South Africa in 2003.
- Mating compatibility was assessed in the laboratory between CM from Canada (CAN) (hand carried via commercial airline) and wild CM from South Africa (SAF).
- We found that CAN CM males were equally attracted to "calling" females from CAN and SAF, despite the fact that CAN CM were transported from Canada to South Africa (about 50 hours in transit) and were between 1-2 days of age at the time of transport.
- Release-recapture field studies showed that at lower temperatures CAN CM females and males were more active than the SAF CM.
- Furthermore, CAN and SAF CM males were equally attracted to CAN and SAF females used as bait in traps.
- In 2004, 4 consignments of CAN CM were air-freighted to South Africa and differences in adult longevity before and after shipping were assessed. Even though transport required as much as 72 hours, we found no effect of transport on longevity. Mating ability after transport also was not affected.
- As such, a season-long SIT validation with imported CAN CM in South Africa should be possible and we hope to initiate this study during 2005.
- Since our studies were conducted with CM from very different time zones and hemispheric locations, it is quite probable that populations of CM from other pome fruit production areas also will be compatible with CAN CM.

Mating Compatibility

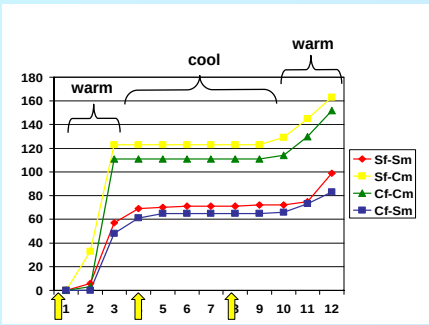
Small cages received 25 CM pairs. Treatments were as follows:

- SAF ♀ + SAF ♂
- SAF ♀ + CAN ♂
- CAN ♀ + CAN ♂
- CAN ♀ + SAF ♂

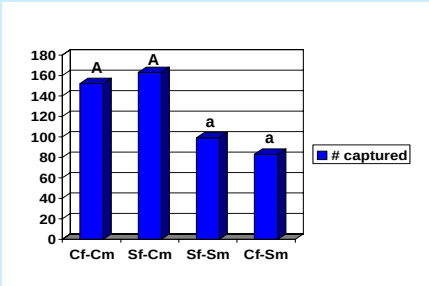


CAN males (Cm) mated equally with both CAN and SAF females (Cf & Sf). CAN males mated with significantly more SAF and CAN females than did SAF males (Sm).

Release/Recapture

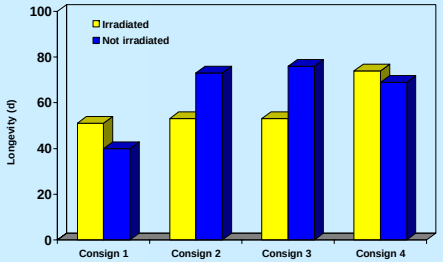


In the field, **cumulative captures** showed that CAN (Cf) and SAF (Sf) females were not significantly different in their ability to attract CAN and SAF males (Cm & Sm) over a wide range of environmental conditions. Both CAN and SAF males responded equally to calling females in the field irrespective of their country of origin. The number of CAN males captured in traps was always higher because large numbers of CAN males were released in the field (arrows indicate release dates). All captures of SAF males represent wild males that were present in the field.



Long Distance Air-Freighting

Adult CAN CM were collected in Petri dishes, packaged in insulated boxes, and air-freighted to South Africa. Visual inspection upon arrival indicated that CM quality was not affected by transport.



Laboratory assays showed that the longevity of CAN CM was not affected by air-freight transport from Canada to South Africa.

Genetic mapping of Z chromosome and identification of W chromosome - specific markers in the silkworm, *Bombyx mori*

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A genetic map of RAPD, SSR and FISSR markers for the Z chromosome- only 2% of molecular information relative to its size being available till date- was constructed using a backcross mapping population. Sixteen Z-linked markers were identified, characterized, and mapped using *od*, a recessive trait for translucent skin as an anchor marker yielding a total recombination map of 334.5 cM. Four RAPD and four SSR markers that were linked to W chromosome were also identified.

The Z chromosome harbours genes for various phenotypic traits expressed in the egg, larva and moth, such as Giant egg (Ge), translucent larval skin (*od*), late maturity (*Lm*) genes, which affect voltinism, moltinism, and quantitative traits such as cocoon weight and cocoon shell weight.

➢Characteristics affecting reproductive isolation and host race formation appear to be predominantly sex-linked in many groups of lepidoptera.

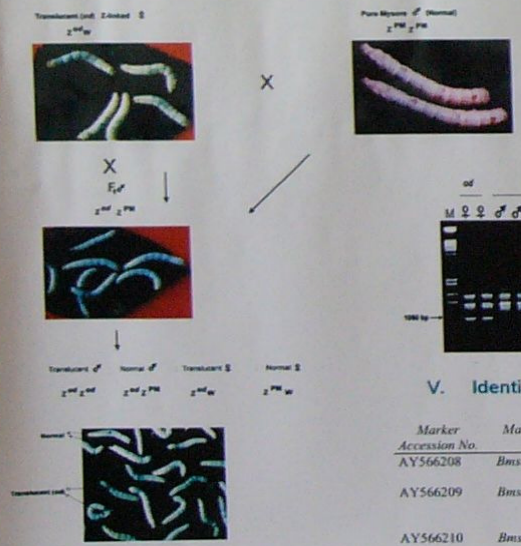
➢Analysis of Z-linked genes especially those controlling maturity, diapause, body size etc., in silkworm will help in evaluating the role of these traits in speciation and evolution.

➢Analysis of the repeat content and the interspersed elements on the Z chromosome and their distribution across the chromosome would help evaluate the status of dosage compensation for genes other than those reported earlier.

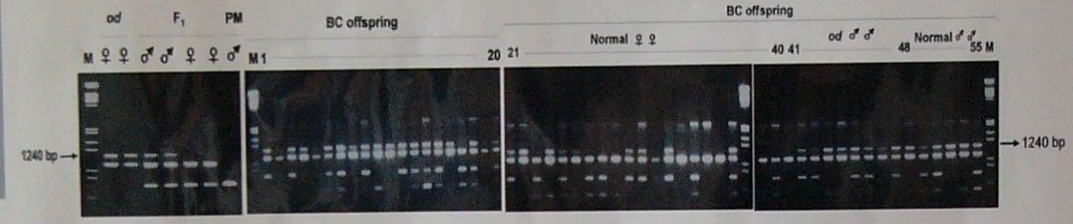
I. Mating scheme for raising the backcross population for the Z chromosome mapping study

	Translucent (od) derived						*Pure Mysore derived*	
	Genotypes		*Z linked*		*W linked*		*Z linked*	
Crosses	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Parental lines	$Z^{PM}Z^{PM}$	$Z^{od}W$	-	+	-	+	+	-
F_1	$Z^{od}Z^{PM}$	$Z^{PM}W$	+	-	-	+	+	+
BC	$Z^{od}Z^{PM}$	$Z^{PM}W$	+	-	-	+	+	+
	$Z^{od}Z^{od}$	$Z^{od}W$	+	+	-	+	-	-

Note: *od*, translucent strain; PM, Pure Mysore strain; BC, back cross offspring of F_1 male mated to translucent (*od*) female; '+' indicates the presence and '-' indicates the absence of RAPD/FISSR-PCR/SSR band.



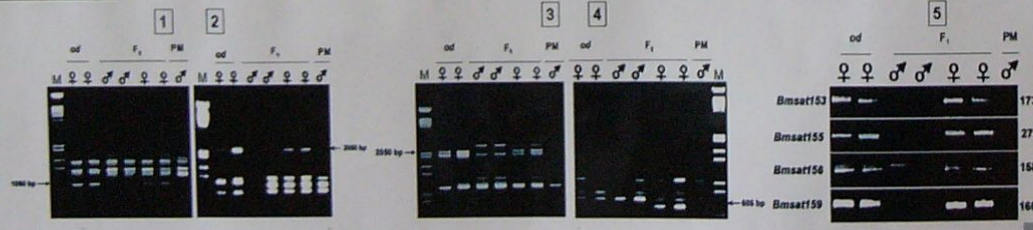
II. An example of inheritance and segregation of Z-chromosome specific RAPD markers generated by primer OPF-02.1240



III. Identification of Z- linked markers in *B. mori* WGS sequence contigs

Marker Acc No.	*Markers*	*WGS Contig*	*Homology (%Identity, Bit Score and E-value)*	*Genes and provisional function (Accession No.)*	*Species*
AY566197	OPU-14.920	BAAB01127123|contig52248	96% / 33aa, 67.0, 1e-09	AP-1γ (FBgn0030089)	*D. melanogaster*
		BAAB01021218|contig147849	No homology		
AY566199	OPR-09.1105	BAAB01048734|contig21903	45% / 68aa, 74.3, 3e-11	CG11851, mannosyltransferase activity	*D. melanogaster*
AY566198	OPG-06.1254	BAAB01209182|contig91100	51% /190aa, 198, 6e-49	ENSANGP00000020972 (XM_315297), unknown function	*A. gambiae*
		BAAB01116360|contig498225	No homology		
AY566201	OPT-04.992	BAAB01106426|contig476977_ 483697	33% / 941 aa, 520, e-145	Reverse transcriptase Osvaldo retrotransposon (CAB39733.1)	*D. buzzatii*
AY566196	OPF-02.1240	gbAADK01008490.1	No homology		
AY566200	OPT-14.583	BAAB01017829|contig1401	92% / 237nt, 319, 6e-83	Non-LTR retrotransposon and Bm1 retroposon	*B. mori*
AY566202	*Bmsat208*	BAAB01155602.1	100%/136 nt, 165,4e-38	*Bombyx mori Bmitin1, Bmitin2, Bmmiple, Bmsys6, BmPM-Scl, Bmsk; Bmsdv1-4* genes, partial and complete cds (dbjAB090308.2)	*B. mori*
AY566204	TA(CAG)$_4$	gbAADK01006369.1	No homology		
AY566203	*Bmsat95*	gbAADK01032857.1	No homology		

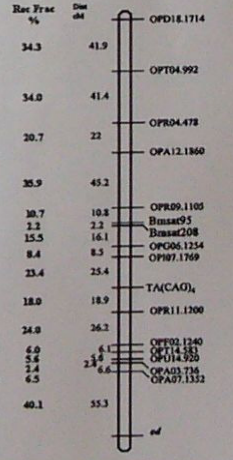
IV. Identification of W chromosome specific RAPD (1-4) and SSR (5) markers



V. Identification of W- linked markers in *B. mori* WGS sequence contigs

Marker Accession No.	*Marker*	*WGS contig*	*Homology (% Identity, Bit Score and E-value)*	*Identified Sequence (Accession No.)*
AY566208	*Bmsat153*	gi|54081682|gb|AADK01028030.1	98%/ 486nt, 658 and 0.0	*B. mandarina* Pao-like LTR retrotransposon Yamato DNA, partial sequence (AB055223)
AY566209	*Bmsat155*	gi|54071509|gb|AADK01038147.1	91%/ 62nt, 84 and 8e-13	*B. mori* Bm1 repetitive DNA element
			90% / 145nt, 168 and 2e-38	*B. mori* non-LTR retrotransposons on chromosome W
AY566210	*Bmsat156*	gi|54079028|gb|AADK01030684.1	95% / 2105nt, 1070 and 0.0	*B. mori* DNA, clone TREST1, partial cds
AY566212	*Bmsat159*	gi|54108487|gb|AADK01001225.1	No homology	

VI. Z-chromosome linkage map- based on the16 markers



SEX CHROMOSOMES IN THE SILKWORM

➢ The male is the homogametic sex (ZZ) and the female is heterogametic (ZW), with no crossing over.

➢ Female sex in silkworm is determined by the presence of a single W chromosome, regardless of the number of autosomes or Z chromosomes.

➢ The classical linkage map of the Z chromosome of *B. mori* contains 15 morphological traits dispersed over a distance covering 50 cM including a number of important traits of economic value.

➢ Recently, 18 Z-linked RAPD markers, and 13 additional genes in a contiguous 320 kb walk of the Z chromosome starting with the sex-linked marker, *Bmkettin*, were identified.

THE Z CHROMOSOME MAP

•The average spacing of the Z linked markers was 20 cM and the total map covered approximately 334.5 cM.

•All of the RAPD markers, except OPA-07.1352 showed linkage to the phenotypic translucent (*od*) marker within 55.3 cM.

•The relatively large gaps by the terminal markers have greatly expanded the total map distance reported here, where relatively high recombination rates were observed.

•The silkworm Z chromosome contains two fold more repetitive elements than autosomes similar to the X chromosomes in mammals and *D. melanogaster*.

•Since the silkworm is reported to lack dosage compensation, which calls for further analysis including many more Z-linked genes, it may be interesting to evaluate the functional significance of such enriched elements on Z-chromosome.

•Since only 2% of molecular information is known for the Z-chromosome, the markers identified in the present study will further aid in the assembly and analysis of Z-linked sequences.

FINANCIAL ASSISTANCE: DEPARTMENT OF BIOTECHNOLOGY, GOVT OF INDIA; INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA

FEASIBILITY STUDY OF USING STERILE INSECT TECHNIQUE (SIT) IN SUN-DRIED FISH INDUSTRY: TEST RELEASE AT SONADIA

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Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, GPO Box No. 3787 Dhaka

Test release at SONADIA
Situation and facts

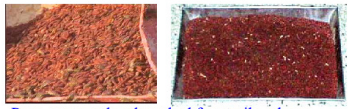
• Fish Drying • Blow Fly
• Fly Strike • SIT •Sonadia

• What we have done so far?
• What we need to do?

Blowfly larvae infesting fish at Sonadia

Fly Strike

•Fly strike cause about 25% quantity loss of dried fish, often with total quality loss
•Farmers use indiscriminate insecticides causing environment and health hazard.
•However the fly could not be reached totally with insecticides.
•Thus it has become a national issue for DOF(MOFL), DOE, MOH of GOB & for MFDA, NGOs



Pupa prouuced and marked for sterile release

Fish Drying

● About 1.2 million peoples are associated directly or indirectly with fish drying (catch, supply, drying and trade)
● About 30,000 Mt. Tons of marine dried fish is produced annually for export
●Inland consumption are much more (no proper estimation available)
● Trash fishes are lost Thrown away due to lack of safe drying technology
● Fish Drying is scattered sporadically among the coastal Islands (Sonadia,Kutubdia, Dublarchor, Ashar chor, Koakata ect.)

Blow fly

•*Lucilia cuprina* is a threat for fish drying at the coastal belt of the bay of Bengal.
• Female lay egg onto the fish & in 8-20 hrs maggot come out that eat up the fleshy part.
• It is a strong flyer, has high fecundity (100-300 eggs/female), 15 days life cycle,
• Rapid population growth, active throughout the year (except Nov. to Jan.)

Thirsty flies resting on wet shoes at high temperature

Sterile Insect Technique (SIT)

Components of SIT

• Major steps of are 1) Mass rearing 2) Radiation sterilization 3) Field release and 4) Assessment

• Each of the components need proper monitoring quality assurance, improvement by research

Sterile adults prior to release

•Our activity

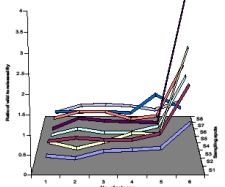
About 4.5 lakhs flies were **reared, radio-sterilized and released,** as test release in SONADIA island in 2002. Reduction in fertility was indicated by reduction in egg hatch.

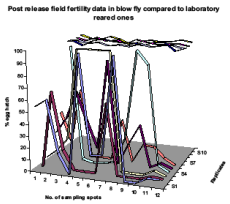
Another 15 lakhs has already been released during September to November, 2003 and in Feb. 2004.

No Wild fly was observed in the post release sampling

Requirement, Rearing facility needs to be upgraded introducing **thermostatically** controlled unit and improved **quality control** measure. **Mobile Irradiation** facility necessary to cut down operational cost, transportation damage.

Increase in the proportion sterile flies after sterile release





Mass Rearing Quality Parameters

%hatch	% Emergence	Mating Comp
Wild 95-99	90-95	1:1
Ster 35.2± 28.7 (0-100)	40.6± 20.1 (30- 70)	3:1, 9:1
Sterility dose 45 Gy		

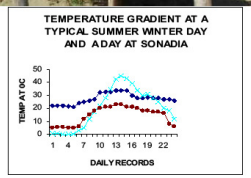
Blowfly egg mass in bovine liver

Dipping fish in insecticide solution

Off-season reservoir population

Pupa transportation plastic racks

General view of fish drying at the target island Sonadia



Different types of traps Sweep nets used in Blow Fly Sampling

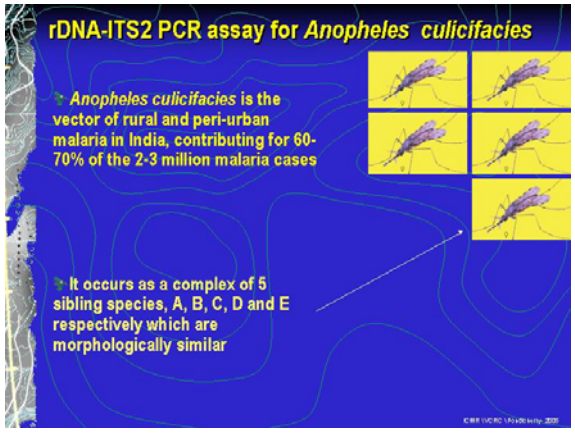
Map and over view of the Sonadia Island

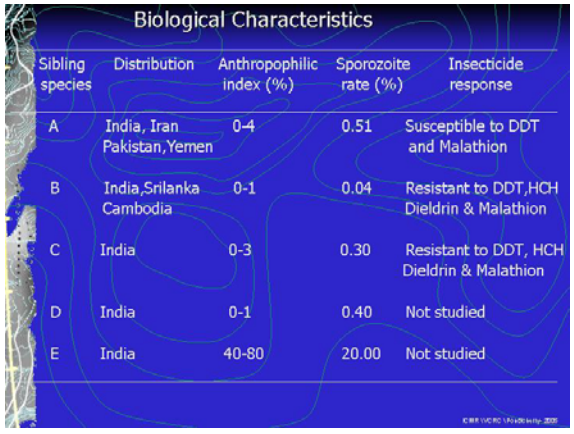
rDNA-PCR assay for the identification of 2 members of *Anopheles culicifacies*

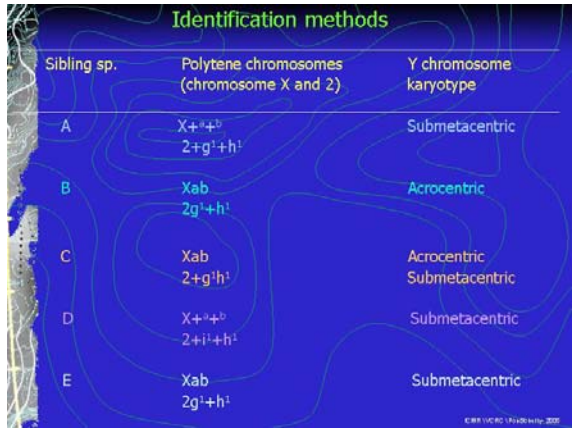
A.M.Manonmani, S. S. Sahu, C. Sadanandane and P. Jambulingam

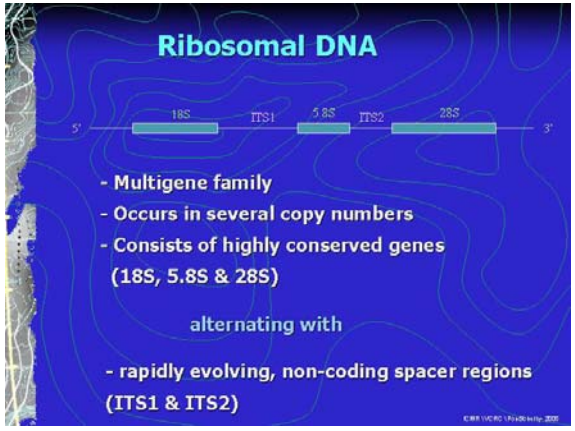
Vector Control Research Centre (ICMR)

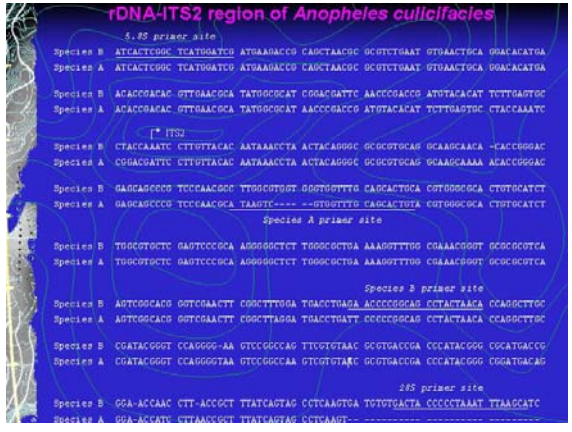
Pondicherry - 605 006 - INDIA

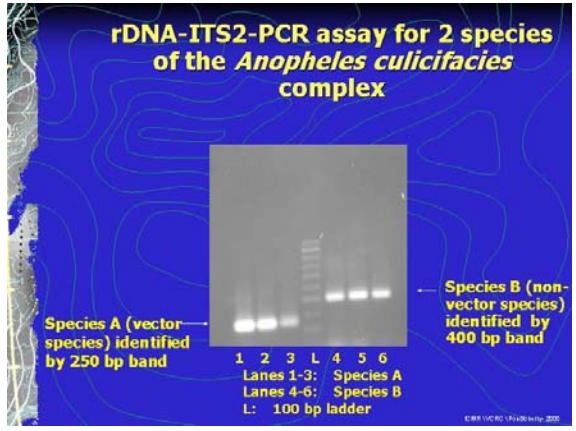


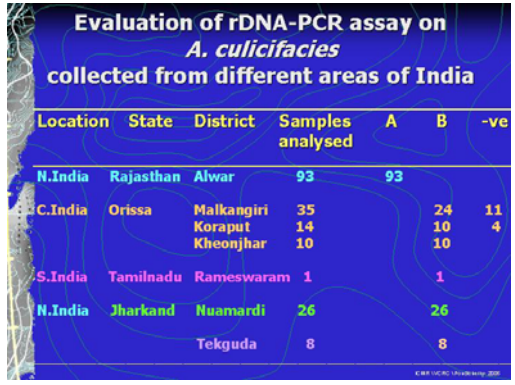


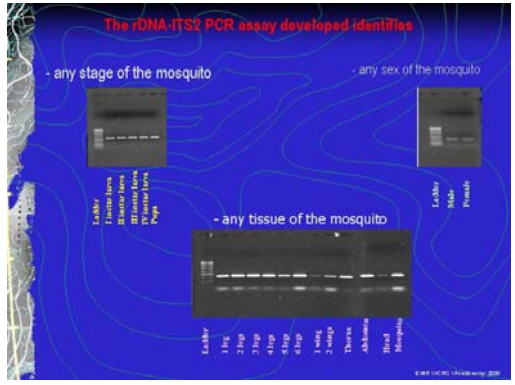


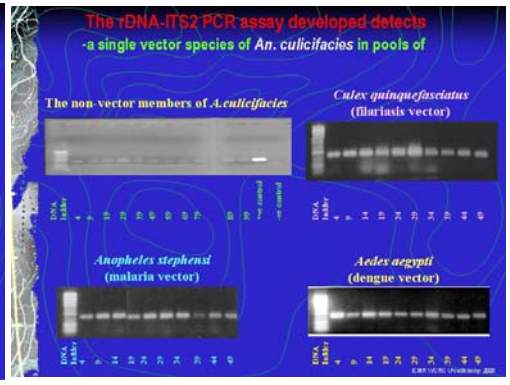












Application of the F1 Sterile Insect Technique (F1SIT) for Field Host Range Testing of the Tortricid *Episimus utilis*, a Candidate for Classical Biological Control of Brazilian Peppertree in Florida.

Onour E. Moeri[1], James P. Cuda[1], William A. Overholt[1], Stephanie Bloem[2], and James E. Carpenter[3]

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[2]Center for Biological Control, Florida A&M University, Tallahassee, FL

[3]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA

BACKGROUND

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), is a dioecious evergreen tree native to Brazil, Paraguay, and Argentina, that was introduced to Florida in 1898 as an ornamental (Austin 1978, Ewel et al. 1982) (Fig. 1). Currently, Brazilian peppertree is distributed widely throughout central and southern Florida, and is listed by the Florida Exotic Pest Plant Council as a "Category 1" invasive exotic species because it is altering native plant communities. It also has exhibited invasive properties in California and Hawaii as well as subtropical regions of at least 20 different countries (Ewel et al. 1982) (Fig.2).

In 1994, several natural enemies of Brazilian peppertree were imported into a quarantine facility in Florida as candidates for classical biological control. One of the candidates was a South American leaf-rolling moth *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) (Fig. 3 A,B). Larvae of *E. utilis* feed by scraping the surface of the Brazilian peppertree leaflets. As they mature, the developing larvae are capable of completely defoliating the plant (Martin et al. 2004) (Fig. 4).

Host specificity tests are used to determine whether or not a potential biocontrol candidate is safe to release in the field. Some biologists believe that these tests often overestimate host range, which leads to the rejection of acceptable candidates (Sands and Van Driesche 2000). As cage testing may inhibit normal behavior, open-field studies can provide a more realistic setting where insects can display an array of behaviors (Clement and Cristofaro 1995). However, open-field studies pose environmental risks in the area of introduction and are prohibited. Through the application of F1 Sterility (F1 Sterile Insect Technique), lepidopteran insects could be safely released temporarily for field host range testing.

Fig. 1. *Schinus terebinthifolius* leaves and fruit.

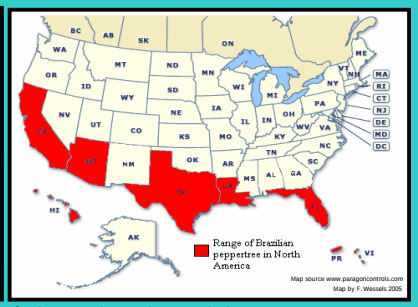

Fig. 2. States where BP is present in the U.S.

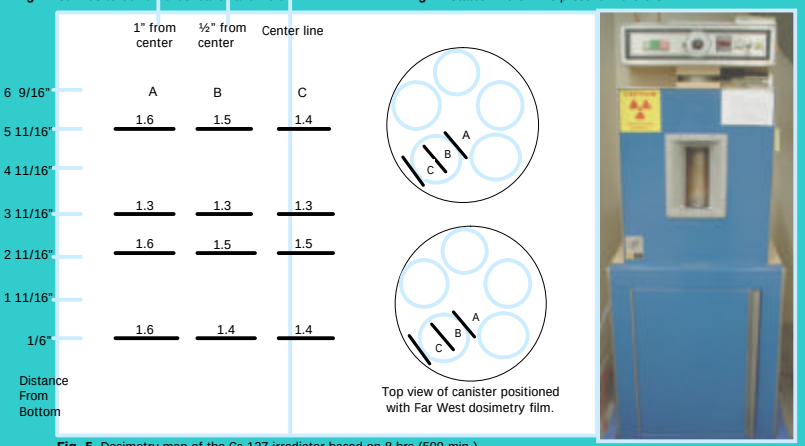

Fig. 5. Dosimetry map of the Cs-137 irradiator based on 8 hrs (500 min.).

Fig. 6A. Top view of irradiation canister.

Fig. 6B. Canisters used to irradiate moths.

Fig. 3A. Adult *E. utilis* male.

Fig. 3B. Larval stage of *E. utilis*.

Fig. 4. Plants showing larval feeding damage.

RESULTS

To date, the detailed radiation biology of *E. utilis* adults is not available. The main problem is acquiring sufficient numbers of adults of similar age. While these issues were being addressed, a dosimetry analysis was conducted at 8 hours (500 min.) using Far West dosimetry film. Values for each position of the Far West film were averaged and then positioned into a dosimetry map representing the Cs-137 irradiator (Fig. 5) (Fig. 6A,B). These values are based on a monthly decay correction factor of 0.998. Differences between the levels of irradiation from the innermost to the outermost position of the vial were not statistically significant (top mean = 6.8; top SD =0.7) ($P > 0.05$, 1-WAY ANOVA) (SAS Institute 2000). Levels of irradiation from the top to the bottom were significantly different (bottom mean = 7.2; bottom SD = 0.6) ($P < 0.05$, 1-WAY ANOVA) (SAS Institute 2000). To minimize the variance between the levels of irradiation, the canister will be positioned at the bottom of the irradiator.

FUTURE RESEARCH

The next phase of the research will involve a radiation biology study that will be based on similar research conducted with the codling moth, *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) (Bloem et al. 1999a,b). Male and female *E. utilis* adults will be collected (24-48 hr old) and exposed to gamma radiation individually in plastic vials within an aluminum lined cardboard canister. Doses of 0, 50, 100, 150, 200, 250, and 300 Gy will be administered by using a Cesium-137 Gammacell® 1000 irradiator with a dose of 13 Gy/min. After irradiation, each treated moth will be placed in a triangular waxed paper oviposition cage (20x12x10 cm) with either a treated or non-treated adult of the opposite sex. The moths will be allowed to mate and lay eggs for 2 intervals of 5 days. The females from each treatment will then be dissected to determine their mating status, i.e. that is spermatophores present in the bursa copulatrix (Ferro and Akre 1975). The oviposition cages will then be incubated for 7 days to facilitate egg development and larval eclosion. The total number of eggs laid (fecundity) and the total number of larvae eclosed (fertility) will be counted per cage for each treatment dose.

Sterility will be expressed as the percentage of eggs that failed to hatch. Ten replicates of each mating combination (i.e. treated female with non-treated male, non-treated female with treated male, treated female with treated male) will be completed for each treatment dose (based on Bloem et al. 1999a). Crosses of non-treated females by non-treated males will serve as the control. For the F1 phase of the study, the F1 larvae will be separated according to each dose and then reared to adults. Emerging adults will be outcrossed with untreated adults of the opposite sex. The sterility in the F2 generation can then be determined based on the collection and incubation of eggs following the same procedures described previously.

ACKNOWLEDGEMENTS AND REFERENCES

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Induced sterility by gamma radiation in Callosobruchus maculatus (F) and sterile insect release ratio to a normal population

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Abstract:

Effects of different gamma radiation doses on Callosobruchus maculatus (F) was studied. In this study doses including 0, 20, 35, 50, 60 and 702 Gy was used in pupal stage. Emerged adults were separated before mating and crossed in treatments including: Normal malexNormal female, Normal malexIrradiated female, Irradiated malexNormal female and Irradiated malexIrradiated female. Comparing means (Duncan, s test, 0.05) showed that in 20Gy dose, all the treatments had significant difference, except Normal malexNormal female and Normal malexIrradiated female. In 35 Gy dose, all the treatments had significant difference. The most sterility was observed in Irradiated malexIrradiated female and it was no significant difference with Irradiated malexNormal female. In 50 Gy dose, treatments which included Irradiated males or Irradiated females had significant difference with treatments containing normal males and females. In 60 and 70 Gy doses, hatchability percentage in the next generation, both for sterile males and sterile females, reduced population significantly. Therefore, 60 to 70 Gy induced sterility in this insect. Sterile insect release to a normal population was studied in three cases including: sterile males, sterile females and sterile males and females release. Results indicated that sterile females release had no effect on reducing population in the next generation. Sterile males release and sterile males and females release had identical effects. Considering to the fact that separating males and females is difficult and time consuming, therefore, it seems that sterile males and females release in the ratio of 10:10:1:1 (Irradiated malexIrradiated female× Normal malexNormal female) produce the best result in population reduction in the next generation.

Introduction:

Pulses with 20-30 percent protein after cereals are the main food source in the world, these crop are related to fabaceae family and papilionoideae sub family [2].

One of the important pests of these crops is a small insect with length of 2-5 mm with the name of Callosobruchus maculatus (fabricius), from Bruchidae family that the larvae will feed from black – eyed cowpea, chick – pea, mung, lentil, bean, etc.

From different variety of pulses, black – eyed cowpea are infested very much. In recent years because of this problem in some areas of Iran the farmers refuse to cultivate this product [1]. The mating duration of this insect is 3-6 minutes and once mating is sufficient to produce eggs, however mating happens a few times. Average life cycle of this insect from eggs to adults is 23-28 days [6]. Nowadays one of the effective and common methods for controlling stored pests is application of fumigants.

Some groups of such compounds may cause undesirable effects, specially on live tissues of fresh fruits and vegetables, and application of fumigants may destroy or decrease germination of some seeds, it should be mentioned the possibility and danger of explosion of some of fumigants too [1]. Methyl bromide is a world- wide fumigant. According to Montreal protocol, developed countries must stop usage of this pesticide due to ozone depletion properties till 2005 and also developing countries till 2015[4]. In US, in 1984, Ethylene dibromide consumption on food staff and agriculture products was banned because it seemed to be carcinogenic [3,8].

At the end of 1930 decade, Dr. E.F. Knipling presented usage of sterile insect release. This innovation presented when he was working on Cochliomyia hominivorax (coquerel). In this method, ionizing radiation is used to induce sterility. Although some chemicals have sterilizing properties, but most of them are carcinogenic and also incomplete sterility may happen. For this reason, it is impossible to apply chemicals in large scale.

Application of ionizing radiation is a physical method and it is possible to calculate the irradiation dose carefully. In this method insects are exposed to gamma irradiation. Irradiation is detrimental to genetically parts of sperms. When sterile males mate with normal females, copulation will happen but leads to no fertilization.

If sterile male release continues for generations, the insect population will decrease drastically [7].

Materials and Methods:

First, some mung seeds were put at 60ºC for 6 hours in oven for disinfestation [5]. Then a population of Callosobruchus adults were released on mung seeds for 24 hours to lay eggs. After that, adults were separated from the mung seeds and seeds containing eggs were put in incubator at 29±2 centigrade degree and 65±5 percent humidity for nineteen days. The mungs containing pupal stage were irradiated with doses 0,20,35,50,60 and 70 Gy in a gamma-cell at NRCAM. After two days emerged adults (males and females) were separated from the mungs before mating and placed in different containers. Experimental treatments prepared as follows: Normal male ×Normal female, Normal males ×Irradiated females, Irradiated males ×Normal females and Irradiated males ×Irradiated females. Each treatment included five pairs of these insects with 50 gram disinfested mung seeds. Every treatment replicated five times. After copulation and egg laying, dead adults were removed and laid eggs were counted. After 21 days, adults of next generation emerged, their population was recorded and by dividing number of adults to eggs, hatchability percentage was calculated. Release ratio experiment was executed in three conditions:

First, when there is a natural population of males and females and sterile males are released. Second, when natural males and females exist and sterile females are released. Third, when natural males and females exist and sterile males and females are released.

Considering first situation including Normal females × Normal males ×Irradiated males, the ratio was 1:1:6, 1:1:7, 1:1:8, 1:1:9, 1:1:10, 1:1:15 and 1:1:20. In the second situation including Normal females × Normal males × Irradiated females, the ratio was 1:1:6, 1:1:7, 1:1:8, 1:1:9, 1:1:10, 1:1:15 and 1:1:20. For the third situation including Normal females × Normal males × Irradiated males × Irradiated females, the ratio was 1:1:6:6, 1:1:7:7, 1:1:8:8, 1:1:9:9, 1:1:10:10, 1:1:15:15 and 1:1:20:20 [5]. Each treatment were put on 50 gram disinfested mung seeds and replicated five times. After mating and completing life cycle they were removed from the diet and number of laid eggs and hatchability was recorded. The experiment was done in a completely randomized design (CRD) and means compared on the basis of Duncan's test, 0.05 error level.

The experiment was done at Nuclear Research Center for Agriculture and Medicine (NRCAM) and irradiation facility was gamma- cell of 4100 Ci, Co60 source, dose rate of 0.64 Gy/s, installed at NRCAM, Karaj, Iran.

Results and discussion:

Comparing means (Duncan's test, 0.05) show that in 20 Gy dose, all treatments have significant difference, except for Normal male × Normal female and Normal male × Irradiated female treatments. Consequently, in 20 Gy dose irradiated and normal females acted similarly and no sterility had taken place.

In 35Gy dose, all treatments had significant difference. The most sterility was observed in Irradiated male × Irradiated female and did not show significant difference with Irradiated male × Normal female. Consequently, sterility was due to irradiated males than females. In 50 Gy dose, treatments which contained irradiated males or females had significant difference with treatments containing normal males and females. It means that in 50 Gy dose, the role of irradiated males and irradiated females for creating sterility is the same. This phenomenon was observed in 60 and 70 Gy doses. 60 and 70Gy doses could decrease percentage of emerged adults significantly both for irradiated males and irradiated females. This result is compatible with Howlader and Rahman (1995) reports. They claimed the range of doses for sterility of callosobruchus chinensis was 80-100Gy and it is a little more than callosobruchus maculatus and it is less than the other stored pests [5].

The results of sterile insect release ratio (70 Gy radiation sterility dose) to a normal population was studied in three cases as follows:

In the first situation only sterile males were added to normal population. The ratio of 1:1:6 till 1:1:20 was statistically the same and all of them had difference with control. In the second situation only sterile females were added to normal population. The result indicated that control and ratio of 1:1:9 was the same and produced high hatchability. The ratio of 1:1:6 was in the second rate of hatchability. Ratios of 1:1:7 and 1:1:8 ranked the third level of hatchability. The ratio of 1:1:20 ranked fourth and the ratio of 1:1:10 and 1:1:15 had minimum hatchability. The result did not show a logical relation to each other. Consequently sterile female release cannot be effective in decreasing insect population actively. In the third situation, sterile males and females were introduced to natural population. In this situation, all treatments which contained sterile males and female had significant difference with control. Among all release ratios except 1:1:7:7 did not have significant difference. The best release ratio was 1:1:10:10, 1:1:15:15 and 1:1:20:20, in which the rate of hatchability decreased significantly.

Due to the fact that release of sterile males alone in comparison with releasing sterile males with sterile females together have the same effect, consequently, releasing sterile males and females can be noted more in decreasing pest population, because in this situation there is no need to separate males and females and leads to save time and labour. To make the long story short, releasing sterile males and females (70 Gy) in the ratio of 1:1:10:10 leads to the best result to reduce the next generation population. This experiment showed that it is possible to reduce insect population in laboratory scale, although it should be done in large scale to understand whether it is applicable or not.

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Table 1: Effect of different radiation doses and mating situations in number of laid eggs, emerged adults and egg hatchability

Dose effect	Treatment effect	Mean egg laid ±SD	Mean emerged adults ±SD	Mean emerged adults in next generation ±SD
20 Gy	NmxNf	380.6 ± 42.3	186.2 ± 28.8	48.9 ± 5.7^{a}
	NmxIf	204.4 ± 34.9	94.8 ± 19.2	46.5 ± 6.6^{a}
	ImxNf	336.6 ± 24.0	50.2 ± 8.8	15.0 ± 1.8^{b}
	ImxIf	158.0 ± 83.8	9.6 ± 8.0	5.1 ± 4.7^{c}
35 Gy	NmxNf	322.4 ± 30.5	179.8 ± 35.3	56.5 ± 13.6^{a}
	NmxIf	91.2 ± 55.5	17.4 ± 11.7	21.0 ± 13.5^{b}
	ImxNf	362.8 ± 76.9	10.4 ± 6.7	3.6 ± 1.8^{c}
	ImxIf	66.8 ± 27.6	0.6 ± 1.3	0.5 ± 1.2^{c}
50 Gy	NmxNf	241.8 ± 29.0	118.8 ± 61.5	48.5 ± 24.8^{a}
	NmxIf	16.4 ± 10.3	1.4 ± 1.5	7.1 ± 7.0^{b}
	ImxNf	254.4 ± 52.5	24.2 ± 32.6	9.8 ± 13.0^{b}
	ImxIf	26.6 ± 18.7	0 ± 0	0 ± 0^{b}
60 Gy	NmxNf	322.0 ± 15.3	194.4 ± 90.7	62.3 ± 9.5^{a}
	NmxIf	49.8 ± 10.7	0 ± 0	0 ± 0^{b}
	ImxNf	446.2 ± 59.7	2.6 ± 2.7	0.62 ± 0.7^{b}
	ImxIf	89.6 ± 23.6	0 ± 0	0 ± 0^{b}
70 Gy	NmxNf	409.2 ± 61.9	146.8 ± 22.4	36.3 ± 6.3^{a}
	NmxIf	7.8 ± 6.2	0 ± 0	0 ± 0^{b}
	ImxNf	337.2 ± 47.1	0.4 ± 0.5	0.1 ± 0.1^{b}
	ImxIf	1.0 ± 1.2	0 ± 0	0 ± 0^{b}

Means having at least one common letter do not have significant difference at 0.05 level

Nm: Normal male *Im: Irradiated male*

Nf: Normal female *If: Irradiated female*

Table2: Number of produced eggs and hatchability percentage related to different release ratios

Treatments	Laid eggs ± SD	Adult emerged percent ± SD
Nf^{1} × Nm^{1}	44.6 ± 19.6	89.2 ± 6.4^{a}
Nf^{1} × Nm^{1}×Im^{6}	60.3 ± 12.7	6.2 ± 9.1^{b}
Nf^{1} × Nm^{1}×Im^{7}	73.3 ± 4.5	14.8 ± 11.3^{b}
Nf^{1} × Nm^{1}×Im^{8}	55.6 ± 3.0	8.4 ± 14.6^{b}
Nf^{1} × Nm^{1}×Im^{9}	55.6 ± 8.3	0 ± 0^{b}
Nf^{1} × Nm^{1}×Im^{10}	32.0 ± 28.1	10.8 ± 18.7^{b}
Nf^{1} × Nm^{1}×Im^{11}	61.6 ± 20.0	3.6 ± 6.3^{b}
Nf^{1} × Nm^{1}×Im^{20}	49.0 ± 22.3	3.2 ± 2.8^{b}
Nf^{1} × Nm	44.6 ± 19.6	89.2 ± 6.4^{ab}
Nf^{1} × Nm^{1}×If	110.3 ± 19.3	73.0 ± 13.2^{bc}
Nf^{1} × Nm^{1}×If	95.0 ± 4.6	70.0 ± 10.1^{cd}
Nf^{1} × Nm^{1}×If	91.6 ± 11.2	69.2 ± 8.4^{cd}
Nf^{1} × Nm^{1}×If	68.6 ± 5.0	92.6 ± 0.5^{a}
Nf^{1} × Nm^{1}×If	312.6 ± 125.7	6.4 ± 7.1^{e}
Nf^{1} × Nm^{1}×If	492.3 ± 96.6	9.8 ± 2.3^{e}
Nf^{1} × Nm^{1}×If	126.0 ± 60.7	54.0 ± 16.6^{d}
Nf^{1} × Nm^{1}	44.6 ± 19.6	89.2 ± 6.4^{a}
Nf^{1} × Nm^{1}×Im^{6} ×If^{6}	143.6 ± 31.6	7.8 ± 12.3^{de}
Nf^{1} × Nm^{1}×Im^{7}×If^{7}	113.0 ± 15.6	25.5 ± 18.0^{b}
Nf^{1} × Nm^{1}×Im^{8}×If^{8}	90.6 ± 14.6	15.6 ± 20.9^{de}
Nf^{1} × Nm^{1}×Im^{9}×If^{9}	77.0 ± 1.7	24.1 ± 7.1^{b}
Nf^{1} × Nm^{1}×Im^{10}×If^{10}	468.3 ± 54.0	0.5 ± 0.9^{e}
Nf^{1} × Nm^{1}×Im^{11}×If^{11}	392.3 ± 91.5	1.4 ± 2.3^{e}
Nf^{1} × Nm^{1}×Im^{20}×If^{20}	254.6 ± 62.0	2.6 ± 4.5^{e}

Means having at least one common letter do not have significant difference at 0.05 level

Nm: Normal male *Im: Irradiated male*

Nf: Normal female *If: Irradiated female*

IAEA-CN-131/130P

Experiments to Measure Sterile Male Medfly Dispersal and Longevity

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CLEANFRUIT
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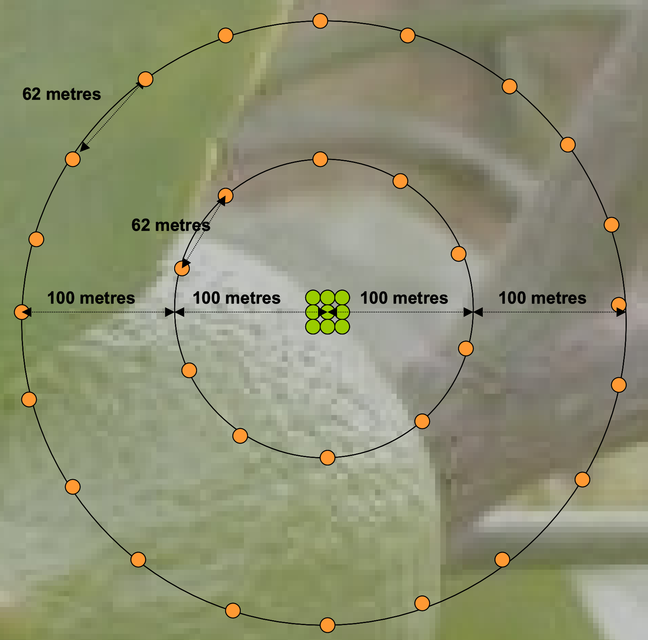
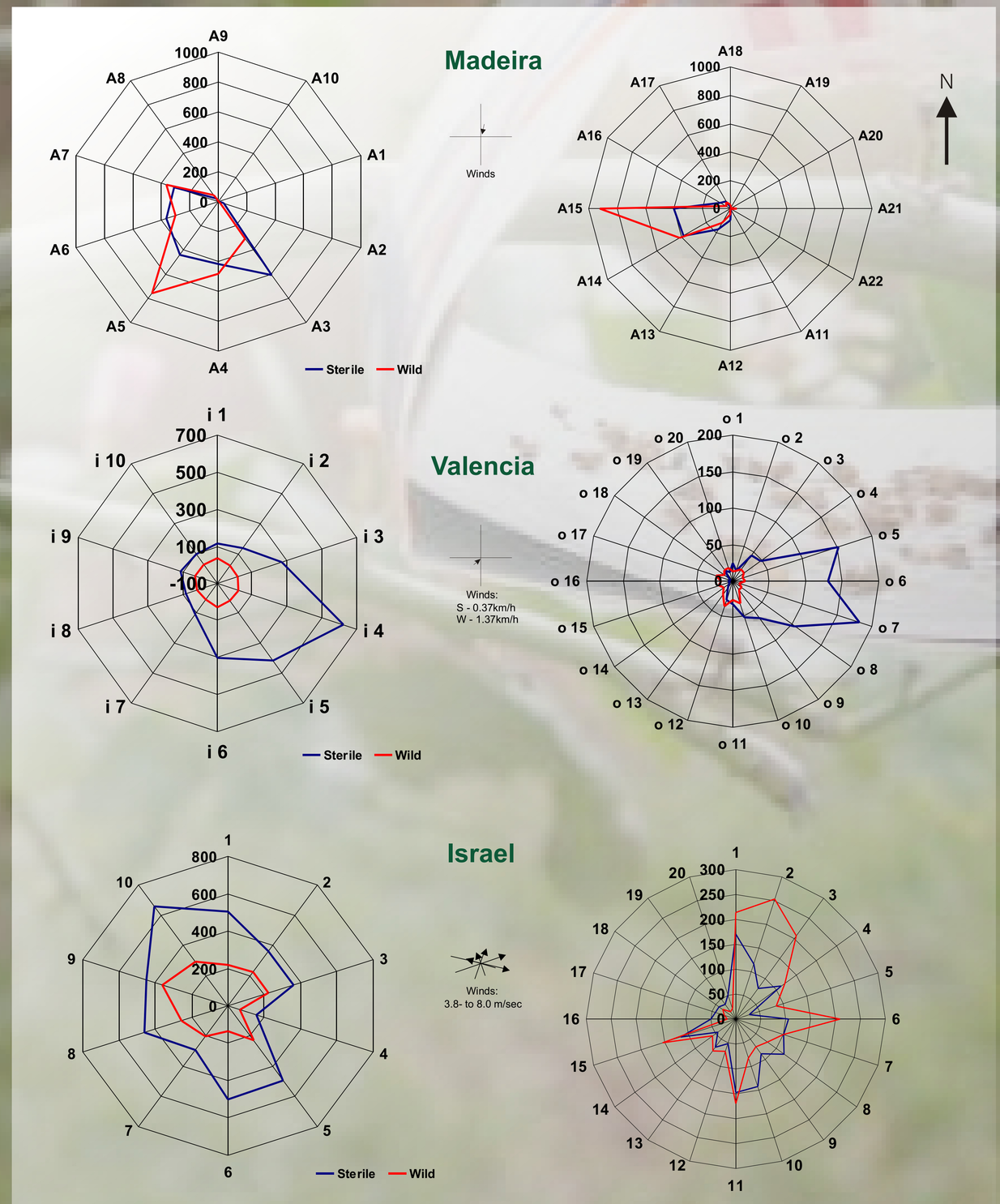
Introduction

As part of the Cleanfruit Project, a protocol to evaluate sterile medfly dispersion and longevity in field conditions was developed to be used in three regions: Madeira Island (Portugal), Valencia (Spain) and Hafetz Hayim (Israel).



Release: 100000 sterile males in a central point

Longevity: Flies with and without food and water in the field conditions.



Sterile and wild flies distribution in the field. Measurement using Jackson traps baited with Trimedlure distributed in two circles around.

Conclusions

The fly dispersion was not homogenous and usually shifted toward one direction. Generally the distribution in orchard of the sterile flies was coincident with wild flies.

The longevity indicated that temperature does affected the fly's longevity without food from less than 48h in warm temperature (Israel) to more than 4 days (Madeira and Valencia).

Food and water supply clearly increased the longevity from 6 days (Madeira and Valencia) to over 10 days (Israel).

Longevity (in days)

Places	Treatments		Orchard
	Food + Water	No food / water	Temp. Average
Madeira	14-15	6-7	18.0ºC
Valencia	14-15	2-3	18.3ºC
Israel	9-10	2-3	22.8ºC

Tsetse Suppression Techniques used in the Southern Tsetse Eradication Project (STEP), Ethiopia

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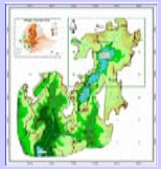
Background



- Project establishment- 1997/98
- Project area size - 25000km^2
- Location - southern rift valley of Ethiopia, between 4° 45' and 7° 15" N latitudes and 36° 40'and 38° 20' E longitudes.
- Institutions involved - IAEA, ESTC, SNNPR state.

Pre-suppression situation of T & T

- High disease prevalence, 17%.
- High tsetse challenge,Max. 69 f/t/d.
- Low animal productivity.
- Reduced draught power,< 2 hrs/day
- High treatment and control cost.
- Congested high lands and under utilized fertile low land areas

Past experience

1. Trap technology

- Introduced to Northeastern part of the STEP area (Bedessa), 1995 by ICIPE.
- Community based tsetse control Pilot project, involved ESTC, RAB, RVL, ICIPE Ethiopia, local government and the farming community.
- 300km^2 area was covered by odor baited NGU traps.
- 95% G.pallidipes population & 90% disease prevalence reduction was reported

2 **Insecticide treated cattle**

- Introduced by NGOs, 1990/91-pilot
- Through revolving fund system
- About 600km^2 area covered
- 99% G.pallidipes population and 95% disease prevalence reduction reported.

Challenges

- Only small area coverage, short lasting effect, temporary relief.
- Lack of coordinated activities.
- Sudden withdrawal of funding.
- Re-invasion.

Tsetse suppression in the STEP

NGU traps

- Baited with cow urine
- Strong community participation in trap deployment and maintenance.

- Strong government support
- Proved to be effective in reducing G.pallidipes population.

Practical problems

- Required frequent visit
- Large number of traps needed to be deployed, affecting farmers agricultural time.
- Reduced community participation at peak agricultural period
- Damage caused by domestic and wild animals, and wear and tear
- Loss of traps to theft, bush fire, vandalism, storms,
- Difficult to deploy in dense bushes and areas far away from settlement
- High professional input (re-visiting, deployment, maintenance, urine replenishment)
- Costly trap material and are cumbersome

Insecticide impregnated targets

- Blue-black-blue vertical panel
- Only the black portion impregnated (attract landing) with Deltamethrin 20% sc diluted to 0.8% concentration.
- Targets impregnated by brushing/paint rolling or dipping and then sun dried.
- Deployment by fixing to wooden and/bamboo pole or tree stem or hanging from branches of trees.
- Trained community members deploy targets (supervision and assistance from project staff).
- 4-5 targets deployed per km^2
- A total of 18,706 targets deployed in 3800km^2
- Proved to be effective to reduce G.pallidipes.

Practical problems

- Black portion fades soon (< 3months)
- re-impregnation was not possible.
- Loss of targets to theft, bush fire, vandalism, storms,
- Damage caused by domestic and wild animals, and wear and tear.
- re-visiting at least every three months
- Difficult to deploy in inaccessible areas.
- Visibility problem in dense and thick vegetation.
- Lack of protective clothing while handling the insecticides.

Insecticide-treated cattle

- Synthetic pyrethroides, Deltamethrin 1%S.C (ready-to-use formulation) and Deltamethrin 1%E.C (diluted with water at the spot of application to 0.005% concentration)
- 20% of the herd (old and larger animals) treated
- Trained spray technicians participate in treatment (supervision by project staff)
- T-bar applicator (for spot on) and Knapsack sprayer (for spray formulation)
- A total of 882,000 cattle (repeatedly treated every fortnight).

Practical problems

- Requires protective clothing (sprayers always complain about irritation and itching).
- Large amount of water required for dilution
- Spare parts of knapsack sprayer are delicate and easily breakable.
- knapsack sprayer are heavy to carry (15-20liters)

Monitoring

- NGU traps were deployed to see the impact of the suppression techniques on tsetse flies population. A significant reduction in fly population density from 4.11F/T/D during the pre-suppression to 0.31F/T/D during the suppression was detected.
- Disease monitoring was done using the buffy coat technique (BCT). This revealed a reduction in disease prevalence from 17%-7%.

Concluding remarks

- Three tsetse suppression techniques were on use in the STEP area. Traps were used at the beginning of the operation but due to cost factor and difficulty in management it was not continued.
- Insecticide impregnated targets have totally replaced the trap technology as they are cheaper,require less maintenance,more durable and easier to make. They are used in areas where cattle do not go.
- Insecticide treated cattle were used for the suppression of tsetse flies gained more reputation by community as they observe dramatic change in the physical performance and production of the animal.
- The combined use of targets and insecticide treated cattle seems more effective to knockdown tsetse population in short period of time. Thus, feasible for area wide insect pest management in the STEP area.
- Some of the STEP areas are not accessible and not reached with the existing techniques. Therefore, the use of alternative techniques like SAT is of great importance before the SIT application.
- Ensure sustainable supply of resources for the suppression to maintain the achievements and further make the area ready for SIT application.
- Strong support from the community, stakeholders and government at all levels was the key factor for the success of the ongoing suppression.

The *tryoni* complex of tephritid flies in Australia

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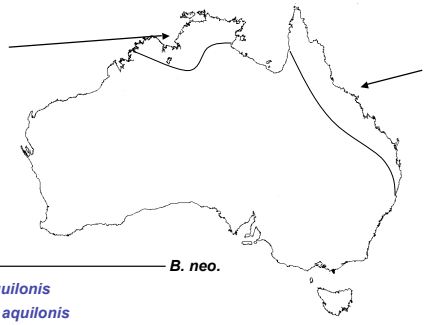
1. How many species

The traditional view is that *B. aquilonis* is a separate non-pest species. However, it is morphologically identical to B. tryoni. Is it a separate species?

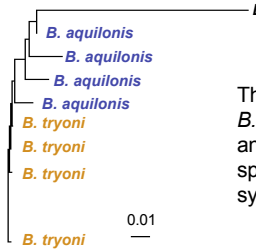
B. aquilonis

Non-pest species?



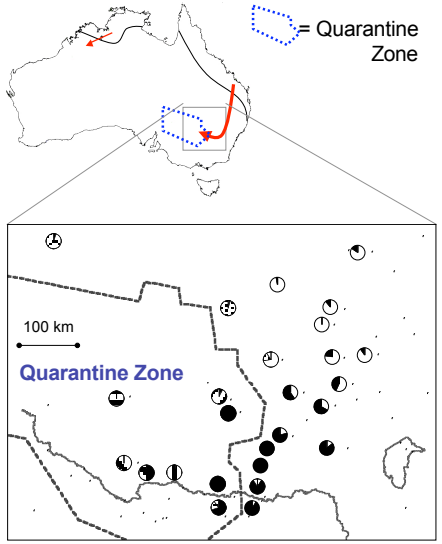
B. tryoni *B. neohumeralis*

Sympatric pest species



This NJ tree, based on microsatellite data, shows that *B. aquilonis* and *B. tryoni* are closely related (F_{ST} ~ 0.02) and likely to be allopatric populations of a single species. *B. neohumeralis*, despite occurring in sympatry with *B. tryoni*, is a genetically distinct species.

2. A structured invasion



Where exotic horticultural crops have been introduced into Australia, *B. tryoni* has soon invaded. Prior to 1996, the Quarantine Zone was free of *B. tryoni.* Since 2000, there have been many serious outbreaks within the Quarantine Zone.

B. tryoni / *B. aquilonis* has also invaded newly developed horticultural areas in NW Australia.

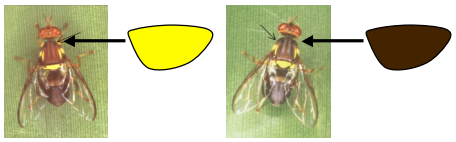
Pie graphs show the genetic constitution of *B. tryoni* sampled near and in the Quarantine Zone:

- as flies have invaded south toward their climatic limit, there is a cline in genetic structuring;
- within the Quarantine Zone, migration from the southern end of the cline predominates at short distances;
- over longer distances, founder effects obscure the source of the outbreak populations.

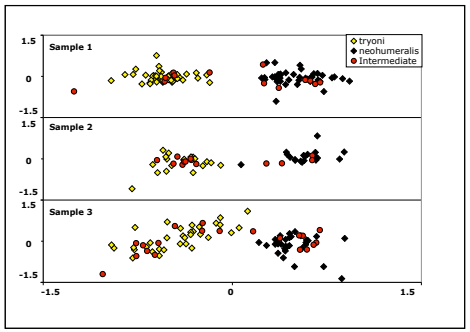
3. Interspecies hybridization?

The two sympatric species are distinguished by their callus colour:

B. tryoni *B. neohumeralis*



Collections of wild flies usually show ~1% with intermediate callus colour e.g.:



For many years,the "intermediate" flies were assumed to be interspecies hybrids. But microsatellite analysis (results shown above) shows that all are likely to be intra-species variants.

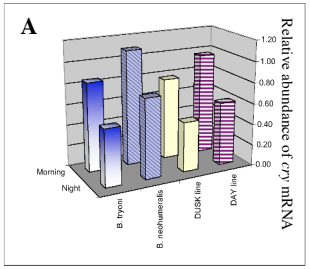
4. Molecular basis of species differences

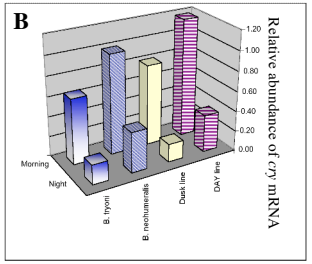
B. tryoni
Dusk-mater

B. neohumeralis
Day-mater

Real time PCR of *cryptochrome* in brain (A) and antennae (B) reveals:

- selection of hybrids for mating time reproduces the appropriate parental pattern of *cry* expression





Implementation of Medfly, Fruit Fly Parasitoids and Codling Moth Rearing Facility in the North East of Brazil

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Antonio S. Nascimento Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil
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The medfly is a major pest for fruit crops worldwide and its presence in the countries means a threat for production and for export. The methodology that has been used in many countries to control/eradicate/suppress medfly population is SIT Sterile Insect Technology that uses irradiation as source for sterilization. Besides medfly, *Anastrepha* species of quarantine importance also is an important pest affecting the fruit crops in Brazil. Parasitoids that are natural enemies of fruit flies also will be reared in the facility for release in the production area. Finally, take the advantage to have large building area, the facility also will produce sterile codling moth, a pest recently introduced in south of Brazil that affect the ever growing apple and pear crops.

The production of sterile medflies, natural enemies to control fruit flies and sterile codling moths all of them using irradiation as sterilization method and their release in the field is the most effective measure to control (suppression and eradication) such pests. The consumption of fresh fruits has increased worldwide and the production of Brazil either for domestic or export has been intensified in the last decade. As food safety is being a major concern for the consumers, the use of environment friendly technologies is always required. The SIT play a major role for control fruit flies and other fruit pests.

The final client for the sterile flies and moths will be the fruit growers. The 200 millions per week of medflies and 10 millions of *Diachasmimorpha longicaudata* wasps will be released in the tropical fruit growing areas in the northern Brazil: Bahia, Pernambuco (Sao Francisco Valley), Ceará, north of Minas Gerais e north of Espirito Santo.

The codling moths irradiated in the facility will be sent by air to the apple and stone fruit production area in the south of Brazil, Rio Grande do Sul and Santa Catarina, where the species is present in the urban area of four municipalities.

Millions/week

Nov 2005 >>	Kick off		
Jul 2006 >>	100		Kick off
Sep 2006 >>		Kick off	
Feb 2007 >>	200	10	15

Strategic Alliances:

USP UESB VALEXPORT CEARÁ GOVERNO DO ESTADO
ADAGRO Embrapa ADAB GOVERNO DA BAHIA
Ministério da Agricultura, Pecuária e Abastecimento BRASIL
Superintendência da Agricultura e Abastecimento da Bahia

OSCAMED BRASIL

IAEA-CN
131/141P

SEMI-FIELD CAGE STUDIES ON THE EVALUATION OF BASIL OIL AS A NEW ATTRACTANT FOR MALE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

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The present investigation deals with the evaluation of basil (*Ocimum basilicum* , Family:Lamioceae) oil as a new attractant for fertile wild type males (EGII Strain), as well as irradiated and non- irradiated mass- reared males from a genetic sexing strain based on temperature sensitive lethal *(tsl)* mutation (Vienna – 7).The males were tested in field cages located at the FAO/ IAEA Agriculture and Biotechnology Laboratory (Seibersdorf, Austria).Jackson trap was used for attraction of males. The results of the first experiment showed that, through 24 hours, the basil oil attracted about 67% of the non- irradiated V- 7 males, while only 54% of the irradiated (100 Gy) of V- 7 males were attracted. A different field cage was used for each of the irradiated and non- irradiated males. The second experiment showed that, about 65% of the fertile wild medfly males (EGII) were attracted, while only about 32% of the irradiated V-7 males were attracted. Both types of males were contained in one cage. The data obtained has shown that basil oil can be used as medfly attractant instead of the highly expensive trimedlure,normally used for this purpose. Opened field test should be conduced to complete the evaluation of the basil oil as a medfly attractant .

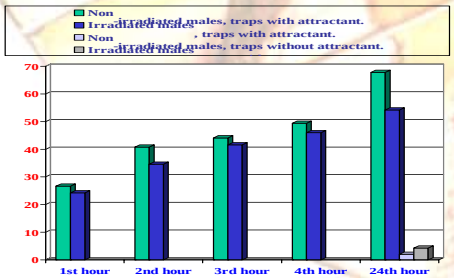

Fig (1) illustrates the percent accumulated attraction of V-7 irradiated and non-irradiated male medfly to traps loaded with basil oil through the first 24 hour. The figure shows that about 68% of the non-irradiated males were trapped at the end of the experiment compared to only about 54 % for the irradiated males. Statistical analysis showed that while the differences between the percent of trapped irradiated and non-irradiated males were significant at the first four hours . Moreover, it was highly significant at the end of the experiment.

Time after release	Percent attracted males / hour + S.E. to traps loaded with basil oil	
	Non-irradiated	Irradiated
1st hour	26 .73 ± 0.82	24.22 ± 0.42
2nd hour	13.99 ± 0.47	10.41 ± 0.31
3rd hour	3.96 ± 0.45	6.94 ± 0.32
4th hour	4.39 ± 0.35	4.40 ± 0.44
24th hour	18.33 ± 0.35	8.24 ± 0.69

Table (1) illustrates the percent attraction of irradiated and non-irradiated males Vienna-7 Mediterranean fruit fly, *Ceratitis capitata* (Wied.) to traps loaded with basil oil throughout the 24 hours in a semi-field cage (Experiment 1). The table shows that 26.73 % and 13.99 % of non-irradiated V-7 males were attracted to the trap at the first and second hours , respectively , compared to 3.96 % and 4.39 % of males at each of the third and the fourth hours respectively. However , 18.33 % non-irradiated males were attracted at the 24th hour. For the irradiated V-7 males , 24.22 % , 10.41 % ,6.94 % , 4.40 % and 8.24 % of males were attracted at the 1st , 2nd , 3rd , 4th ,and 24th hours, respectively .

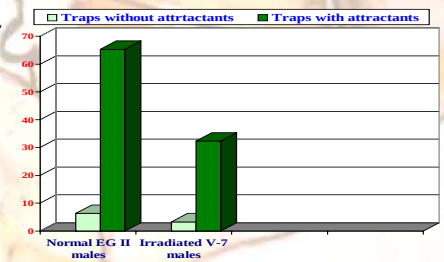

Figure 2 demonstrate the percent attracted normal EGII and irradiated (100 Gy) V-7 medfly males to basil oil as male attractant using sticky Jackson traps in a semi-field cage (Experiment 2).

The figure shows that while about 65 % normal EGII males were attracted to traps loaded with basil oil through 6 hours , only 6% of the males were stacked to traps without basil oil . This result indicates the relatively high attraction power of basil oil to EGII males compared to the control traps . Moreover , the same figure showed less attractabilty as about 32 % of the irradiated V-7 males were attracted compared to only 3% of males were stacked to traps without the attractant

This result is encouraging the use of basil oil as male attractant before any release of sterile males for controlling the medfly. This will lead to a suppression of the wild population by about 68 % throughout 24 hours , subsequently the number of released irradiated males can be reduced , saving in cost and efforts in a SIT program .

Using basil oil in different sites of field (without sticky traps) during irradiated medfly male release in SIT program will help in aggregation of both wild males and irradiated V-7 males to mate with wild female .

A model of diffusion of *Glossina palpalis gambiensis* (Diptera: Glossinidae) in Burkina Faso

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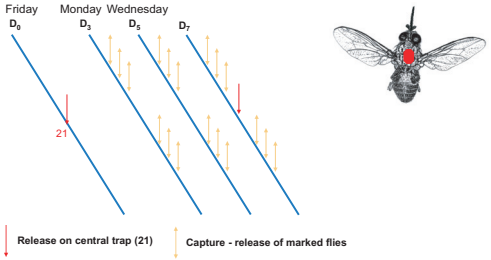
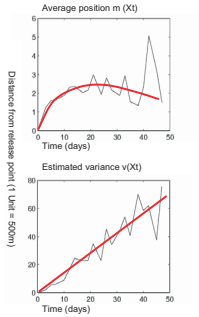
2 Centre international de recherche-développement sur l'élevage en zone subhumide, Bobo-Dioulasso, Burkina Faso.
3 Institut Sénégalais de recherches agricoles, Laboratoire national d'études et de recherches vétérinaires, Dakar, Sénégal.
4 Gigean, France

Field Data

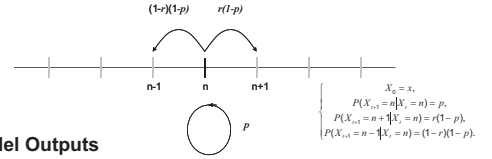
Mark release recapture protocol on the Dienkoa River, Burkina Faso (Cuisance 1983)
8683 released and 3228 re-captured males *Glossina palpalis gambiensis* Vanderplank
May to August 1981





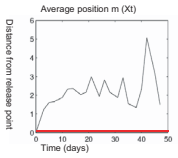
Step 1

First model based on a naturalistic description (Buxton 1955)
"The fly belt occupied by *G. palpalis* is nearly always along the waterside.[…] It is known that the insect moves very freely up or down stream or up a tributary. […] Evidently then the width of the zones varies, but we may say that spontaneous movement of the insect is so closely confined to the vicinity of water that it is almost linear."
One-dimensional random walk on a straight line
Simplest model to describe the movement of insects (Okubo 2001). Succesfully apllied to savannah tsetse species in two dimensions (Hargove 2000).
Stochastic process : individuals jump to the neighbouring positions.

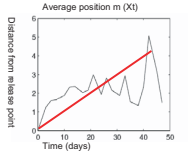


Model Outputs

Case of the isotropic random walk (r = 1-r)

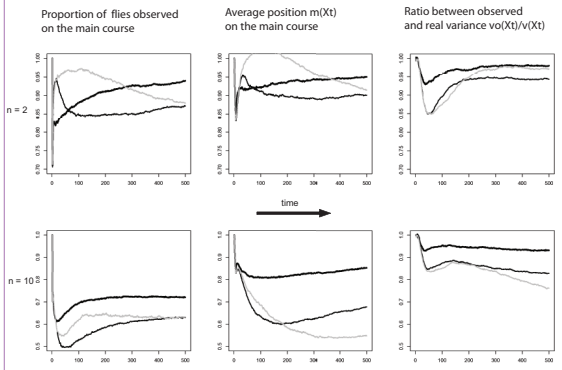


Case of the non isotropic random walk (r > 1-r)

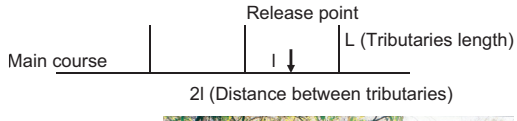


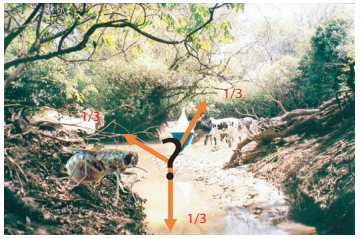
Step 2

Random walk on a tree : does partial observation matter?
The traps were located on the main course but the river is rather a network than a straight line.
Diffusion process with death on a tree (D, diffusion coeficient and μ, daily mortality rate)



Symmetric networks used for the simulations

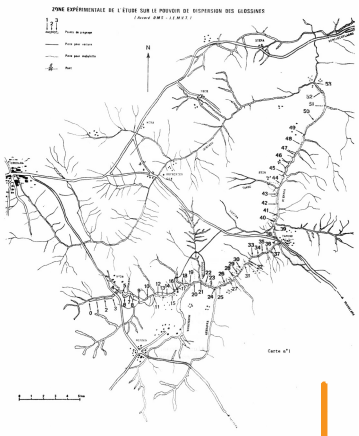




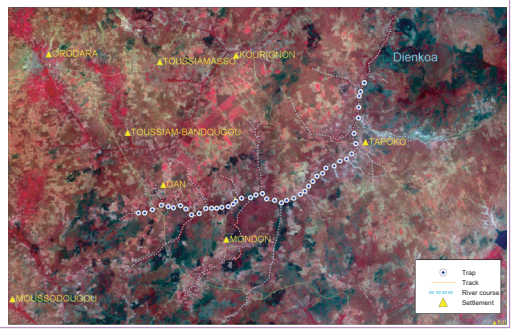
Model outputs
Partial observation can lead to an over-estimation of mortality rates, up to 50% at day 40.
The variance in position (proportional to the diffusion coefficient) can be under-estimated by 20 % in the case of complex networks, highlighting the necessity to take the real hydrographical network structure into consideration in dispersal studies.

Step 3

Taking the space complexity into account in the diffusion model



Digitalization of the real hydrographical network and trap location on a Landsat TM image (pixel 30*30m), from the authors original map (Cuisance 1981).

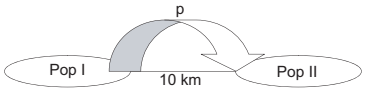


Step 4

Estimating the model's parameters (Diffusion coefficients (D) and mortality rate (μ)) for two models:
- diffusion on a straight line with complete observation (a),
- diffusion on a network with partial observation (b).

Two "summary statistics" were estimated in an hypothetical case with two populations (I and II) at 10km distance from each other on a straight river course without tributaries and for the two models:
- the distance exceeded during 10% of the flies lifespan (**Dmax10**),
- the probability for a fly to pass from I to II (**p**).

Necessity to take the network structure into consideration in isolation studies and eradication campaigns using Sterile Insect Technique.



	(a)	(b)
D	0.29km^2/j	0.46km^2/j
μ	6.5 %	4.4 %
Dmax10	4 km	13 km
p	< 0.01	> 0.1

References

Buxton, P. A. (1955) The natural history of tsetse flies. An account of the biology of the genus Glossina (Diptera). London, Lewis H.K. & Co Ltd, 816.
Cuisance, D., Février, J., Filledier, J. & Dejardin, J. (1983) Etude sur le pouvoir de dispersion des glossines. Bobo-Dioulasso, CRTA/IEMVT/OMS, 71.
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Mass-rearing, Quality Control of Olive Fruit Fly: Replacement of Cellulose in larval Diet: Mating Time Asynchrony between Wild and Lab Flies

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Introduction

Olive fruit fly *Bactrocera oleae* (Gmel.), is one of the key insect pests for the olive tree cultivation in Mediterranean region, and more recently in California, where it has been introduced recently. Currently, control of *B. oleae* is based on the use of insecticides either in bait or in cover sprays (Tzanakakis and Katsoyannos, 1998), resulting in adverse effects on the environment (Ferreira & Tainha, 1983). Availability of an improved method for olive fly mass rearing is a prerequisite for successful control of the species with the use of alternative control strategies, such as SIT (Economopoulos, 1977).

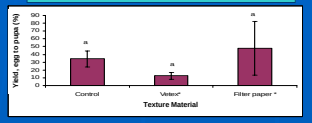
It had been known that mating activity in laboratory strains spreads in a period of 4-5 h before scotophase, as compared to wild strains which exhibit mating activity almost exclusively within 1 to 0 h before scotophase (Loher and Zervas, 1979, Zervas and Economopoulos, 1982).In the field this could lead to mating activity strain separation, in case both sexes of laboratory strain are sterilized and released (Economopoulos, 1972). It had been known although that laboratory conditions and photoperiod (Tsitsipis, 1981) are strongly affecting the vital rhythms of the olive fly.

Results-discussion

Extensive effort has been undertaken to replace or reduce the use of cellulose as larval medium bulk material by cheaper, readily accessible material(s). Paper and synthetic "vettex" gave promising results. Larval diet recycling (Tzanakakis,1969) and use of starter-finisher technology (Economopoulos *et al*,1987) suggested practical re-use of larval diet, and possibility for young larval high density exploitation of medium.

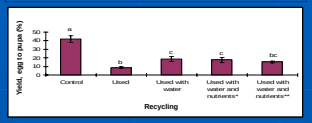
In recent experiments it has been found that, although short photophases result in mating activity suppression towards the end of photophase in both strains, the above described mating-time pattern in the 2 strains is always observed. Furthermore, no matter the L:D regime used with the laboratory strain, shift to a changed L:D regime of longer or shorter photophase than before results in mating activity adaptation within 24h,i.e.the lab strain exhibits mating activity over longer period before scotophase as compared with the wild strain. An attempt to select for early- late mating pattern within the lab strain did not lead to success in spite of 6 generations of selection so far.

New inert materials, Recycling, Starter-finisher.



*In certain treatments, larval food preservatives (potassium sorbate, and nipagin) were added at 75% of standard larval diet quantity

Figure 7. Substitution of chromatography cellulose powder of olive fly standard artificial larval diet by other texture material. Three replicates per treatment. P<0.05 (Tukey test).



*All nutrients (soy hydrolyzate, brewer's yeast, sugar, olive oil, tween-80) were added at 100% of fresh larval diet quantity. Significant fungal development was observed.
** As above, without the addition of olive oil and tween-80. Significant fungal development was observed.

Figure 8. Reuse of olive fly's artificial larval diet. Three replicates per treatment. P=0.05 (Tukey test).

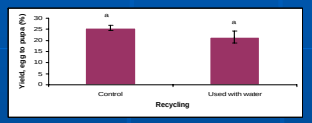
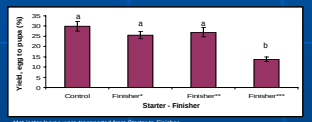


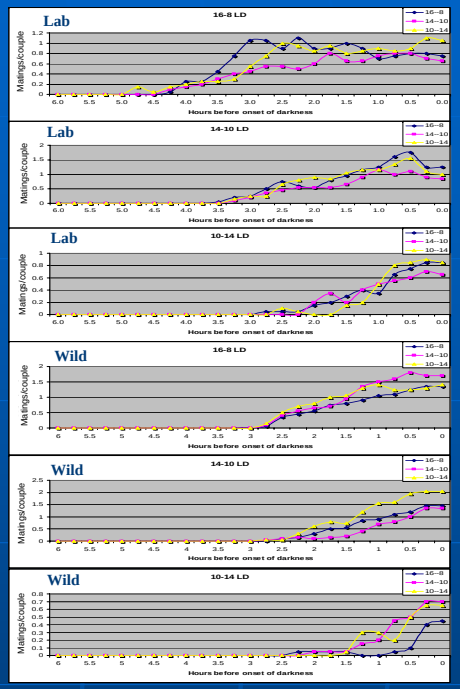
Figure 9. Reuse of olive fly's artificial larval diet. Five replicates per treatment. P=0.05 (Tukey test).



*1st instar larvae were transported from Starter to Finisher
** 2nd instar wwe were transported from Starter to Finisher
*** 2nd-3rd instar larvae were transported from Starter to Finisher

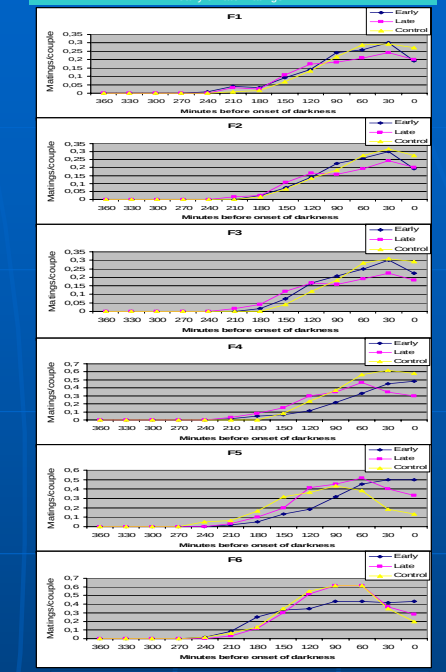
Figure 10. Starter-Finisher method applied to olive fly rearing (Starter: high density of 1st or 2nd instar larvae in standard artificial diet, Finisher: optimal density of 2nd or 3rd instar larvae in standard artificial diet). Three replicates per treatment. P=0.05 (Tukey test).

Mating activity adaptation of laboratory and wild olive flies after transfer to new photoperiodic regime



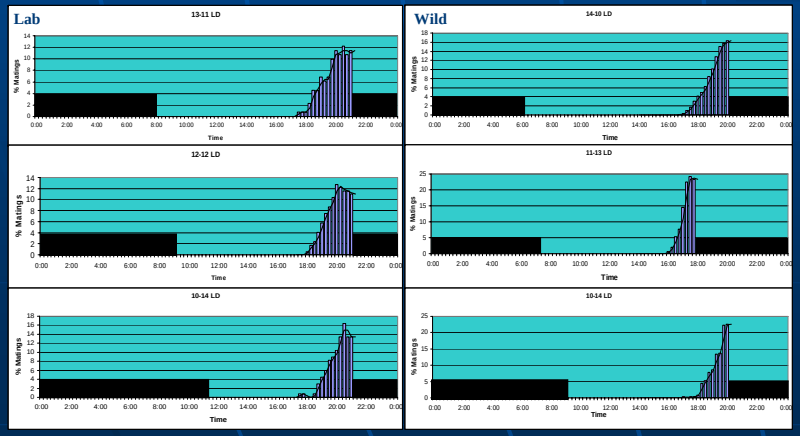
Figures 11-16. Observations on insects kept for 5 days on initial photoperiod and transferred for 48 hours to new one. Final photoperiod where observations conducted is shown on the top of the figure, and previous exposure for each population is shown on the right. Laboratory conditions of 25±1 C, 65±5% R.Humidity. 30 individual cages (couples) per treatment.

Mating activity of laboratory populations selected in every generation for early or late matings.



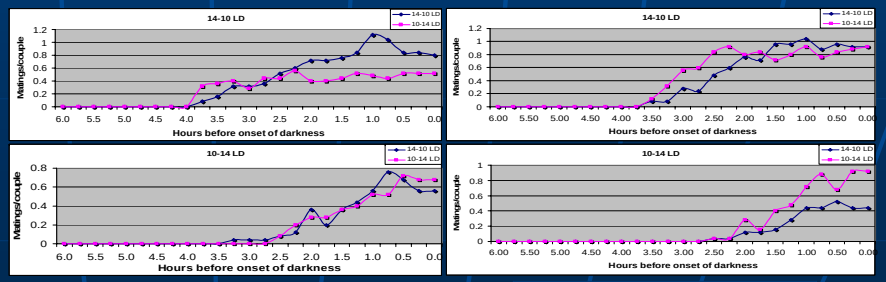
Figures 21-26. Observations on selected populations of olive fly. From every generation ~200 insects were selected on the first hour of matings (Early) and on last hour (Late). These insects gave progeny for next generation. Experiments conducted in laboratory conditions of 25±1 C, 65±5% R.Humidity, photoperiod 14:10 LD. 20 individual cages (couples) per treatment.

Mating activity of laboratory and wild olive flies under various photoperiods



Figures 1-6. Mating timing during the photophase as observed in populations of individual cages of laboratory and wild olive fruit flies. Observations conducted under laboratory conditions of 25±1 C, 65±5% R.Humidity and photoperiod as denoted on each figure. Dark rectangles denote the scotophase length. For each experiment 30 couples of insects were observed with 15 min intervals.

Mating activity adaptation of laboratory olive flies after transfer to new photoperiodic regime



Figures 17-18. Observations on insects kept for 5 days on initial photoperiod and transferred for 24 hours to new one. Final photoperiod where observations conducted is shown on the top of the figure, and previous exposure for each population is shown on the right. Laboratory conditions of 25±1 C, 65±5% R.Humidity.30 individual cages per treatment.

Figures 19-20. Observations on insects kept for 10 days on initial photoperiod and transferred for 24 hours to new one. Final photoperiod where observations conducted is shown on the top of the figure, and previous exposure for each population is shown on the right. Laboratory conditions of 25±1 C, 65±5% R.Humidity.30 individual cages per treatment.

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Activity of some Insecticides Against Adult New World Screwworm Fly,

Cochliomyia hominivorax

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Screwworms , *Cochliomyia hominivorax* and *Chrysomya bezzina* , are considered of the most destructive insect pests of livestock and other warm-blooded animals . It is not feasible to experiment with the reproductively mature insect under conventional laboratory conditions because of the potential risk of escape of such highly reproductive insects especially in situations where an eradication campaign or a control program are in progress.

In earlier studies (Elowni *et al* . 1994) , sexually sterilized *C. hominivorax* derived from irradiated mass-produced pupae were used in *in vitro* tests to evaluate the basic toxicity of seven insecticides against the adult fly . Pyrethroid insecticides (cypermethrin , deltamethrin , cyfluthrin) and the organophosphate trichlorfon were shown to have a rapid action against the fly. They produce their 99% knockdown effect in less than 5 min . It is established that a female screwworm fly invading a host requires a period of about 6 min before it lays its eggs on the host . An invading fly probing on an animal treated with such rapidly acting insecticides would therefore be expected to be eliminated before ovipositing and the host will eventually be protected .

In the present study , protective activity of three of these rapidly acting insecticides was examined in an experimental model simulating primary infestation of animals by the adult fly . Sexually sterile flies were exposed for 5 min to the body of sheep treated with recommended doses of either deltamethrin spot - on (1mg / kg) , cyfluthrin pour - on (2mg / kg) or cypermethrin squirt - on (4 mg /kg) preparations applied onto the withers (deltamethrin) or along the backline between the withers and rump (cyfluthrin , cypermethrin) . Fly mortality was assessed on the withers , abdomen and flank on various days post-treatment for up to day 24 (deltamethrin , cyfluthrin) or day 28 (cypermethrin) . Deltamethrin produced 100% mortality on day 1 post-treatment on the withers compared with a mortality rate of 97% and 86% for cypermethrin and cyfluthrin treatments , respectively (Fig.1) . Mortality (Y) caused by the various insecticides on this site showed a decreasing trend with increase in days (X) . This relationship was expressed by best - fit regression equations of :

$Y = 102.76 - 3.71 X$ (deltamethrin) ,

$Y = 16.18 + 81.18 / X$ (cypermethrin) and

$Y = 89.26 - 1.95 X$ (cyfluthrin) .

In contrast to detamethrin and cyfluthrin , cypermethrin appeared to have been distributed rapidly over the body (Figs 2 & 3) and it produced on the abdomen and flank mortalities which were statistically indistinguishable from those produced on the withers.

Observations on the behaviour of screwworm (Mangan *et al*. 1990) indicate that sterile females are attracted activity to wound sites and they exhibit an oviposition behaviour similar to that of the reproductively mature insect . Sexually sterile screwworms may therefore be utilized effectively as a model for evaluation of protective properties of insecticides .

Fig 1. Mortality of adult sexually sterile *Cochliomyia hominivorax* exposed to the withers (insecticide application site) of sheep treated with pyrethroid insecticides

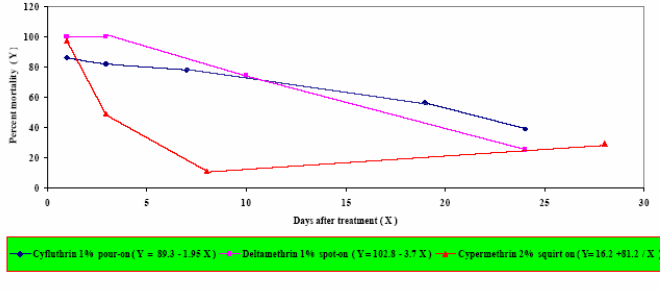


Fig 2. Mortality of adult sexually sterile *Cochliomyia hominivorax* exposed to the abdomen of sheep treated with pyrethroid insecticides

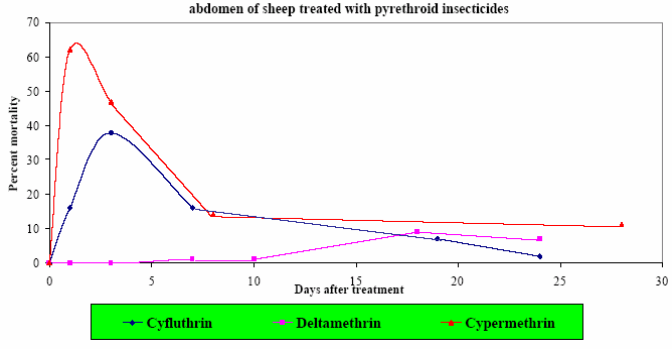
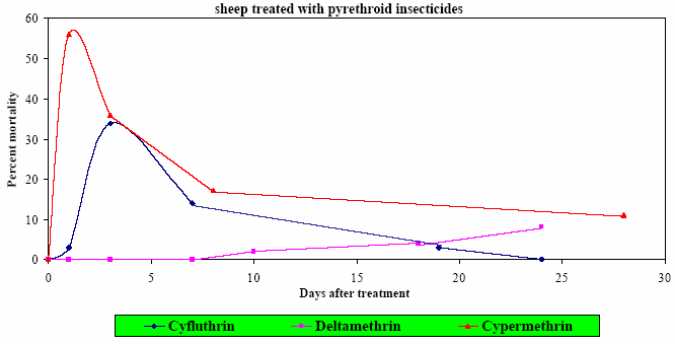


Fig 3. Mortality of adult sexually sterile *Cochliomyia hominivorax* exposed to the flank of sheep treated with pyrethroid insecticides



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Comparison of different attractants for monitoring and control of the olive fruit fly *Bactrocera oleae* in Greece

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This study aimed at developing new trapping systems for the olive fruit fly. The experiments were conducted in the Greek island of Chios using a randomized complete block design and various attractants, including an aqueous solution of the protein NuLure plus Borax, dispensers of Ammonium bicarbonate, Ammonium acetate, Putrescine or Trimethylamine, an aqueous solution of ammonium sulfate and ammonium phosphate, and pheromone dispensers of spiroketal.

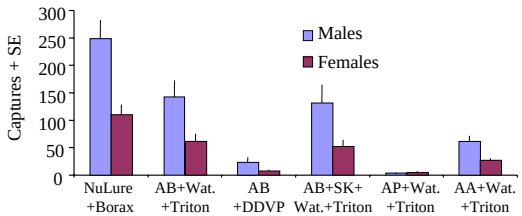
McPhail type trap (PMT)

Elcophon trap

Vioryl stick pheromone trap

NuLure vs ammonium lures and pheromone attractants in PMT traps

	Attractant and retention system
1	NuLure + borax
2	Ammonim Bicarbonate (AB) + water - triton 300 ml of water + 0.01 Triton
3	AB + DDVP (Vapona; 15x15x15 mm plug)
4	AB + Spiroketol (AgriSense) + water – triton
5	Ammonium phosphate (aques solution
6	Ammonium Acetate



PMT with NuLure vs local traps, Ammonium Phosphate and Ammonium Sulfate

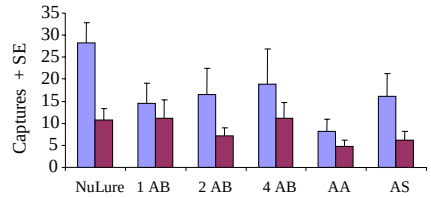
NuLure baited PMT traps were 2 and 5 times more attractive for males and females respectively than the local trap Elcophon baited with entomela (Phytophil, Athens, Greece). Ammonium sulfate baited McPhail traps were much more effective than similarly baited ammonium phosphate traps both for males and females.

NuLure vs pheromone traps

PMT NuLure baited traps were 70 times more attractive for males than spiroketal pheromone baited, sticky, green panel traps (Vioryl), and 200 times more attractive than PMT traps baited with spiroketal (Vioryl) dispensers.

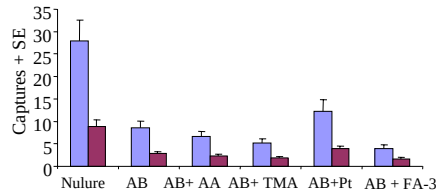
NuLure vs various quantities of ammonium dispensers in PMT traps

	Attractant and retention system
1	NuLure + borax
2	One Ammonium Bicarbonate (AB) + water - triton 300 ml of water + 0.01 Triton
3	2 AB + water – triton
4	4 AB + water – triton
5	Ammonium Acetate + water – triton
6	Ammonium sulfate (aqueous solution)



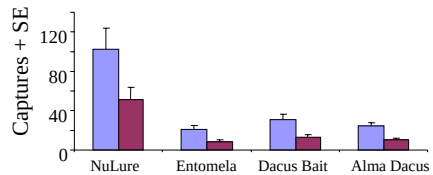
NuLure vs combinations of various other attractants in PMT traps

	Attractant and retention system
1	NuLure + borax
2	Ammonim Bicarbonate (AB) + water - triton 300 ml of water + 0.01 Triton
3	AB + Ammonium acetate + water - triton
4	AB + trimethylamine + water - triton
5	AB + putrsescine + water - triton
6	Ammonium Acetate



NuLure vs local proteins in PMT traps

	Attractant and retention system
1	NuLure + borax
2	Entomela
3	Dacus bait
4	Alma dacus



Conclusions

- The most effective of the trapping systems tested was the PMT trap baited with NuLure. Ammonium bicarbonate seems to be the most promising of the attractants.
- Surprisingly pheromone traps performed very poorly in our tests.
- More research is needed for developing synthetic attractants for the olive fruit fly.

Can ISSR markers reveal anything new about genetic structure of *Ceratitis capitata species*?

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INTRODUCTION. The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most devastating fruit pests worldwide. In Spain, it is a major pest of citrus and fruit crops. The knowledge of the within and between populations genetic variability would constitute a key aspect of its ecology. Futhermore, molecular markers provide genetic information that could be crucial for the implementation of appropriate medfly control programmes.

OBJECTIVE: Test the usefulness of ISSR technique for the study of the genetic structure of medfly populations.

MATERIAL. Five Spanish samples:

Málaga (MAG)
Valencia (VAL)
Castellón (CAS)
Tarragona (TAR)
Laboratory strain (LAB)

1 from laboratory strain

4 wild from Mediterranean area

TECHNIQUE. The ISSR-PCR technique (amplification of inter-simple sequence repeats). We have developed our own protocol based on Zietkiewicz *et al.*, 1994 (Genomics 20: 176-183).

Genomic DNA extracted according to Aubert & Lightner, 2000 (Mar. Biol. 137: 875-885) with some modifications.

120 individuals were analysed employing three different primers (814, 846 and 849. Set # 9 UBC, University of British Columbia).

RESULTS AND CONCLUSIONS

In this first approach to test the application of ISSR to *C. capitata* samples, a total of 33 different DNA bands were scored with the three primers used. All amplifications were consistently reproducible.

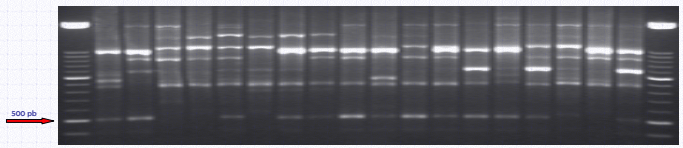

Figure 1. Agarose gel (2%) showing ISSR profiles of medfly samples with primer 814. Lanes 1 and 20 contain a 100 bp ladder molecular weight marker. Lanes 2-5: MAG sample. Lanes 6-9: LAB. Lanes 10-14: CAS. Lanes 15-19: VAL.

No differentiation among populations. Intrapopulation similarity indices (SM) ranged from 0.62 to 0.73 (mean= 0.69±0.04) and are similar than interpopulation values (mean= 0.66±0.05).

MAG	0,6825				
CON	0,6308	0,6273			
COV	0,7255	0,6214	0,7374		
MON	0,6922	0,6163	0,7361	0,7111	
AMP	0,6639	0,5849	0,7155	0,7024	0,6938
	MAG	CON	COV	MON	AMP

Table 1. Interpopulation (below diagonal) and intrapopulation (on diagonal) similarity indices for medfly samples based on ISSR bands.

High intrapopulation differentiation. AMOVA analyses revealed that more than 75% of the total genetic variation was within samples.

Source of variation	d.f.	SSD	Variance component	p	% total variance
All populations: 1 GROUP					
Among populations	4	131.69	1.19	< 0.001	21.74
Whitin populations	115	494.61	4.30	< 0.001	78.26
Two groups: LAB/WILD					
Among groups	1	82.66	1.66	< 0.001	25.67
Among populations within groups	3	49.03	0.51	< 0.001	7.86
Whitin populations	115	494.61	4.30	< 0.001	66.47

Table 2. Analysis of molecular variance (AMOVA) for 120 individuals in 5 populations of *C. capitata*.

Usefulness of ISSR-PCR markers to detect genetic variability in the medfly. Polymorphism indices ranged from 54 to 80% (mean 0.69). These values are higher than those detected with isozymes and slightly more elevated than values obtained with RAPD, probably owing to different nature of markers.

Development of an embryonic lethality system for transgenic SIT in the fruit pest *Ceratitis capitata*

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Agricultural and Forestal Pest Problems

Insects of Economic Importance to Agriculture and Forestry

Worldwide about 25% potential harvest lost because of insects

Pests of stored products and farm animals — Damage by feeding

Problems of Pest Control by Insecticides

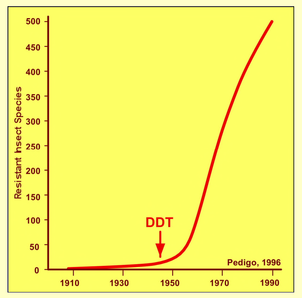
Insecticide-Nonspecifity

- Kill also beneficial insects

Insecticide Resistance

- Insecticide treadmill
- Escalating costs for developing novel compounds

Potential Health Hazard



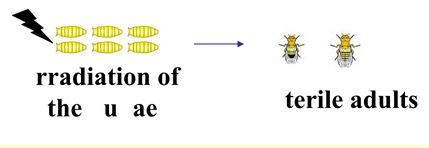
SIT: Sterile Insect Technique (Dr. Edward F. Knipling, 1955)

Pest management by inundation of a pest population with sterilized males

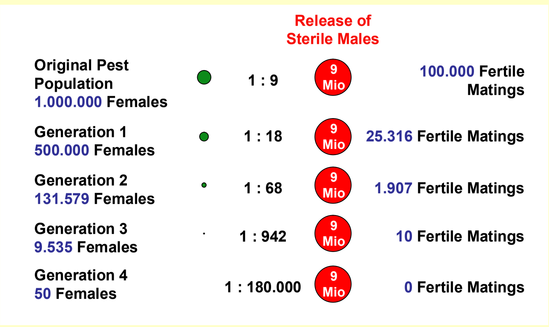
Matings then result in non-fertilized eggs or embryos that cannot survive

Sterile Insect Technique

Common Procedure of Sterilizing



Efficiency of SIT-Program



Advantages:

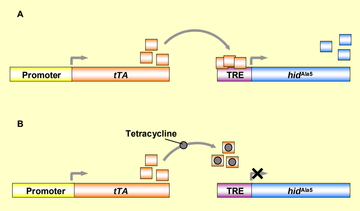
- Ecologically safe, since absolutely species specific
- Effectivity increases during application of SIT

Problems:

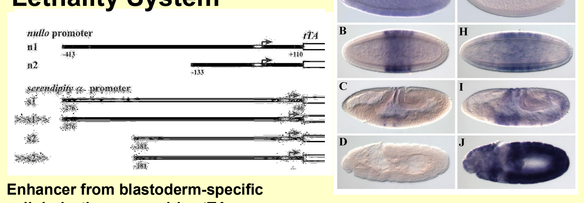
- Releases of sterile females that damage fruit by oviposition or transmit diseases by biting domestic animals and humans
- Males sterilized by radiation often non-competitive (especially in many lepidopteran species)
- Marking of sterile insects is technically and logistically difficult but necessary for monitoring progress in operational SIT

Embryo Lethality System for Transgenic SIT

Tetracycline-Repressible Transactivator System



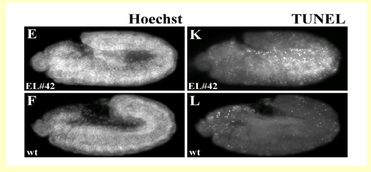
Embryo-Specific Lethality System



Enhancer from blastoderm-specific cellularization genes drive tTA

tTA activates effector gene *hid*

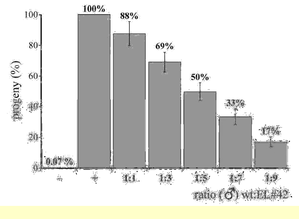
Pro-apoptotic gene *hid* causes cell death



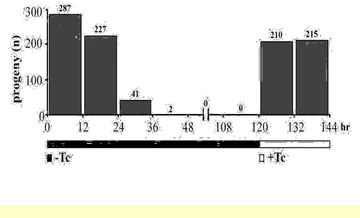
Efficiency of Embryonic Lethality System

Temperature	Hatch rate	
18 °C	0.07%	(n = 18000 eggs)
25 °C	0.01%	(n = 23000 eggs)
29 °C	0.00%	(n = 21000 eggs)

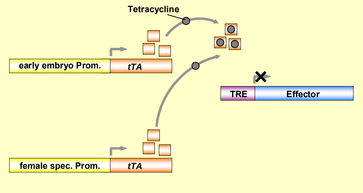
Competitiveness of sterile males against wild type males



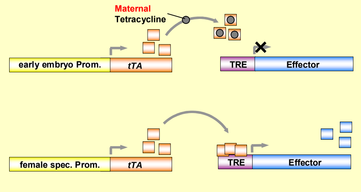
Maternal Suppressibility of Embryonic Lethality



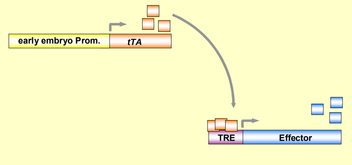
Rearing of Strains on Tetracycline



Generating only Males due to Female Lethality



Killing Progeny by Induced Embryonic Lethality



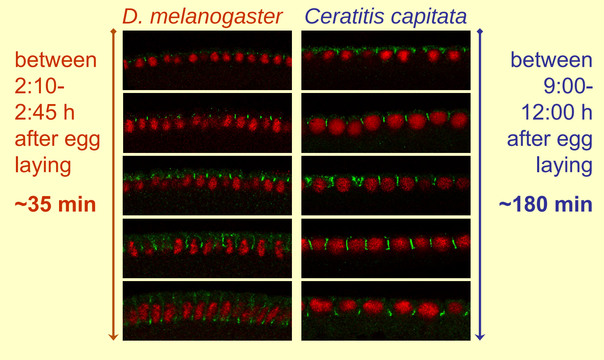
Transfer of System to an Insect Pest: The Medfly *Ceratitis capitata*

Ceratitis capitata

Female *Ceratitis capitata*

GFP expression in the compound eyes of transgenic *Ceratitis capitata*

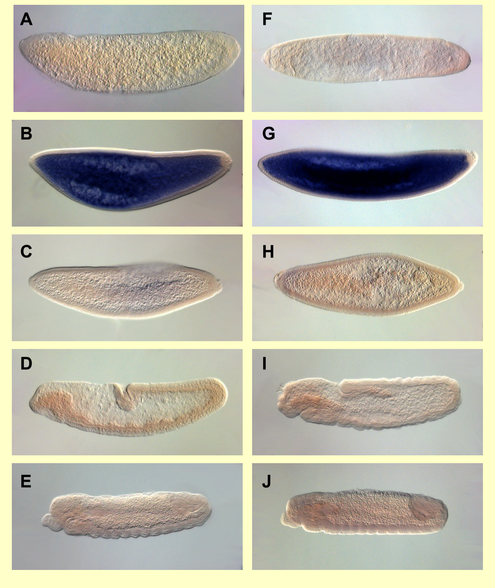
Cellularization: *D. melanogaster* vs. *C. capitata*



Cellularization of *Drosophila melanogaster* (left side) and *Ceratitis capitata* (right side).

The immune fluorescence staining with primary armadillo antibodies and secondary Alexa488 antibodies (green) displays the invagination of the cell membrane in both species. The cell nuclei were stained with propidiumiodide (red).

Cellularization-specific *C. capitata* Genes

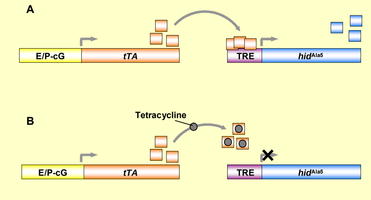


Cellularization specific expression patterns of *Ceratitis capitata* genes isolated by cDNA-subtraction.

A) no staining; F) beginning weak expression in the preblastodermal embryo; B/G) strong expression at beginning of cellularization; C/H) very weak expression at the end of cellularization; D/E/I/J) no staining in gastrulation, germ band elongation and germ band retraction.

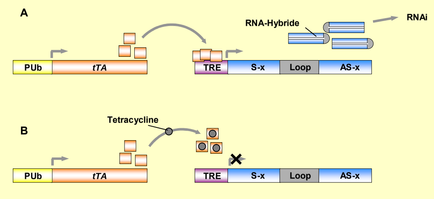
Schemes of Embryo-specific Lethal Transgenes in *Ceratitis capitata*

A) The tetracycline-controlled transactivator (**tTA**) mediates gene expression by binding to the tTA-response element (***TRE***).
B) The main advantage of this system is that targeted gene expression can be further controlled by a food supplement. Tetracycline (Tc) and tTA form a complex that prevents tTA from binding to its response element, which therefore becomes inactive. Supplementing the diet with Tc makes it possible to switch off the system and so allows a control beyond that imparted by the cellularization-specific enhancer/promoter (**E/P-cG**).



A) Binary expression system driving cellularization gene mediated RNA interference. This would provide a species-specific system as RNAi requires high levels of sequence identity.
B) Supplementing the diet with Tc makes it possible to switch off the system and so allows a control beyond that imparted by the RNA interference.

PUb:polyubiquitin promoter, **S-x**: sense of gene x, **AS-x**: antisense of gene x.



Research is supported by the Robert Bosch Foundation

Some regularities in the dynamics of spatial and territorial distribution of insect population density

IAEA-CN-131/149 P

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Institute of Ecology of Vilnius University, Lithuania

Population density of various insect species in any ecosystems is determined not only by parameters of the population itself, but also by the influence of population density dynamics of the interrelated surrounding species of interacting ecosystems. The most significant redistribution of populations occur during the formation and subsequent disappearance of the primary foci of mass reproduction of hosts. At the time of mass reproduction of phytophagous insects, increase in their population density within a territory of the primary foci did not occur simultaneously on the whole territory. In the beginning, a very high increase in population density occurs only in several to some tens of trees in the primary mass reproduction foci. Obviously, this is a suitable time for the control of population density of phytophagous and entomophagous insects in order to limit host population density. During the following year, the movement and redistribution of the definite population density level remains the same in the primary foci and surrounding territories within 100-500 meters radius. In just a year's time, the movement of the definitive level of the insects population density across the territory occurs at large distances (Fig 1). Over the last three decades, the primary foci of mass reproduction of the species investigated in Lithuania shifted at a 160 km per year rate and mostly from west to east.

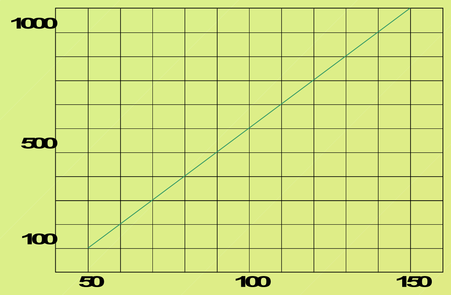

Fig. 1. The relation between the rate of territorial movement of the definite population density level across in the primary foci of mass reproduction and the absolute insects population density: on absciss – the distance of movement across the territory (km), on ordinate – population density of insects 1000/ha

During the last three decades, three outbreaks of *Yponomeuta evonymella* L. occurred (fig. 2). The most abundant populations were detected in 1977-1981, in 1991-1995 and 2004. The last period still continues. The main parameter that helps to forecast increase or decrease in the dynamics of ermine moth population is the mean number of adults in a spider web nest of ermine moth. The critical point of increase in the average number of adults per nest was reached in 1985 and 1997. In 1990, the average number of ermine moth parasitoids per nest on bird cherry in other territories exceeded the corresponding number in the primary foci. In 1991, the density of parasitoids per nest considerably decreased. In 1992, the average number of parasitoids per nest increased and it was the same in the primary foci as in other territories. In the 1992-1993, the accumulation of parasitoids in other territories was followed by increase in their population density in the primary foci in 1992. Since the beginning of nests accumulations on bird cherries in the primary foci, a rapid increase in the average number of parasitoids per nest was observed. Increase in population density at the time of the outbreak of ermine moths on bird cherry trees did not occur simultaneously within a territory of the primary foci and across the entire territory. During the last outbreak of ermine moths on bird cherry that resulted in very high densities the primary foci was developing along with the increase in the number of nests per tree (fig. 3).

In 1991-1993, increase in the number of nests per tree was observed on the other most populated bird cherries. In 1994, the ermine moth population on bird cherries expanded across the entire territory. A new outbreak of ermine moths on bird cherries occurred in 2004.

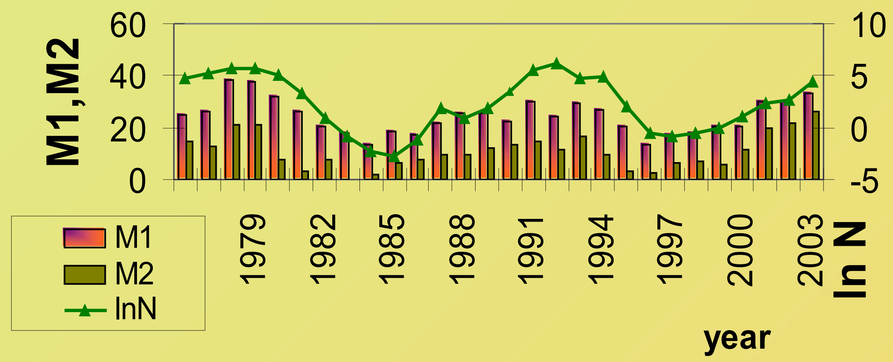

Fig 2. The long-term dynamics of the first instars caterpillars and adult populations density or ermine moths on bird cherry in 1977-2004: M1 - the average number of caterpillars per nest; M2 - the average number of adults per nest; N - the average number of nests per tree

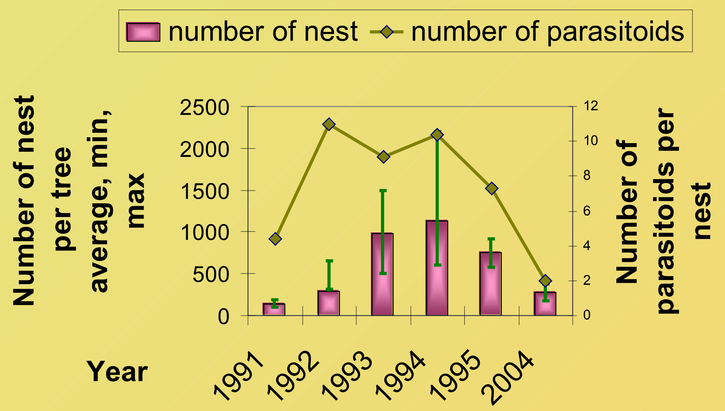

Fig.3. The dynamics of the number of nest per tree on the most populated bird cherries in the primary foci during the mass reproduction of ermine moths

Outbreaks of *Panolis flammea* Schiff. covered 44000 ha pine forests in Lithuania in 1999-2002. The formation of the primary foci of mass reproduction proceeded from some tens of pines in small areas. The highest population density was recorded in 2001. Here, the mean abundance of eggs on one pine-needle varied from 1.9 to 4.8. Thus, the egg population density and death were very different in various localities of pine beauty outbreak.The significant correlation between percentage of death and average number of eggs per tree were observed in 2001 (fig. 4-5).

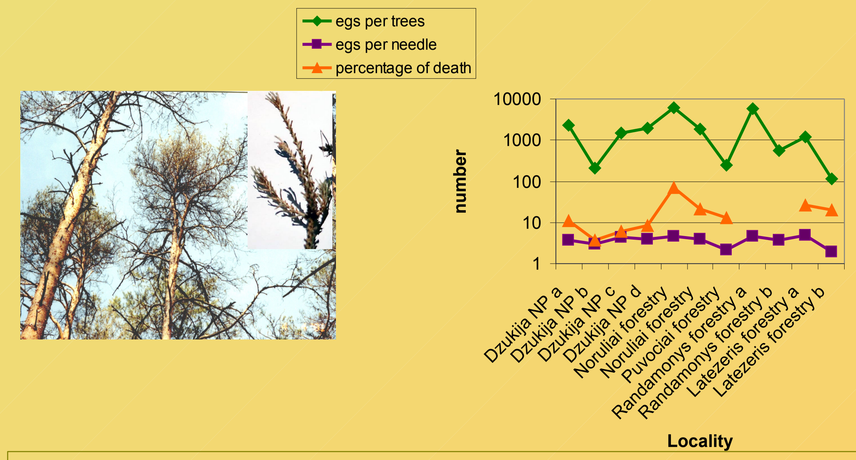

Fig 4. The relation between egg population density of the pine beauty in various pine forest in Lithuania in 2001.

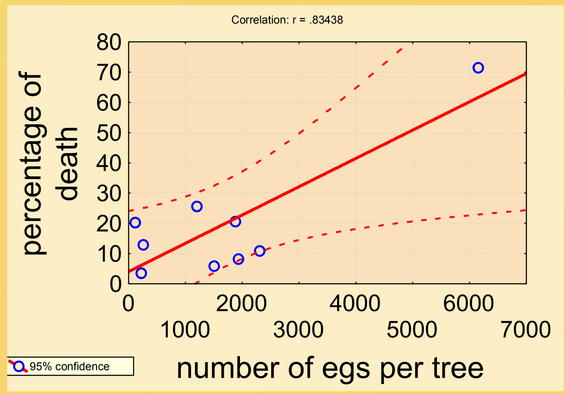

Fig 5. The correlation between egg population density of the pine beauty and percentage of death (Lithuania, 2001).

USE OF GAMMA RADIATION FOR ECONOMIC PRODUCTION OF AN EGG PARASITOID, *TRICHOGRAMMA CHILONIS* (ISHII).

POSTER NO. IAEA- CN 131/150

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OBJECTIVES:

To economize the production of bio-control agents and their application for management of the sugarcane borers

STEPS INVOLVED

- Irradiation of *Sitotroga cerealella* eggs
- Mass rearing of egg parasitoid, *T. chilonis*
- Management of sugarcane borers by the use of biological control agents

ACHIEVEMENTS

Effect of Gamma Radiation on the eggs of *S. cerealella*

- Irradiation significantly reduced the hatch percentage of the host eggs,
- Sex ratio was skewed in favour of males at higher doses.

Table I.Effect of Gamma Radiation on the eggs of *S. cerealella*

Dose (Gy)	Hatch (%)	Pupae recovered (%)	Adult emerged (%)	Sex ratio	
				Male	Female
5	88.6	64.3	65.32	57.14	42.86
10	80.0	62.0	64.52	55.00	45.00
15	81.6	66.6	63.06	64.29	35.71
20	76.3	51.3	66.28	73.53	26.47
25	72.3	50.3	65.61	72.73	27.27
30	68.0	40.3	59.55	75.00	25.00
35	40.3	27.6	68.84	73.68	26.32
40	19.6	12.3	56.91	100.0	0.00
45	3.6	2.0	50.00	100.0	0.00
50	0.6	0.6	0.00	0	0
Control	90.3	68.3	68.81	53.19	46.81

mass rearing of the parasitoids

Effects of irradiation of the *Sitotroga cerealella* eggs on parasitization by *Trichogramma chilonis*

- The parasites preferred the fresh eggs for parasitization and parasitization decreased as the age of the host eggs increased.
- Parasitization was recorded up to 6-days of age on irradiated eggs whereas, on normal eggs it occured up to 4-days of age.
- Results revealed that radiation doses of 20 and 25 Gy could be effectively used to enhanced parasitization as well as to decrease the age effect of host eggs of the parasitoids.
- Parasitization was comparatively higher at 20 and 25 Gy dose of radiation and decreased the age effect.

Table II.Effect of irradiation on host eggs for parasitization of *Trichogramma chilonis*.

Dose (Gy)	Parasitization potential in host eggs at different age (days)						
	1	2	3	4	5	6	7
5	19.2	16.4	11.2	6.4	0.6	0	0
10	23.2	21.0	17.6	11.0	0	0	0
15	20.2	17.0	12.6	11.8	0.8	0	0
20	23.6	20.0	15.2	13.2	9.0	3.8	0
25	24.4	19.8	13.2	9.2	4.2	1.4	0.8
30	19.2	16.6	13.2	9.4	4.8	1.8	0
35	17.8	15.6	14.6	5.4	0.8	0	0
40	15.2	13.4	8.4	1.4	0	0	0
45	13.4	7.6	5.4	0.2	0	0	0
50	11.6	7.6	1.4	0	0	0	0
Control	18.8	11.2	7.2	2.0	0	0	0

CONCLUSION

- Radiation increased the incubation period of the eggs of *Sitotroga cerealella*, which proved useful to increase the parasitic potential of *T. Chilonis*.
- Use of nuclear techniques in the mass rearing of the parasitoids also enhanced the production potential of females who play an important role in the augmentative bio-control programme.

Optimising seeding rates for mass rearing Old World Screw-worm fly

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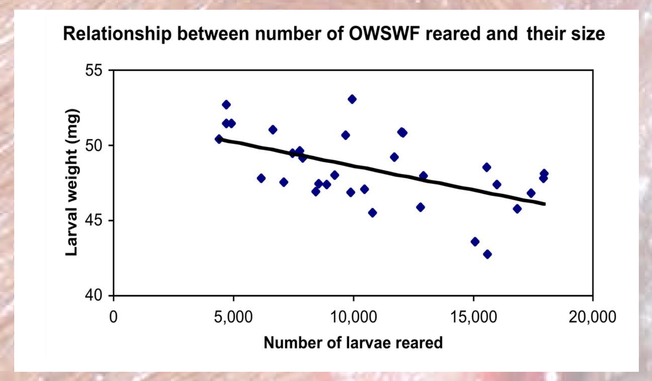
Two species of screw-worm fly are found in tropical areas, the New World screw-worm fly *Cochliomyia hominivorax* and the Old World screw-worm fly *Chrysomya bezziana* (OWSWF). Both are obligate myiasis flies. The larvae infest wounds of vertebrates including livestock and man. Australia is free from both pests; however the Old World screw-worm fly is present in neighbouring Papua New Guinea and Indonesia. If this pest enters Australia it would devastate large portions of Australia's livestock industries. Extensive research and development has occurred in order that the Sterile Insect Technique (SIT) can be employed to eradicate the fly if it ever becomes established in Australia.

In collaboration with the Malaysian Department of Veterinary services, the Australian Department of Agriculture Fisheries and Forestry constructed an experimental mass rearing facility in Malaysia to optimise mass rearing of the OWSW as an important component of Australia's preparedness program.



One of the factors that affect the fitness of mass reared insects is their size. Here we examine two factors, seeding rates and the accumulation of ammonia that influenced the size of the insects produced.

Figure 1. Size and number of larvae reared per tray.
A strong relationship was observed between the weight of eggs (essentially the number of eggs) reared in a tray and the size of the larvae produced. This was significant, as size may well determine the competitiveness of the sterilised males when released into the wild.

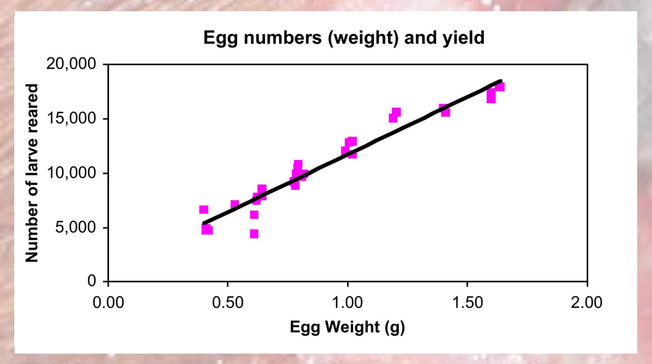


Figure 2. Egg numbers (weight) and yield.
While the size of larvae produced was influenced by the number reared on a tray, mortality was not. The linear relationship indicates that mortality was not affected by the density of the larvae.

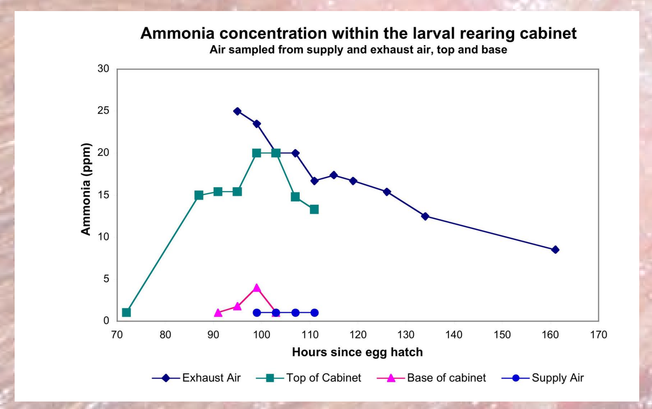
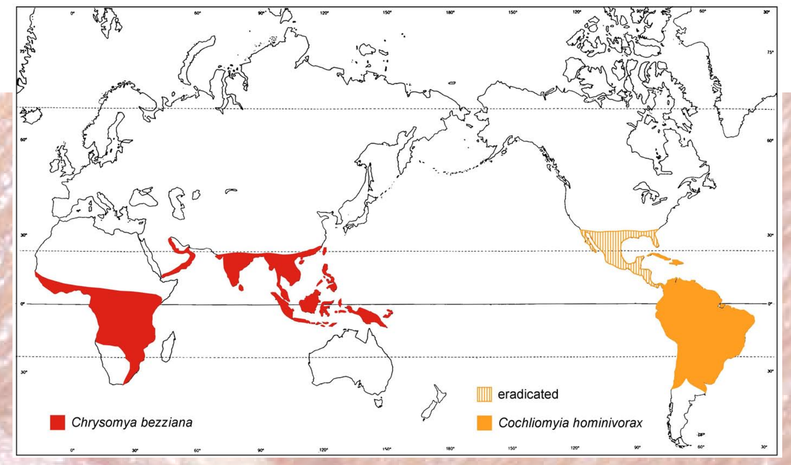


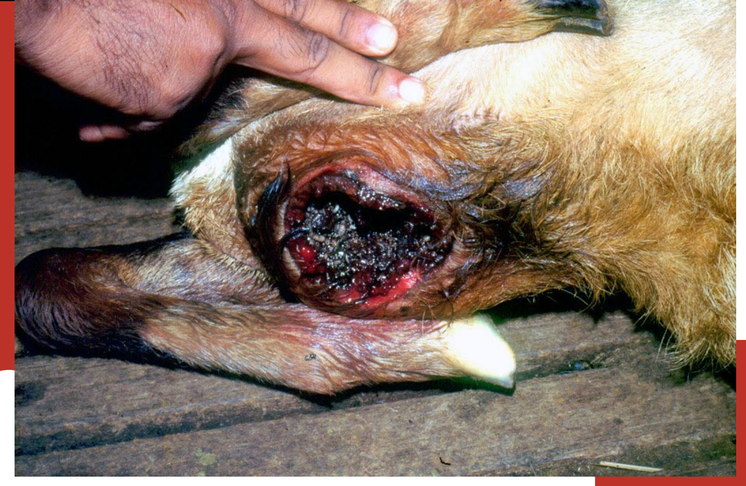
Figure 3. Ammonia concentration
An unexpected environmental factor proved to impact on larval size. As the larvae matured, ammonia was released into the atmosphere. It was shown to pool near the top of the rearing cabinet (note difference in concentrations at the top and base of the cabinet) with the exhaust air (leaving from the top of the cabinet) of a similar concentration to that at present between the upper trays. It was found that if ammonia was allowed to accumulate, larvae prematurely ceased feeding and exited the feeding tray as undersized larvae. Improved air circulation proved important to producing large (and hopefully fit) larvae for use in SIT.



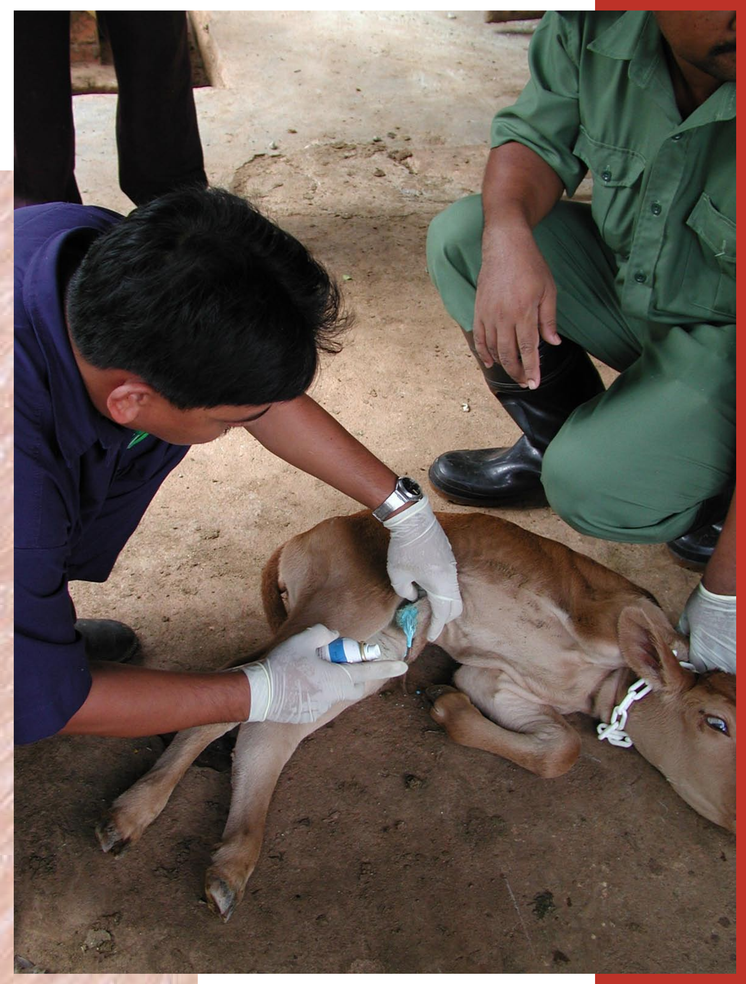
Distribution of Old and New World Screw-worm Fly.

The Institut Haiwan Screw-worm Fly Laboratory, Kluang, Malaysia was constructed to refine mass rearing techniques.

Larvae were reared on an artificial diet. Diet trays were housed within rearing cabinets that accurately controlled temperature and humidity for developing larvae. Trays containing diet were seeded with eggs, and larvae were fed additional diet as they matured.

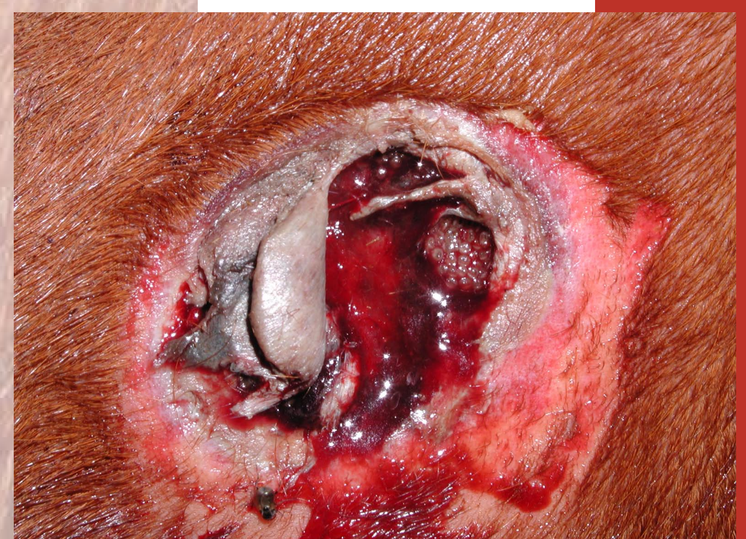


Large myiasis on the chest of a sheep.



Prophylactic treatment of the navel of a calf. The navel is a favoured oviposition site for OWSWF. Such myiases often lead to the death of the calf through peritonitis following the invasion of the body cavity by larvae.

This myiasis probably started as a wound around an ear tag. Over a period, much of the pinnae has been eaten by fly larvae.



Horn wound on the flank of a cow.
A cluster of large larvae can be seen in the upper right of the wound. Only the rear end of the larva bearing the spiracles is visible, with the larva in its normal head-down feeding position.

Field Assessment of 3 Years of Releases of sub-sterile males of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

IAEA-CN-131/153P

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**** : FAO, biological control expert.**

Introduction

The carob moth, *Ectomyelois ceratoniae* Zeller (Pyralidae), is a serious polyphagous pest of both stored products and field crops in the Mediterranean basin and near East regions. In Tunisia, it is the most important and destructive insect that attacks pomegranate and dates and causes great economic losses.

Many control methods were investigated against this pest in order to keep the wild population below economic levels.

Chemical control of this insect is not efficient because its larvae feed and develop inside the fruit (Dhouibi, 1989). The research of more effective control methods against this pest was then suggested. In this respect, the use of the autochthon parasitoids, the bio-insecticide and the genetic control method, namely the Sterile Insect Technique, are experimented against this pest (Dhouibi,1982; Jammazi, 1994; Charni, 1995; Omrane, 1996; Abderahmane, 2002; Mediouni et *al*., 2004)

The SIT research program is based on the radiation-induced inherited sterility and consists of releases of partially sterile males and fully sterile females. Field assessment of some parameters related to the ecology and behavior of irradiated insect showed a great interest before the decision of the application of such technique. Field's male and female behaviors after irradiation have to be evaluated and adjusted before and even during the releases.

Pomegranate infested with carab moth

Material and Methods

Experiments were undertaken in a 30 hectares pomegranate field near Tunis (Tunisia). Releases were done during the entire seasons with an approximately rate of 1000 irradiated insects/hectare/week. Releases were done for three successive years. The effective gamma dose used is 400 Gy. Radiation was carried out using a ^{60}Co irradiator (CNSTN, sidi thabet Tunisie ; dose-rate 460 Gy/min). Male's field dispersion was studied using a series of Delta traps baited with the synthetic pheromone of the insect and hanged to trees. From a release point chosen in the field, six levels of male capture were selected. These levels were 20, 40, 60, 80, 100 and 120 m from the release point. Insects' counting is done once a week.

Released insects were recognized from wild ones according to their red marked body (digestive duct is stained in red with the Calco-red mixed with the diet).

Two large field-cages (3x2.5 m) covered with a mosquito-net were placed onto two middle size trees in order to study male field competitiveness. In each cage, we hung a Delta trap baited with two virgin non- irradiated females. Three ratios of irradiated to normal males were investigated: 1:1, 2.5:1 and 5:1. Experimentations were replicated five times for each ratio.

Field cage

Delta trap hanged to pomegrenate tree

Results and discussions

Table 1: Weight and Quantities of irradiated and released insects during the 3 years of releases.

2001		2003		2004	
Weight (g)	Quantity	Weight (g)	Quantity	Weight (g)	Quantity
1735	86750	2120	106000	765	30250

1- Sub-sterile Male's Dispersion

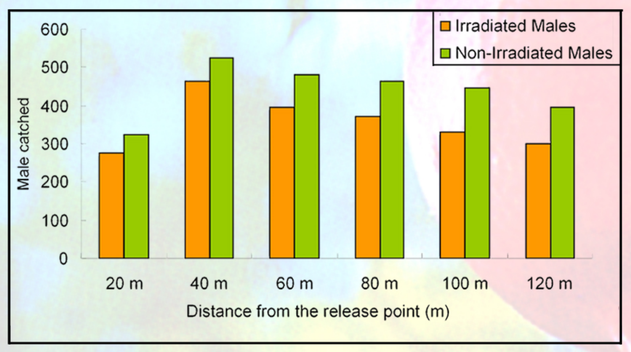

Figure 1: Field dispersion of irradiated and non- irradiated males of the Carob moth (2001,2003,2004)

Results showed that irradiation at the sub-sterilizing dose 400 Gy don't affect the male's ability of flying. Indeed, we observed that irradiated males are able to reach traps placed at 120 m from the release point.The number of captured males depends on the importance of the distance that separated them from the release point. The maximum of the captured irradiated males is obtained between 40m and 80m. In fact, traps placed at these distances presented the highest average of captures per week.

The area includes between 40 and 80 m from the release point is the region where the irradiated males disperse the most. Moreover, results showed that the dispersion of irradiated males is similar to the dispersion of non-treated ones. Nevertheless, the captures of normal males are slightly more important

Statistical analyses didn't show any significant differences for the distances 40, 60 and 80m.This result has a practical implementation.In fact, in order to get a homogeneous dispersion of irradiated males into the field, distances between 40 and 80 m should be kept between the release-points to cover the completely treated area and to get an unvarying dose of released insects.

2- Sub-sterile Male's Competitiveness in the field

Table 2: Field Competitiveness of irradiated males of *E. ceratoniae*

Ratio IM : NM (Repetition)	Total/cage	IM captured	NM captured
1 :1 (5x)	10 : 10	24 *a* (48.0%)	26 *a* (52.0%)
2,5 :1 (5x)	25 : 10	88 *b* (70.4%)	37 *a* (29.6%)
5 :1 (5x)	50 : 10	221 *b* (88.4%)	29 *a* (11.6%)

IM : Irradiated males, NM : Non-irradiated males

The field irradiated males competitiveness estimated if they are able to compete with wild ones and if they are able to fertilize females. According to our research, we note that when the ratio of irradiated to normal males increases in the cage, more their competitiveness is better. Indeed, the rate of capture was about 48% for the ratio 1 to 1 and reached 88% for the ratio 5 to 1. For the ratio 1 to 1, the difference between the percentages of captured males was not statistically significant despite the slight superiority observed with normal males. This result showed that irradiated males of *E. ceratoniae* with the sub-sterile dose 400 Gy remain attracted by virgin females and are able to compete with normal males in order to de copulate and fertilize these latest. We deduce consequently that this Gamma ray dose hasn't any undesirable effect on the reduction of competitiveness of irradiated males under field conditions.

Conclusion

After three years of weekly long-season releases, a preliminary assessment could be made for the possibility of the application of the SIT against the carob moth *Ectomyelois ceratoniae*. Indeed, results showed that irradiation at the sub-sterilizing dose 400 Gy don't affect the male's dispersion. Irradiated males disperse normally in the field and are able to reach traps placed at 120 m from their release point. The maximum of insect capture is obtained between 40m and 80m. This result has an important practical implementation to get a uniform and homogeneous dispersion in the field.

Results showed that sub-sterile males of *Ectomyelois ceratoniae* are competitive under field conditions at all the ratios tested. Besides, at the ratio 1: 1 irradiated males were as competitive as were the non- treated males. Moreover, in order to get a good level of field competitiveness, a ratio of 2.5 to 1 should be adopted during the releases. This suggests that before starting releases, an estimation of the wild population density has to be made.

IMPLEMENTATION OF IPM BY *Ceratitis malgassa* IN CITRUS GROVES IN MADAGASCAR

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In Madagascar the endemic fruit fly *Ceratitis malgassa* cause important damage particularly high in citrus where it is estimated between 50 to 70%. The scattering of cultivated and sub spontaneous fruits ,the spreading out of harvest, the transport and the sale of wormy fruits , the bad phytosanitary status in orchard and the multiplicity of hosts allows to the females of this fruit fly to find available fruits in the nature throughout the year. The producers control this pest by using chemical insecticides; they don't respect neither the dose of the chemical product nor the time of the application. The implementation of Integrated Pest Management is the monitoring of *Ceratitis malgassa* population.

In preliminary step, short term experiments should be engaged in order to precise the optimum trapping system. Three experiments are carried out in citrus groves.

Experiment 1: Determination of the response of *Ceratitis malgassa* to Torula Yeast:

It consists to compare the attractiveness of Torula Yeast to that of Bimunal.

Experiment 2: Definition of optimal trap for the planned attractants (Trimedlure and Torula Yeast pellets): Local Nadel type (cylindric yellow plastic box with 4 lateral opening), Tephry trap and Mac Phail trap will be tested.

Experiment3: Response of *Ceratitis malgassa* to the composite 3 lures.

In view of monitoring methods in the future

RESULTS AND DISCUSSION

For all experiments the orchards selected is square, homogeneous in terms of variety and age of tree, each plots is separated from other by a distance of 50 m, it is necessary to minimise the risk of attraction of flies from adjacent plot.

The result is based on counting of adults (female and male) per trap.

We used 2 pellets of Torula Yeast in 200 ml water per trap vs 250 ml of Bimunal.

Experiment 1: it included 5 replicates and lasted 2 weeks. The traps are set up on 07 Mars 2004. Dome traps are used and observations are made every week. The bait is replaced at every observation.

The result in Table 1 shows that Torula yeast is more significant attractive, the number of captured fruit flies in trap with this attractant is high, the response of female is distinctly appreciable.

Table 1: Number of *Ceratitis malgassa* adult captured per trap
TY = Torula yeast
B = Bimunal
f= female m=male

Location	14 Mars 2004		21 Mars 2004	
	TY	B	TY	B
Plot1	35f, 15m	18f,2m	30f,5m	13f,2m
Plot2	20f,12m	15f,3m	25f,10m	12f,3m
Plot3	40f,10m	15f,3m	45f,12m	15f,3m
Plot4	25f,15m	14f,2m	35f,10m	19f,3m
Plot5	22f,16m	15f,3m	25f,8m	14f,3m

Experiment 2: in this experiment we want to study what is the type of trap is optimal for the planned attractants. The trial started on 1 April 2004

2 types of traps are compared:

- For trimedlure we choose for local Nadel type trap and Tephry trap, both traps are baited with trimedlure dispenser and DDVP strips.
- For Torula Yeast, Tephry trap and Mc Phail trap are compared, both traps are baited with Torula yeast.

5 replicates are done for each trial during 2 weeks.

The results on table 2 and table 3 displays that for both attractants there are not significant difference, the number of fruit fly adult captured is nearly equal.Nadel trap is cheapest and the producers can make it easily.

Table 2: number of adult captured per trap baited with Trimedlure
NT = Nadel trap
TT = Tephry trap
f=female m= male

Location	8 April 2004		15 April 2004	
	NT	TT	NT	TT
Plot1	25f, 15m	20f,12m	20f,5m	23f,10m
Plot2	30f,12m	29f,10m	25f,10m	30f,12m
Plot3	28f,10m	30f,13m	31f,12m	35f,3m
Plot4	33f,5m	29f,2m	38f,10m	25f,3m
Plot5	25f,6m	28f,3m	25f,8m	34f,3m

Table 3 number of adult captured per trap baited with Torula Yeast
Mc P= Mc Phail trap
TT = Tephry trap
f=female m= male

Location	8 April 2004		15 April 2004	
	TT	Mc Phail	TT	Mc Phail
Plot1	35f, 15m	30f,12m	38f,5m	35f,10m
Plot2	40f,12m	39f,10m	42f,10m	38f,12m

Experiment3: this experiment is set up to determine the response of both sex of Ceratitis malgassa to the composite 3 lures (Ammonium Acetate, Trimethylamine, Putrescine), the result would be quite useful for monitoring methods in future control programmes and could be of use for control purposes (mass trapping in apple productions). Multi lure trap are used for this experiment.the trial started on 9 April 2004 and lasted for 2 weeks

the observation is done every week.

The result of capture appears in Table 4. We can notify that male are not attracted.If we compare with the result of other attractant the number of captured fruit flies is high. This kind of lure is interesting for monitoring because of its avilability and its cost the producers can not use it for control.

Table4: result of capture in multilure trap
F: female

Location	16 April 2004	23 April 2004
Plot 1	35 f 0 m	25 f 0 m
Plot 2	28f 0m	31f 0m
Plot 3	20f 0m	45f 0m
Plot 4	30f 0m	15 f 0m
Plot 5	40f 0m	37 f0m

Developing IPM Components for Leafminer Fly in the Cañete Valley of Peru

155P

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INTRODUCTION

The leafminer fly (LMF) *Liriomyza huidobrensis*, which is endemic to the neotropics, rapidly spread in the 1970s to other regions, and is reported today as an invasive pest in countries of Africa, Asia and Europe.

On the Peruvian coast, the Cañete valley is an important agricultural production area where LMF causes serious damage and losses in potato (*Solanum tuberosum*) and other vegetable crops (Fig.1). The short life cycle of LMF of only 2-5 weeks allows the development of up to 15 generations per year. Damage is caused by feeding and oviposition lesions of females but mainly by the mining of larvae in the potato foliage (Fig.2). Without insecticide application 90% and 41% LMF larval damage was recorded at harvest in the local potato varieties Revolution and Canchan, with yield losses of 53% and 50%, respectively. Farmers try to manage this pest with up to 13 pesticide applications. Therefore, the insecticide use presents the highest input costs with an average of US$ 600 per ha.

Fig. 1. LMF infestation on potato

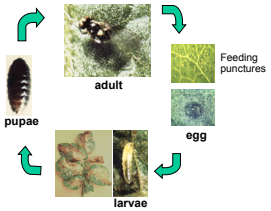


Fig. 2. Life cycle of LMF

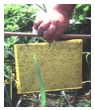
OBJECTIVES

We followed the hypothesis that pesticide use could be tremendously reduced by Integrated Pest Management (IPM). Therefore, our overall objective was to enhance our understanding of those factors that influence the population dynamics of LMF (both abiotic and biotic) and to test alternative control measure which included the evaluation of commercial cultivars for tolerance and the development of resistant potato clones, trapping devices, the selective use of insecticides, and biological control agents.

RESULTS

Population dynamics

Climatic conditions are favorable for the development of the fly. Monthly average temperatures fluctuate between 23.6 ºC (18.4 -28.7) in summer and 15.6 ºC (13.2 - 18.0) in winter. The highest LMF population and infestation occurred during the winter season (July to October), with peaks of over 5,000 flies/yellow sticky trap (20 x 20 cm), weekly. The autumn season (March to May) proved to be the best cropping period to escape infestation (Fig. 3).



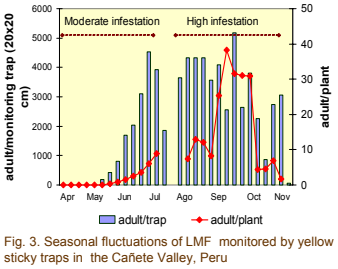


Fig. 3. Seasonal fluctuations of LMF monitored by yellow sticky traps in the Cañete Valley, Peru

Natural enemies

Parasitoids play a very important role in the natural regulation of the LMF population (Table 1 and Fig. 4), but are suppressed by insecticide applications. In fields with insecticide spraying, parasitism levels are low (<20%) but high in non-treated fields (up to 80%) (Fig.5).

Table 1. Diversity of parasitoid species of LMF at the coast of Peru, 2003

Superfamily	Family	Species	n	%
Ichneumonoidea	Braconidae	*Phaedrotoma* sp	411	2.7
		spp A	3	0.0
		spp B	70	0.5
Cynipoidea	Eucoilidae	*Ganaspidium* sp.	715	4.7
Chalcidoidea	Eulophidae	*Diglyphus begini*	517	3.4
		Diglyphus websteri	1512	10.0
		Chrysocharis caribea	1248	8.2
		Chrysocharis flacilla	2532	16.7
		Chrysocharis brethesi	92	0.6
		Chrysonotomyia sp. A	141	0.9
		Chrysonotomyia sp. B	69	0.5
		sp. A	12	0.1
		sp. B	44	0.3
		sp. C	22	0.1
		sp D	32	0.2
	Elachertinae	*Zagrammosoma* sp.	3	0.0
	Pteromalidae	*Halticoptera arduine*	7746	51.1
			15169	100.0

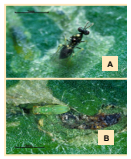


Fig 4. Adult (A) and pupae (B) of the ectoparasitoid *Diglyphus begini*

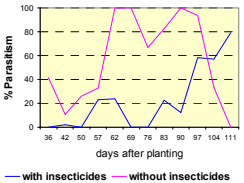


Fig 5. Differences in parasitism rates in fields with and without insecticide applications, Cañete, Peru

Paecilomyces fumosoroseus, a fungal insect pathogen, is considered a very promising biological control agent (Fig. 6). 60% mortality of LMF adults was recorded in bean (*Phaseolus vulgaris*).

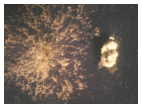


Fig. 6. LMF adults infected with entomopathogenic fungi at the coast of Peru, 2004

Plant resistance

Breeding work at CIP resulted in genotypes with reasonable levels of resistance against LMF (Fig. 7). In a participatory selection by farmers, two of the resistant potato clones perceived best ratings in terms of yield and tuber characteristics (Fig. 8).

Fig. 8. Participatory selection of potato clones resistant to LMF at field level (1 and 2) and culinary quality assessment (3), Cañete Valley, Peru 2003.

Fig. 7. CIP's potato clones with resistance (level between 1-25% foliar damage) to LMF larval damage selected under high LMF infestation, at 75 days after planting, Cañete, 2001.

Physical control

Yellow sticky traps (80 fixed traps/ha) and 9 applications of mobile traps compared to chemical control (three treatments) in potato cropping reduced LMF adult populations more efficiently (Fig. 9) and decreased insecticide application costs by 56% (Fig.10). Farmers quickly accepted the use of sticky traps as an LMF management component in potato and other crops (Fig.11).

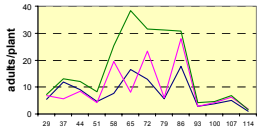


Fig. 9. Dynamics of LMF adults in potato after the use of yellow stick traps and insecticide applications, Cañete, Peru.

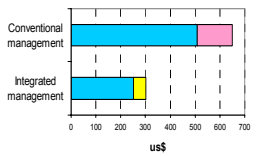


Fig. 10. Economic evaluation of LMF management in the Cañete valley, Peru

Fig. 11. The use of fixed and mobile sticky traps by farmers in the Cañete valley, Peru

Chemical control

Cyromazine, followed by abamectin and spinosad, controlled LMF larvae population by more than 75%, with a residual effect of more than 20 days (Fig. 12). However, LMF parasitoids were strongly affected. In contrast, abamectin had a much less detrimental effect (Fig.13). Reducing the costs of abamectin while keeping its efficacy was achieved by plant oil mixtures of low dosages of abamectin. The use of this selective but expensive compound has become an economically viable option for farmers (Fig.14).

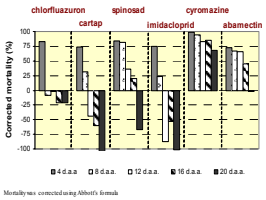


Fig.12. Efficacy of insecticides on the mortality[1] of LMF larva under field conditions, Peru, 2003.

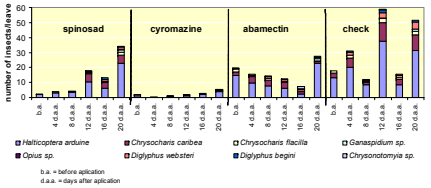


Fig.13. Effect of insecticides on the abundance of LMF parasitoids, Peru, 2003.

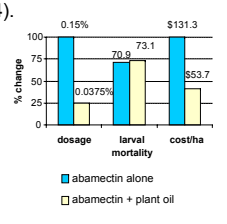


Fig.14. Effect of abamectin alone or mixed with plant oil mixed plant oil on LMF larvae, Peru, 2000.

CONCLUSIONS

- The introduction and use of IPM in the Cañete valley has reduced LMF infestation and pesticide application costs but LMF damage in potato and vegetable production is still considerable.
- Future research will focus on biological control either by inundative releases of parasitoids or the use of entomopathogens to control LMF adults as well as on the use of Sterile Insect Technology (SIT).
- The Peruvian coastal valleys with limited production areas surrounded by extended deserts should favor the use of SIT.

Insecticidal effects of oils extracted from aromatic plants on *Ceratitis capitata* Wied. In Lebanon

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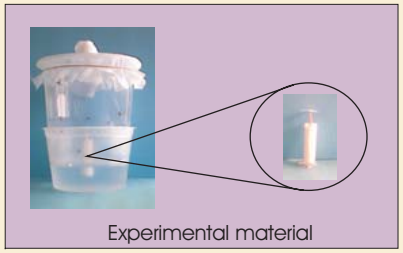


Experimental material

Artemisia herba alba

Mortality
After 24 h: 47.8%
After 48 h: 56.7%

Foeniculum vulgare

Mortality
After 24 h: 83.3%
After 48 h: 95.5%

Ruta chalepensis

Mortality
After 24 h: 34.4%
After 48 h: 51.1%

Mortality
After 24 h: 34.4%
After 48 h: 35,6%

Lavandula stoechas

Mentha microphylla

Mortality
After 24 h: 35.6%
After 48 h: 42.2%

Mortality
After 24 h: 41.1%
After 48 h: 61.1%

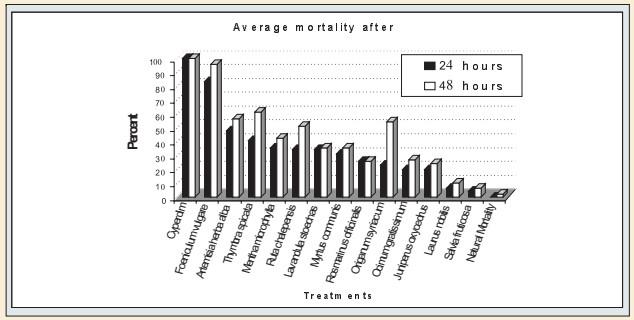
Thymbra spicata

Mortality
After 24 h: 31.1%
After 48 h: 35.6%

Myrtus communis

Natural mortality
After 24 h: 0.0 %
After 48 h: 2.2%

Mortality after pesticide application (cyperdim)
After 24 h: 100%
After 48 h: 100%



Treatments	Average mortality after 48 h (%)	Homogenous subgroups
Cyperdim (pesticide)	100	A
Foeniculum vulgare	95.5	A
Thymbra spicata	61.1	B
Artemisia herba alba	56.7	B
Origanum syriacum	54.4	B
Ruta chalepensis	51.1	B
Mentha microphylla	42.2	B C
Lavandula stoechas	35.6	C
Myrtus communis	35.6	C
Ocimum gratissimum	26.7	C D
Rosmarinus officinalis	25.6	D
Juniperus oxycedrus	24.4	D
Laurus nobilis	10.0	E
Salvia fruticosa	6.7	E
Natural mortality	2.2	E

List of the aromatic plants, their distillation yield (mL Ess.oil/100g of dry weight) and the major constituents of their essential oil

Foeniculum vulgare	1.108	Anethol, fenchone, fenchol, limonen fenchyl accetate.
Thymbra spicata	1.989	Thymol, camphor, camphene, borneol
Artemisia herba alba	0.625	Camphor, camphene, cymen,terpinolene
Origanum syriacum	1.269	Carvacrol,thymol,pinene,terpinene,carene
Ruta chalepensis	0.706	Orthophenylphenol, eucalyptol, santhene methylisoquinolene, camphor, borneol
Lavandula stoechas	0.207	Camphor, fenchone, myrtenol, borneol
Salvia fruticosa	2.054	Camphor, carene, terpineol, terpinene
Mentha microphylla	1.088	Menthol, menthone, sabinen, myrcene, terpineol, pinene,terpineol, cineol
Juniperus oxycedrus	0.027	Myrcene, limonen, fenchone, pinene
Rosmarinus officinalis	0.770	Borenol, camphor, carene, pinene
Myrtus communis	0.266	Myrtenyl acetate, myrtol, eucalyptol,cineol
Laurus nobilis	0.893	Methyl eugenol, eugenol,terpineol, cineol
Ocimum gratissimum	0.602	Thymol, para-cymene, linalool, myrcene

Abstract: The excessive use of chemical pesticides to control agricultural pests is becoming alarming particularly in Lebanon. The objective of this study is to search for biopesticides of plant origin that could be used to control one of the major pest of fruit production the Mediterranean Fruit Fly (*Ceratitis capitata* Wied.).
A colony of the Lebanese wild strain of this insect was reared under laboratory condition to provide biological material. The insecticidal activity of the essential oils extracted by hydrodistillation from thirteen aromatic plants spread in Lebanon was assessed.
The constituents of these essential oils were analyzed using a Gaz Chromatography/Mass Spectrometer (GC/MS) apparatus. Results show that essential oils having promising insecticidal potential are isolated from *Foeniculum vulgare, Thymbra spicata, Artemisia herba alba, Origanum syriacum* and *Ruta chalepensis*

Poster IAEA-CN-131/158P

FAO/IAEA International Conference on Area-Wide Control of Insect Pests : Integrating the Sterile Insect and Related Nuclear and Other Techniques

Control trial on *Glossina morsitans submorsitans*, *G. palpalis gambienis* and *G. tachinoides* in the Sudanese-Guinese zone of Mali, using deltamethrin impregnated traps with rural communities participation

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Funded by E.U. / PROCORDEL / CIRDES

Abstract: *Glossina palpalis gambiensis* and G *tachinoides* infest the Baoulé river and its tributaries in all the localities, with average apparent densities often higher than 30 flies per trap per day. *Glossina morsitans submorsitans* is widely distributed in all the biotopes. It's average rate of infection by the trypanosomes is 27.27%. 41.66% of the infections are due to *Trypanosoma vivax*, against 16.66% for *T congolense*. The low average rate of infection 3.20% observed with the BCT test on N'Dama cattles, is explained by their frequent treatments by trypanocidal drugs. The serologic ELISA test gave an average prevalence of 83.01%.

The installation of the impregnated traps with deltamethrin by the local communities of 10 villages, in zones at risks of trypanosomosis caused a drastic fall of the density of the flies populations. A total average reduction rate of 97.00% was obtained in villages of the southern part on the one hand, against 93.00% in villages of northern part on the other hand. In forest gallery an average reduction rate of 91.40 -100% was observed on *Glossina palpalis gambiensis*, against 88.75 – 97.75% on *G tachinoides*. In woody savannah, the average reduction rate of *G morsitans submorsitans* was 98.26 – 99.62%.

The installation of impregnated traps on other zones at risks to be identify by the space analysis (GIS) will increase without any doubt, the effectiveness of this control trial action against *G morsitans submorsitans*, the most dangerious sub species.

The tsetse flies and trypanosomosis control activities had a very positive impact on the livestock and the human population. Cattle population was increased by 24.0% between 2000 and 2004, despite the reduction of the purchases ; abortions and mortalities have also been considerably reduced. During this same period, the production of milk per cow passed from 0.5 liter per day to 1.5 liters per day, and donkey population was multiplied by 13. In 2000, before the control activities, the total loads for the cattle of the 10 villages were estimated at 8,301,120 Fcfa (1 EURO = 655.93 Fcfa) and the expenditure in trypanocidal drugs accounted for approximately 80%. In 2004 in favour of the control, the total expenditure of the 10 villages for the cattle fell to approximately 5.8 millions Fcfa (reduction of 30%). The expenditure in trypanocidal drugs accounts for only 45% of the total expenditure.

From 2000 to 2004 the added value of the cattle passed from approximately 74 million to 148 million Fcfa, in other words an increase of 100%. The total agricultural added value of the 10 villages passed from 137.7 million in 2000 to 368 million in 2004 (increase of 167%). The annual average cost of the control activities is approximately 85,000 Fcfa per village, that is about 3,251 Fcfa per farm. The control activities gives a marginal profitability rate of 6,593%, i.e. each 1 Fcfa invested in the anti-vectorial control is recovered with a benefit of 6,593 Fcfa. That shows that rural communities tsetse flies and trypanosomosis control is a very profitable economic activity.

The zone of intervention is located in the district of Yorobougoula of the Yanfolila "cercle" (Region of Sikasso) where the animal trypanosomosis constitutes a major constraint. The zone with semi-wet tropical climate of "soudano-guinéen" type (annual average rainfall = 1,300 mm), is infested by the three species of tsetse flies : *Glossina morsitans submorsitans*, *G palpalis gambiensis* and *G tachinoides*.

1. OBJECTIVES

: The general objective of this project is to support the effort of the village communities in the extention of the new methods of tsetse flies and animal trypanosomosis control and, consequently, to lead to an improvement of animal health and agricultural production by an increased use of the harnessed culture and the manure in a system of production integrated in the south of the country. Consequently, an increase in the incomes drawn from agriculture and breeding and a progressive attenuation of the poverty of the farmers may be achieved.

Photograph 1 : N'Dama Cattle

Photograph 2 : Cotton field

2. MATERIEL AND METHOD

: The entomological studies were carried out on the level of the points of controls indicated by the village communities like zones at risks of trypanosomosis transmission. The apparent densities (a number of tsetse flies/trap/day) were estimated by biotopes and locality. The proto-zoological investigations were carried out into 565 bovines of 9 villages. Blood drawn from the jugular vein using tubes containing EDTA for the research of the trypanosomes by the BCT and the harvest of plasma intended for the serological tests. K Othrine pm 25 (pyrethrinoid of synthesis, SOFACO, Roussel Uclaf Groups) is the insecticide used for the impregnation of the traps to the amount of 200 active matter mg for one square meter of fabric. The zootechnical studies concerned: annual evolution of the number of the herds, various sources of entry and exit of animals, appearance of animal species very sensitive to the trypanosomosis, and facility of access to the pastures lands. For the socio-economic impacts of the control activities, the analyses concerned: the characterization of the farms, variable loads and the added value of the cattle, the agricultural added value and the total added value, the annual cost and the marginal profitability rate of the control activities.

Photograph 3 : Mono conical trap « Vavoua »

Photograph 4 : Biconical tra CHALLIER-LAVEISSIERE

Table 1: Principal sources of entry and causes of exit of the animals in the herds (%)

	Year		Mean ± standard error	Level of significance
	2000	2004		
Entry	77.5	92.5	85.0 ± 2.9	HS
Birth in the herd	22.5	7.5		HS
Purchase			15.0 ± 2.9	
Exit	34	14	11.7 ± 2.9	VHS
Mortality				
Sale	50.2	69.0	59.6 ± 6.6	NS

Photograph 5 : Dam of water reserve of Gouna

3. RESULTS

Glossina palpalis gambiensis and *G morsitans submorsitans* are present in the localities of Nyomassala, Linfara and Bourakala villages, with an apparent average densities often higher than 30 tsetse flies/trap/day. Very high densities of *Glossina sp* infest the Ranch of Madina-Diassa. They reached 21 for *G tachinoides* and 54 for *G palpalis gambiensis* along the Baoulé river, and 122 for *G morsitans submorsitans*. The average infection rate of *G morsitans submorsitans* by the trypanosomes is 27.27%. 41.66% of the infections are due to *Trypanosoma vivax*, against 16.66% due to *T congolense*.

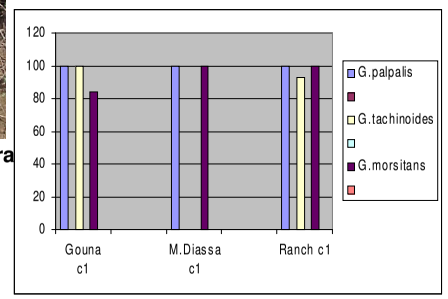


Figure 3: The apparent density reduction rate of *G.palpalis gambiensis* and *G.tachinoides* in gallery and *G morsitans submorsitans* in woody savannah, one year after the installation of the impregnated traps on the level of the villages of Gouna, Madina-Diassa and ranch of Madina-Diassa

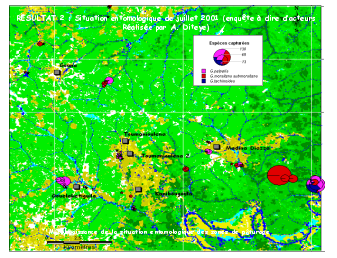


Image 1 : Apparent density of tsetse flies species

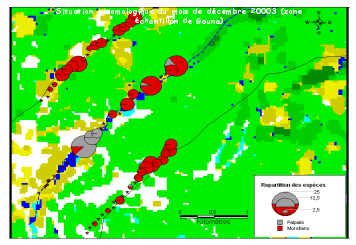


Image 2 : Ecodistribution of the tsetse flies along the transects

On 593 samples examined in the 9 villages, only 19 positive cases were detected with the BCT (3.2%). We found a broad prevalence (89.5%) of the infections with *Trypanosoma congolense* (17/19) compared to *T vivax* (2/19 or 10.5%). The serological results indicate an average prevalence of 83.01% (varying from 69.56% to 90.90%).

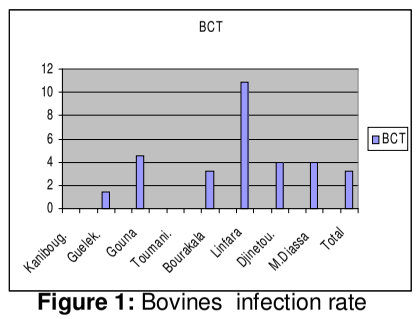


Figure 1: Bovines infection rate by trypanosomes, detected with BCT

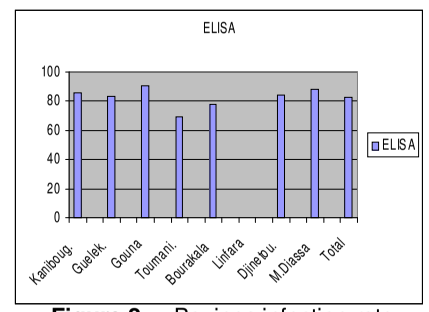


Figure 2 : Bovines infection rate by trypanosomes, detected with ELISA

Before the installation of the impregnated traps the average apparent density of *Glossina sp* was 15.71 tsetse flies/trap/day at the level of the villages of Gouna, Madina-Diassa and of the Ranch. After the control activities it fell to a value between 0.32 and 0.57, what corresponds to a total reduction average rate of 97.96% and 96.37% for the first and second surveys, respectively (Figure 3 and Figure 4).

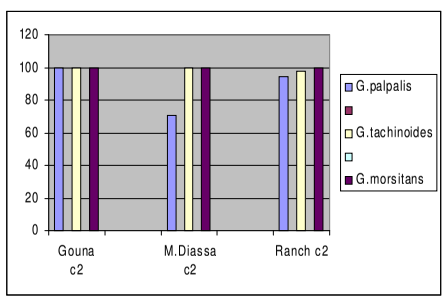


Figure 4: The apparent density reduction rate of *G.palpalis gambiensis* and *G.tachinoides* in gallery and *G morsitans submorsitans* in woody savanna, two years after the installation of the impregnated traps on the level of the villages of Gouna, Madina-Diassa and ranch of Madina-Diassa

4. CONCLUSION AND PROSPECTS

The high reduction rate of the apparent density of the tsetse flies populations is explained by the effectiveness of the strategy used and the perfect knowledge of the zones at the risks by the local communities. It is necessary to also announce the weak rainfall of 2002 which caused a big concentration of the tsetse flies on the level of the permanent rivers, target zones, making them more vulnerable to the control action. The relatively weak increase of the apparent density during the second evaluation, is explained by the rate of recolonisation, because control was carried out immediately after the rains, before the installation of the traps for the following control activities. The installation of traps impregnated of deltamethrin by the rural communities caused a drastic reduction in the populations of tsetse flies during two years of control. The continuation of the anti-vectorial control will decrease the risks of re-infestation of the cleansed zone and will contribute to consolidate the assets. The installation of impregnated traps in other zones at risks are to be identified by the space analysis (GIS) wihich will increase without any doubt, the effectiveness of this control trial action against *G morsitans submorsitans*, the most dangerious sub species.

From 2000 to 2004, the increase of 100% of the added value of the cattle (of 74 Fcfa million to 148 Fcfa million), and in 167% of the agricultural total added value (of 137,7 million to 368 Fcfa million), is due mainly to the action of tsetse flies control activities, as well as to other activities undertaken by ONDY and CMDT (training, herd and ranch management, construction of dams, improvement of the farming techniques, ect..).

Socio-economic analysis of the control activities: In 2000, before the control, the total expenditure for the cattle of the 10 villages amounted to 8,301,120 Fcfa and the expenditure in trypanocidal drugs accounted for approximately 80% of this total amount. In 2004 in favour of the control, the total expenditure of the 10 villages for the cattle droped to 5,791,240 Fcfa (reduction of 30%). The expenditure in trypanocidal drugs accounts for only 45% of the total expenditure. From 2000 to 2004 the added value of the cattle passed from 73,684,380 Fcfa to 148,146,760 Fcfa, that corresponds to an increase of approximately 100%. The minimum of increase recorded in Guélékétiguila is of 82%, while the maximum reched of 165% is recorded in Linfara (figure 5). This increase is explainde by the anti-vectorial control activities which allowed an increase of number of bovines and the production of milk per cow which passed from 0.5 liter per day in 2000 to 1.5 liters per day in 2004.

Table 2: The marginal profitability rate of the anti-vectorial control (in %)

Years	Total costs (Fcfa)	Benefit (Fcfa)	Marginal Profitability Rate
2000	6 451 441	137 735 095	6,593 %
2004	9 946 882	368 206 969	

Developing an SIT Program for False Codling Moth: *Effect of Different Overflooding Ratios on the Incidence of Damage under Controlled Conditions*

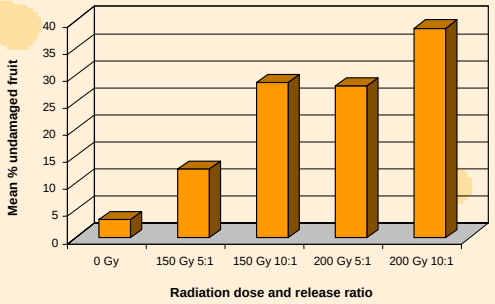
J. Hendrik Hofmeyr, Citrus Research International, South Africa
James E. Carpenter, USDA-ARS-CPMRU, USA
Stephanie Bloem, Consultant - IAEA, USA

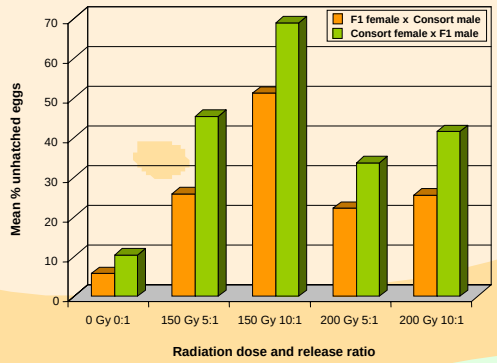
- The false codling moth (FCM) is the **key pest** of many citrus cultivars in southern Africa and a serious pest of cotton and maize in tropical Africa. FCM has developed resistance to pesticides and other control options have had limited success.
- We investigated the effect of different doses of radiation (150 Gy and 200 Gy) and overflooding ratios (5:1 and 10:1) on the incidence of fruit damage in field-cages.
- All treatments **significantly reduced** the number of **larval injuries** to fruit and the number of **damaged fruit**. In cages receiving irradiated FCM at a 10:1 (T:N) ratio, the mean number of undamaged fruit was 8-10 times greater than in control cages.
- In addition, we saw **significant reductions** in …
  - the mean **number of F1 adults** produced when the cage treatment included the release of irradiated FCM.
  - the **fertility of F1 adults** produced in cages receiving irradiated FCM, indicating that irradiated males competed successfully for un-irradiated females.
- Furthermore, the mean number of fertile males and fertile females produced in the F1 generation was significantly greater in the control cages than in cages receiving irradiated FCM, resulting in a higher rate of increase from the P1 to the F1 generation in the control cages.

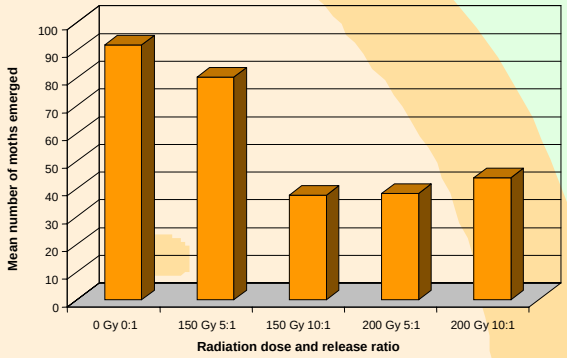
  The potential impact of releasing 150 Gy treated FCM was projected for several generations to simulate the typical SIT scenario in which irradiated FCM would be released continuously from the beginning of the growing season when wild FCM populations would be low.
- The increase in the number of fertile FCM in a control population was compared with the increase in the number of fertile FCM in a population subjected to releases of 150 Gy treated FCM. The control population began with 10 pairs of fertile FCM in generation 1 with a mean reproductive rate of 4.52× per generation. The treatment population also began with 10 pairs of fertile FCM, and was subjected to a release of 100 pairs of 150 Gy treated FCM (10:1 overflooding ratio) at the beginning of the first three generations, which reduced the mean reproductive rate for males and females to <1× per generation (0.935×). In this model based on the data collected in our field-cage study, the fertile population receiving 150 Gy treated FCM declined slightly while the fertile population in the control cages increased by more than 9,000%. These results support the further development and assessment of the SIT as a control tactic for the FCM.

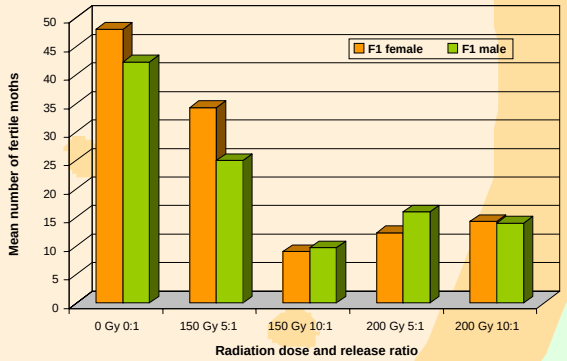
Single orange trees were enclosed in insect-proof nylon mesh cages. Fallen fruit were collected daily and kept individually in covered containers to allow larvae to develop.

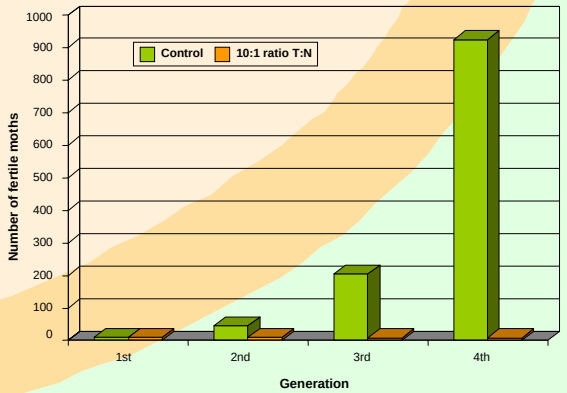
Dose (Gy)	# irradiated (T) pairs	# unirradiated (N) pairs	Release ratio T:N
0	0	10	0:1
150	50	10	5:1
150	100	10	10:1
200	50	10	5:1
200	100	10	10:1











Estimated increase in the number of fertile FCM in a control population compared with a population subjected to releases of irradiated (150 Gy) FCM.

Radiation Biology and Inherited Sterility in False Codling Moth (Lepidoptera: Tortricidae)

J. Hendrik Hofmeyr, Citrus Research International, South Africa
Stephanie Bloem, Consultant - IAEA, USA
James E. Carpenter, USDA-ARS-CPMRU, USA

- The false codling moth (FCM) is an indigenous pest to southern Africa, the Ethiopian Region and several African islands. It is the **key pest** of many citrus varieties and attacks many other annual and perennial crops.
- FCM is currently **not present in the USA**. Many Federal and State Agencies have expressed concern that FCM could soon be introduced into the USA as a direct result of increased trade and tourism. Because FCM infests so many different hosts, and because it would be a quarantine issue for important commodities, establishment of FCM in the USA could result in economic losses in the billions of dollars.
- FCM has documented resistance to pesticides and other control strategies have had limited success and cannot be used as stand-alone tactics.

We are developing the sterile insect technique for FCM that could be used as an area-wide suppression tactic in South Africa and as an eradication tool should FCM be introduced into the USA.

- We examined the effect of increasing doses of gamma radiation on the **fecundity and fertility** of FCM **(Figs. 1 & 2)**.
- We determined the minimum dose at which irradiated FCM females were 100% sterile when mated to un-irradiated (fertile) males **(A)**.
- Four doses were chosen for documentation of inherited sterility effects in the F_1 generation. Mortality during development **(B)**, sex ratio distortions **(C)**, and fecundity and fertility **(Figs. 3 & 4)** of the F_1 generation resulting from irradiated (P_1) males and un-irradiated (fertile) females are presented here. Finally, the minimum dose at which irradiated FCM (P_1) males produce 100% sterile F_1 offspring is also reported **(D)**.
- As a result of these findings, we conducted research in field-cages to determine the effect of different doses and overflooding ratios on fruit damage **(poster 160P)**. A season-long SIT validation trial is planned for August 2005 in South Africa.

Results – Parental (P_1) Generation

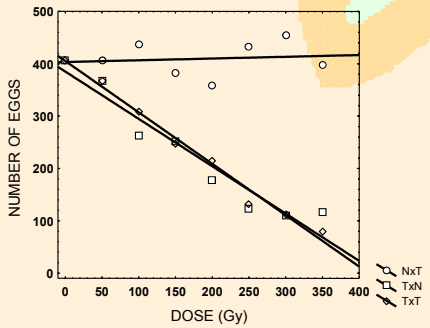


Fig. 1. Effect of dose on **fecundity** (mean number of eggs laid) per mated female. Males and females were irradiated (T) with 0, 50, 100, 150, 200, 250, 300, & 350 Gy and inbred (T♀ x T♂) or out-crossed to un-irradiated (N) moths (T♀ x N♂, N♀ x T♂).

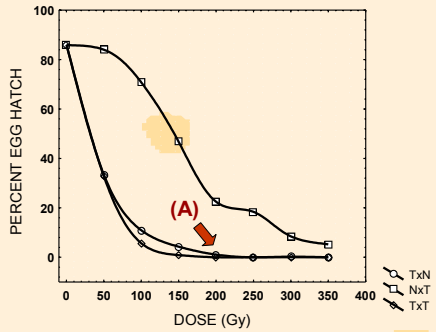


Fig. 2. Effect of dose administered to FCM P_1 adults on the **fertility** (mean percentage of eggs that hatched).

Results – First Filial (F_1) Generation

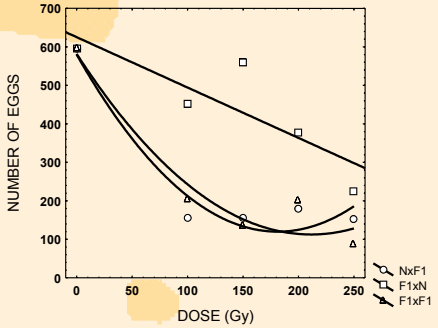


Fig. 3. Effect of dose on **fecundity** of the F_1 generation. Irradiated males were mated with un-irradiated females (N♀ x T♂). The F_1 progeny obtained were inbred (F_1♀ x F_1♂) and out-crossed (F_1♀ x N♂, N♀ x F_1♂) to un-irradiated moths.

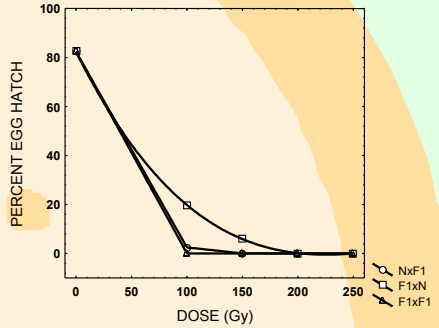


Fig. 4. Effect of dose on **fertility** in the F_1 generation.

FCM Inherited Sterility data using an N♀ x T♂ mating producing 500 eggs as an example.

Dose (Gy)	% eggs hatched	# F_1 eggs hatched	% mortality during F_1 rearing	# of F_1 adults emerging	Sex ratio of F_1 ♂: ♀	# of F_1 ♂: ♀ emerging
0	81.7	**409**	44.7	**226**	0.80:1	**100:126**
100	67.4	**337**	57.7	**143**	1.72:1	**91:52**
150	55.6	**278**	82.6	**48**	3.72:1	**38:10**
200	37.8	**189**	86.0	**26**	5.90:1	**22:4**
250	25.3	**127**	91.5	**11**	29.3:1	**10.6:0.4**

(B) (C)

FCM Induced & Inherited Sterility for Parental (P_1) and First Filial (F_1) generations.

Dose (Gy)	% sterility for P_1 ♂	% sterility for F_1 ♂	% sterility for F_1 ♀
0	18.3	13.7	13.7
100	32.6	99.2	75.3
150	44.4	100	93.9
200	62.2	100	99.7
250	74.7	100	100

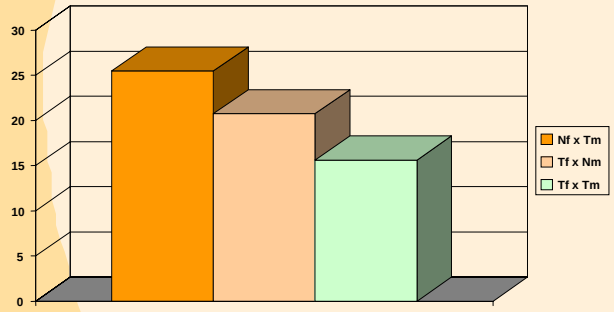
(D)

Acceptability and Suitability of Eggs of False Codling Moth from Irradiated Parents to Parasitism by *Trichogrammatoidea cryptophlebiae*

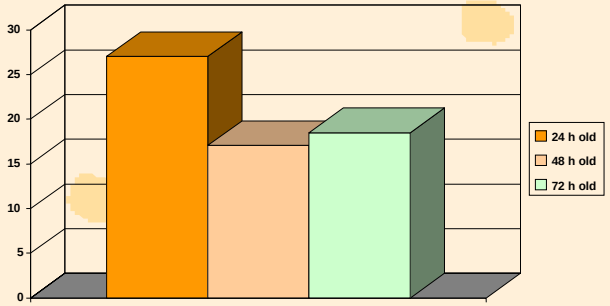
J. Hendrik Hofmeyr, Citrus Research International, South Africa
James E. Carpenter, USDA-ARS-CPMRU, USA
Stephanie Bloem, Consultant - IAEA, USA

- The false codling moth (FCM) is the key pest of citrus in South Africa as well as a serious pest of cotton and maize in tropical Africa.
- Presently, the augmentative biological control program using the egg parasitoid *Trichogrammatoidea cryptophlebiae* is insufficient to treat all FCM susceptible citrus in South Africa.
- We are conducting research to develop an area-wide pest management program for FCM that would employ a combination of SIT, releases of *T. cryptophlebiae* and orchard sanitation.
- In SIT programs for Lepidoptera both irradiated males and females are released into the environment resulting in a large number of sterile host eggs being laid in areas under SIT. These sterile eggs might serve as host material for the egg parasitoid. As such, knowledge of the compatibility of *T. cryptophlebiae* and the release of irradiated FCM is crucial to the evaluation of the combined use of these tactics.

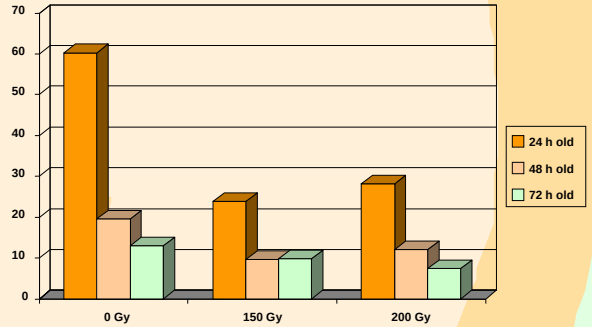
- We examined the acceptability and suitability of FCM eggs from irradiated and untreated moth pairs to parasitism by *T. cryptophlebiae* in the laboratory under no-choice and choice situations.
- Male and female FCM adults were treated (T) with 150 or 200 Gy of gamma radiation, inbred or out-crossed to untreated (N) counterparts, and eggs laid by different FCM pairs were offered to *T. cryptophlebiae* as host material. Newly laid (24 h old) eggs, as well as eggs that were 48 h and 72 h old were evaluated.
- All treatments in the no-choice experiments were acceptable for oviposition and suitable for parasitoid development.
- Significant differences in the number of parasitized eggs were found when one member of the FCM pair, particularly the female, was irradiated, or when the FCM egg age was greater than 24 h.
- We found significant interactions between dose of radiation and FCM cross with respect to the number and fitness (as measured by tibial length in female wasps) of emerging wasps.
- Our results suggest that *T. cryptophlebiae* would accept, successfully develop in, and emerge from eggs laid by the different FCM pairs that would theoretically occur in the field under an SIT program (N♀ by T♂, T♀ by N♂, T♀ by T♂) for FCM. We also suggest that further evaluations in the field combining releases of irradiated FCM and parasitoids are warranted.



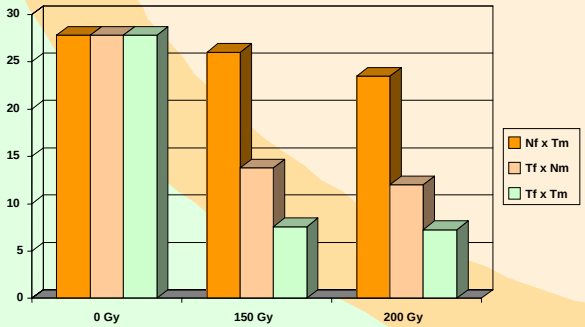
Effect of FCM cross on the **mean number of FCM eggs parasitized by *T. cryptophlebiae***. N = untreated adult moths and T = moths treated with gamma radiation (150 or 200 Gy).



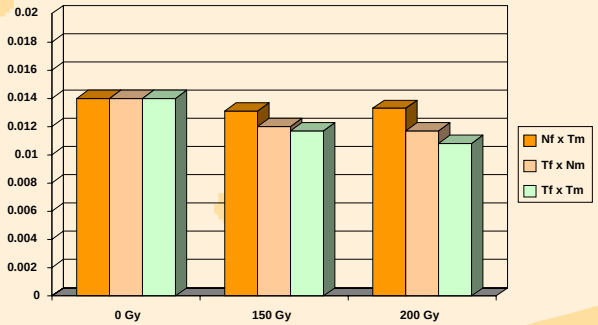
Effect of host egg age on the **mean number of FCM eggs that were parasitized** by *T. cryptophlebiae.*



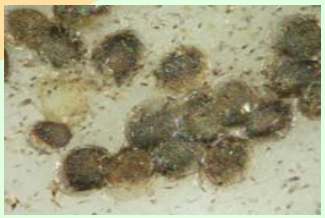
Percentage of FCM eggs parasitized by *T. cryptophlebiae* as influenced by FCM egg age and dose of radiation.



Mean number of parasitoids emerging as influenced by type of FCM cross and the dose of radiation used to treat the female (f), the male (m) or both members of the host pair.



Mean tibial length (mm) of female parasitoids emerging from FCM eggs as influenced by cross and dose of radiation.



Sexual Competitiveness of Irradiated Male *Glossina palpalis gambiensis* Reared *in vitro* for more than 20 years

IAEA-CN-131/163P

I. Kaboré[a], H. Kam[ba], and A.M.G. Belem[b]

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Introduction

The sterile insect technique (SIT) was successfuly applied in 1984, in the pastoral zone of Sideradougou (3500 km2), West of Burkina Faso, where *Glossina palpalis gambiensis*, *Glossina tachinoides* and *Glossina morsitans submorsitans* were occurring. Since this date, colonies of the three species are maintained on an *in vitro* system at CIRDES.

The present study dealt with the assessment of the quality of reared *G. p. gambiensis* males. The effects of irradiation and the number of successive matings on sexual potency, sperm transfer ability and the survival of these males flies are evaluated.
In a second phase, mating competitiveness tests were carried out between these males irradiated with a dose of 112 Gy and unirradiated wild males captured from the field in Mali.

Feeding system

Materials and Methods

Maintenance of the flies

The *G. p. gambiensis* colonies are maintained at 23 -25 °C and 70 - 85% RH on a 6 days per week feeding regimen, using fresh defibrinated bovine and porcine blood.

Irradiation of the flies

Three experimental groups each of 90 males aged 6 days were irradiated from a gamma source, *Cæsium*^{137}, at doses of 102, 112 and 122 Gy.

The control group comprised of 90 males, aged 6 days and unirradiated.

Successive matings of the flies

The 3 experimental groups and the control are successively, six times, introduced into mating cages with 2-3 days females, at 48 hours interval and at a ratio of 1/1.

Mating competitiveness tests

Reared Males:
-In vitro feeding
-Marked with a dot of polymer paint
-Irradiated at 112 Gy

Wild Males and females:
-In vivo feeding
-Not marked with polymer paint
-Unirradiated

Field cage, IAEA, MUTIKA, 2002

Conclusion

- The rates of survival of the irradiated and unirradiated males are similar with about 76% in 2 weeks, and 20% after 45 days.
- The irradiated males have shown a good mating activity and sperm transfer capacity.
- The induced sterility during the six successive matings varied from 89,83 to 95,22%.
- Irradiated and unirradiated males had better mating perpormance.
- Sterilization and the six successive matings had any significant effect on the survival, behavior or sexual performance of the reared fly.

Acknowledgements: We would like to thank IAEA, CIRDES, the Colleagues from Mali and Burkina Faso for financing and supporting this work.

Pre-mating period, mating duration and spermathecal fill of G. p. gambiensis females from Mali mated with irradiated, reared males and wild males

	Pre-mating period (min)		Mating Duration (min)		Spermathecal fill (%)	
Males	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated
Mean s.e.	88.66 8.43	78.47 8.69	49.45 4.59	49.82 2.76	76.65 7.22	74.65 6.35
Maxim.	124.80	117.20	73.80	66.62	100.00	100.00
Minim.	52.00	45.71	29.80	37.00	22.50	34.00

	Dose (Gy)	Induced Sterility (%)	Pupea number	Pupea Weight (mg)	% EM
Cont	0	0	1346	24,35	97,25
Gp 1	102	89,83	141	22,10	85,82
Gp 2	112	94,25	81	22,07	83,95
Gp 3	122	95,22	70	22,15	92,86

Induced sterility, Pupea production and Emergence rate

	Cont	GP1	GP2	GP3
A1	100	98,39	100	100
A2	100	98,36	98,59	96,43
A3	96,36	98,59	94,74	94,20
A4	85,71	86,76	86,15	89,71
A5	84,75	60,66	67,19	63,16
A6	76,36	76	85,45	78,43
Mean	90,53	86,46	88,69	86,99

Female insemination rates

Degree of spermathecal fill

	Cont	GP1	GP2	GP3
A1	97,8[a]	92,7[a]	95,75[a]	88,95[a]
A2	92,1[a,b]	92,7[a]	95,55[a]	92,85[a]
A3	86,35[b]	94,55[a]	86,5[b]	86,75[a,b]
A4	64,05[c]	68,2[b]	71,75[c]	81,45[b]
A5	57,4[d]	39,75[c]	35,4[d]	48,7[c]
A6	47,05[e]	47,00[d]	49,00[e]	56,15[d]
Mean	72,92*	73,65*	74,01*	75,61*

Mating Indices for *G. p. gambiensis*

	Relative Mating Index		Mating propensity	Relative Mating Performance
Day	Irradiated	Unirradiated		Irradiat. vs Unirrad.
1	0,125	0,875	0,267	-0,0938
2	0,643	0,357	0,467	+0,0204
3	0,500	0,500	0,357	0,0000
4	0,619	0,381	0,700	+0,0113
5	0,519	0,481	0,900	+0,0014
6	0,563	0,438	0,533	+0,0078
7	0,556	0,444	0,300	+0,0123
8	0,769	0,231	0,433	+0,0414
9	0,467	0,533	0,500	-0,0044
10	0,294	0,706	0,708	-0,0242
Mean s.e.	0,505 0,058	0,495 0,058	0,517 0,063	

SIT for control of the malaria vector, *Anopheles arabiensis* in Northern State, Sudan: An historical view

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INTRODUCTION

Historical data is presented relevant to current research on a field site in Northern state, Sudan (Figure 1), where control of the malaria vector *Anopheles arabiensis* by the sterile insect technique is planned. The target area is primarily the Dongola Reach of the Nile, between the Fourth and Third Cataracts. The mosquito is restricted to the Dongola Reach by desert and inhospitable terrain at the cataracts. A new dam below the 4th Cataract will isolate it further. Here at the edge of its distribution, the survival of *An. arabiensis* is linked to human activity. Therefore historical data on human settlement patterns can be used to infer its likely presence or absence. As this region corresponds to Upper and Middle Nubia, it has a very rich and ancient history with many important archaeological sites.

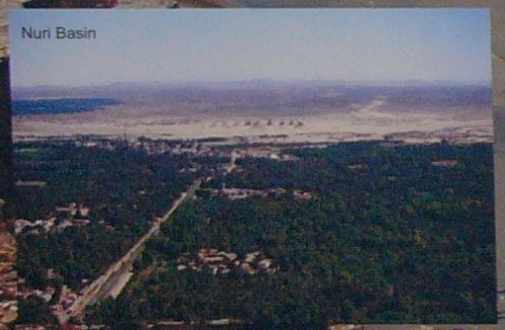
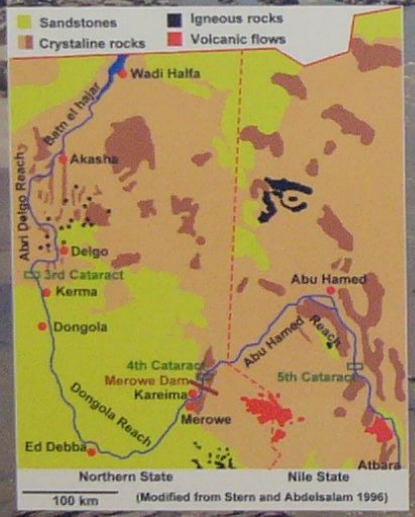


FIGURE 1 Geology Northern State



18,000 – 3,500 years ago

Mosquitoes will not have survived the hyper-arid northern Sudan of 18,000 YA, but presumably flourished 10,000 years later when the area was tropical grassland and human settlements were re-appearing (Figure 2). By the Neolithic (7,000 YA) agropastoralism and livestock management was increasing alongside expanding occupation of the eastern palaeochannels of the northern Dongola Reach.

The area was densely populated from 5000 to 3500 YA (the Kerma Period), forming the centre of the Kingdom of Kush, which extended to settlements in the Wadi Howar and 4th Cataract region (Figure 3). Livestock prevailed and large wild animals including giraffe, hippopotamus, lions, monkeys, antelope and elephant were still present. A sizable mosquito population appears certain and probably not isolated from outside the region, although the area was by now reverting to semi-desert and so riverine links will have been critical for migration.

3500-1500 YA

New Kingdom Egyptians conquered the Kushites, Kerma was destroyed, the palaeochannels dried up, and the settlements along the Dongola Reach disappeared. The Kushite Kingdom then underwent a revival 2100 YA that was to last over 1000 years and include the conquest of Egypt. However the settlement pattern remained one of relatively isolated urban centres (Figure 3), which will still have promoted fractured and isolated *An. arabiensis* populations.

1500-200 YA

If the vector survived, conditions progressively improved in medieval times following the introduction of the *saqia* waterwheel. Improved farming methods allowed settlements to spread in a less interrupted pattern, as exists today. In this period, droughts, major floods, the associated famines, conflict and population movement, and major upheavals caused by political change, will all have taken their toll on humans and mosquitoes alike, but for periods of years or decades, rather centuries or millennia.

FIGURE 2 Vegetation

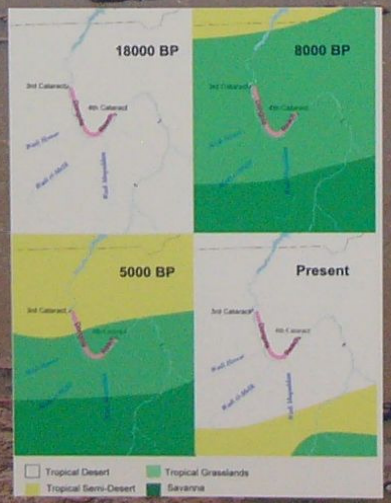


FIGURE 3 Settlement Patterns

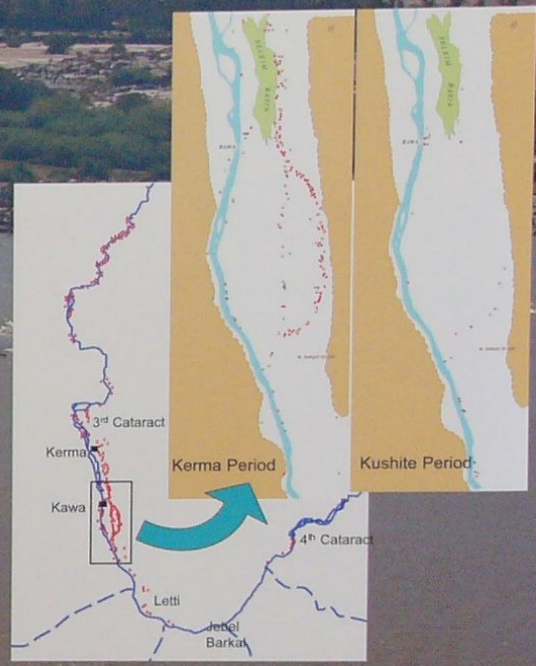
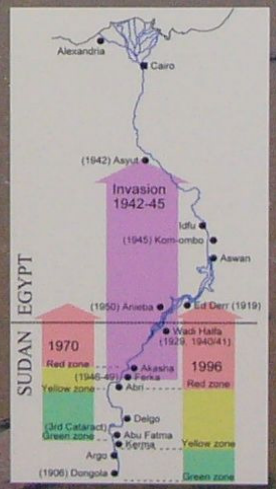


FIGURE 4 Records of An. arabiensis



LAST 200 YEARS

There is no archaeological evidence for malaria in Northern State yet, but there is from neighbouring Nile State from 1500 YA and much earlier from Egypt. Various 19th century visitors to Dongola recorded malaria-like illness, so the vector, previously known as *An. gambiae*, was presumably present, but it was not directly recorded until 1906/07. Figure 4 gives dates (shown in brackets) next to the location of the most northerly record of *An. arabiensis* at that time. In 1942 it reached Askut 850 km into Egypt causing a major malaria outbreak. In 1976 the Aswan High dam became operational creating Lake Nasser. In the 1970s the Egyptian Sudanese Gambiae Control Project started annual campaigns to try and push the vector back upriver.

Development and Field Release of Genetic Sexing Strains of the Melon Fly, Bactrocera cucurbitae, and Oriental Fruit Fly, B. dorsalis, in Hawaii

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EXOTIC FRUIT FLY SYMPOSIUM RIVERSIDE, CALIFORNIA

Introduction:

The melon fly, *Bactrocera cucurbitae* (Coquillett)and the oriental fruit fly, *B. dorsalis* (Hendel), are serious economic pests in Asia and the Pacific. Control of tephritid fruit flies has traditionally been carried out by chemical means, such as protein bait sprays or male annihilation, but more recently biological control techniques, especially the sterile insect technique (SIT), have gained wider use due to their environmentally benign nature.

It is known that the efficiency of the SIT can be improved several fold with genetic sexing strains in which only males are released (McInnis et al. 1994, Rendon et al. 2004). A pupal color sexing strain for the oriental fruit fly was developed by McCombs and coworkers (1995), while one for the melon fly was developed recently by McInnis and coworkers (2004). In each of these cases, chromosomal translocations link the gene for normal brown pupae color to the male sex (Y chromosome) resulting in brown pupae males, while females have the mutant white pupal color. Using this sexing system, photoelectric sorting machines are used to separate males from females at high speed in order to obtain the large numbers of males for release in SIT programs.

In this study, we report the discovery and development of the first pupal color-based sexing system for the melon fly and the first large-scale production and all-male sterile fruit fly releases for both species. A series of quality control studies were completed under both laboratory and field conditions. These studies indicated that both sexing strains were very competitive against wild flies; therefore, we proceeded to mass-produce and field release both species in several test sites in Hawaii between 2002-2005. Both sexing strains performed very well, inducing significant egg sterility into wild populations under conditions of low sterile:wild fly ratio, resulting in high population suppression.

Sexing System:

Both species can be sorted by sex using pupal coloration as the mechanism- males emerge from brown pupae and females from white pupae (Fig. 1). Genetic recombination to produce brown pupae females or white pupae males occurs only rarely in these species- less than 1 per 50,000 or 10,000 for melon fly and oriental fruit fly, respectively.

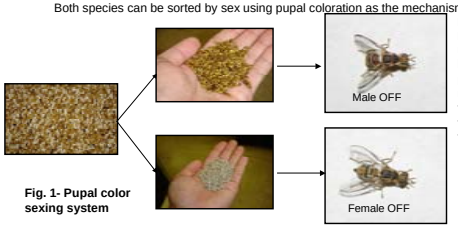


Fig. 1- Pupal color sexing system

Quality Control Tests:

Prior to committing to mass production, we tested the two sexing strains in field cages for survival and mating competitiveness compared to wild or wildish (recently colonized) strains (Fig. 2). We have found males of the sexing strains to be equally competitive with their wild counterparts in both survival and mating ability. A significant result of the mating cage tests was that for melon flies an adult diet of sugar only led to zero matings, while for the oriental fruit fly very few matings were recorded (T. Shelly, personal communication). This dramatic result for sugar-only fed males contrasts sharply with the case for the medfly in which normal mating can take place for sugar-only fed males under similar conditions. The performance of the sterile melon fly males receiving low or moderate amounts of protein were not significantly different (Tukey's t-test) (P= 0.11).

Fig. 2- Field cage for mating and survival ability

Mass-rearing, dying and irradiation:

Following the encouraging initial small scale rearing and field cage studies, we initially focused on the melon fly as the main target pest for the ongoing USDA/ARS IPM program in Hawaii. Flies were reared to pupation in Honolulu then pupal color sorted using high-speed photoelectric sorting machines (Fig. 3 photos). At ca. 2 days prior to emergence the all-male pupae were dyed with a standard fluorescent dye to mark emerging adults, irradiated in a Gammacell 220 unit at 100 Grays, then shipped to the field test sites (Fig. 4 photos).

Fig. 3- Larval diet, mixed brown and white pupae, photoelectric sorter, sorted pupae

Fig. 4- Dying, bagging, and irradiating pupae

Melon and oriental fruit fly test sites:

The first area of study was against the melon fly on the Big Island of Hawaii in 2002 (Fig. 5 photos). Fly releases were made inside a 12 sq. km. residential area. A control area free of sterile flies was maintained also for baseline sterility and wild fly population density estimates. Subsequently, sterile flies (500,000-1,500,000 once per week) were released on Maui (2003) and Oahu (2004) with good results (Fig. 9). Egg sterility rose to above 70% in the treated area compared to ca. 10% in the control area, while the sterile:wild fly ratio was only

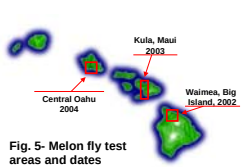
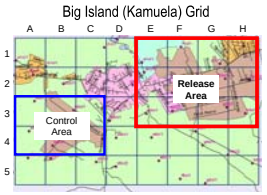


Fig. 5- Melon fly test areas and dates



10-20:1, very low compared to the ratios needed in normal medfly SIT programs. The sterile fly release program against the oriental fruit fly began in 2004 on Oahu (Fig. 6 photos). The first effort took place in a small citrus orchard with encouraging results (Fig. 10). In this test, egg sterility rose to ca. 65% after 5-6 weeks of releases. Sterile: wild fly ratios increased from less than 1:1 to ca. 30:1 over the 4 month study. Egg sterility started relatively high, likely due to a prior intense male annihilation test in the same field, and maybe did not rise above 65% due to immigrant females moving into the small area. For this latter reason, we have shifted back to the much larger test area in Waimea, Big Island, near the site of the previous melon fly test. Initial fly releases were made in February, 2005 and will continue at least for 5-6 months. We began releasing ca. 200,000 flies/wk, and have increased to ca. 500,000/wk.

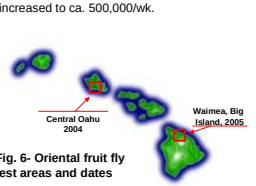
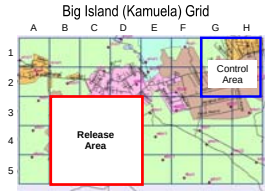


Fig. 6- Oriental fruit fly test areas and dates



Fly release and trapping methods:

The irradiated and dyed pupae for each species were packaged into boxes and shipped to the Big Island for the 2002 melon fly test and 2005 oriental fruit fly test, to Maui for the 2003 melon fly test, and simply held in adult emergence trailers for the 2004 melon fly and oriental fruit fly tests. Upon arrival at destination, the pupae were then dispensed into 5L 'chicken' buckets or 70L plastic tubs and held with food and water (agar) for 3-5 days under ambient conditions prior to release in the field (Fig. 7 photos).

Fig. 7- Packaging dyed pupae, food, and water into buckets, then releasing by point releases or roving trucks

Fly release and trapping methods (cont'd.):

Fly release methods include point releases with small or large containers, or roving releases from slow moving vehicles along roads. Once released, the sterile flies may be trapped back in male traps containing the strong male lures, cuelure for melon fly and methyl eugenol for oriental fruit fly (Fig. 8a). Traps were serviced weekly to obtain both wild fly density levels and the sterile:wild fly ratio- both statistics are vital to adjust the flies being released each week to focus on active wild fly sites. Also, host fruit was sampled routinely for both species in all the test sites to provide dissected eggs and sterility estimates comparing treated and control areas (Figs. 8b,c).

Fig. 8a- Male trapping

Fig. 8b- Fly eggs in fruit host

Fig. 8c- Infested fruit

Chemotherapy studies:

Efforts to improve the SIT with chemotherapy treatments from the male attractants, cuelure (melon fly) and methyl eugenol (oriental fruit fly) have been carried out both in the laboratory and in field cages (Shelly et al. 2000; McInnis et al. 2004). Cuelure has not been shown to have a lasting beneficial effect, while methyl eugenol has been shown to have a very significant effect on boosting the male mating ability of oriental fruit flies. When applied on a small scale to males held in cubical cages (1ml/300 males, 10 day old mature flies, 1 day prior to testing in field cages), a six-fold increase in male competitiveness (C values based on the proportions of sterile matings (RSI values)) was observed (Table 1). When the methyl eugenol was provided to males in the aforementioned 5L "chicken buckets" or 70L plastic containers, a ca. 2-fold benefit was realized (Fig. 11).

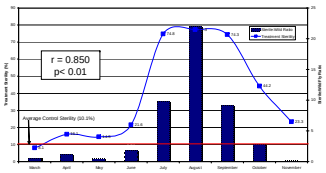


Fig. 9- Sterile:wild fly ratio vs. egg sterility, Maui, Hawaii.

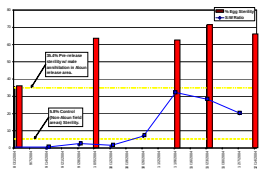


Fig. 10- Sterile:wild ratio vs. egg sterility. Sterile OFF releases, Aloun Farm, 2004.

	Date	Rep	Treated # Pairs	RSI	Control # Pairs	RSI
1.	8/5/04	1-2	83 79	0.843 0.860	73 ---	0.808 ---
2.	10/7/04	3-4	14 16	0.653 0.938	15 12	0.267 0.667
3.	10/13/4	5-6	35 43	0.886 0.767	34 25	0.441 0.440
4.	11/3/04	7-8	56 48	0.839 0.854	61 44	0.590 0.364

RSI Range: 0.653 – 0.938

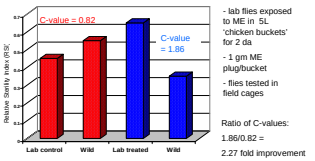
Average RSI: 0.830 Average: 0.447

Treated Comp. "C" value = 0.830/0.17 = **4.88**

Control Comp. "C" value = 0.447/0.553 = **0.81**

$$\frac{\text{"C" Ratio w/ME}}{\text{No ME}} = \frac{4.88}{0.81} = \mathbf{6.02}$$

Table 1- Competitiveness "C" values based on the proportions of sterile matings (RSI values).



- lab flies exposed to ME in 5L 'chicken buckets' for 2 da
- 1 gm ME plug/bucket
- flies tested in field cages

Ratio of C-values: 1.86/0.82 = 2.27 fold improvement

Fig. 11- Methyl eugenol-based aromatherapy effects on C-values ratios.

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Monitoring of Lepidopteran Species by Means of Pheromone Traps in Tobacco Stores in Bursa, Turkey

IAEA-CN-131/ 167 P

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INTRODUCTION: Turkey ranks 6 th in the world in tobacco production with 151,860 tons annual productions in 2003 (Fao 2003). Tobacco pests prefer Turkish tobacco due to its higher sugar content and cause widespread infestations.

MATERIAL& METHODS: This study was carried out at weekly intervals in Tekel's leaf-tobacco stores located in Mudanya and Osmangazi districts of Bursa, northwestern Turkey during 2000-2002. Pherocon II type traps (Trécé, Salinas, CA, United States of America) and STORGARD IMM+4 (*Plodia interpunctella* pheromone lure) pheromone capsules were used for capturing adults and monitoring the fluctuations in population.

RESULTS & DISCUSSION:

Lepidoptera species captured in the tobacco stores

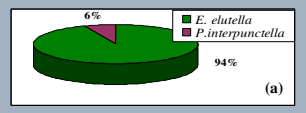
There were two main species; *Ephestia elutella Hübner* and *Plodia interpunctella* (Hübner) in the tobacco stores in Bursa. *E. elutella* was the most abundant species captured during monitoring period in the tobacco stores (Fig 1 and 2). Erakay (1979) reported that *E. elutella* has been recorded as the main pest in the tobacco stores in the Aegean Region of Turkey.

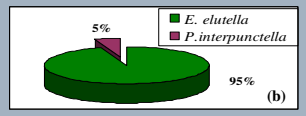
The first adults of *E. elutella* were observed between April-May, whereas adult *P.interpunctella* first appeared in early June in the tobacco stores during 2001-2002. Moreover, flights of *E. elutella* adults were monitored until the end of November and these flights declined gradually towards the end of November (Fig 1 and 3). Sannino et al. (2003) stated that *E. elutella* flights continued from April to November, the population reaching a peak in May and June, in the tobacco stores in Italy.

Population Fluctuation of Male Ephestia elutella

In store(1) in Mudanya, *E.elutella* exhibited four peaks of seasonal activity; the first one in May, the second one in June, the third one mid-August, and the last one at the end of September-early October in 2001. However three peak points were observed in 2002. The first one in late May, the second one mid-August and the last one in mid-September(Fig 3).

In store (2) in Osmangazi during 2001-2002, Tobacco moth catches indicate that the flight period ocurred from April to November, with peaks between April-June, July- August and late September(Fig 4).





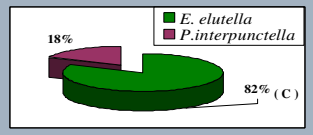
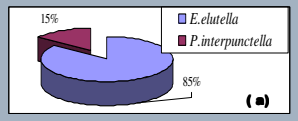
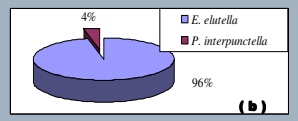


Figure 1. In Mudanya Tobacco Store, abundance of Lepidopter species : (a) 2000 (b) 2001 and (c) 2002





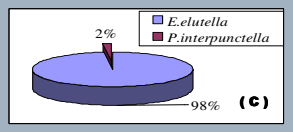


Fig2. In Osmangazi Tobacco Store, abundance of Lepidoptera species : (a) 2000 (b) 2001 and (c) 2002

These results are similar to those of Erakay (1979) and Buchelos (1998) who reported that *E. elutella* gave three generations.

Population Fluctuation of Male Plodia interpunctella

The adults of Indianmeal moth showed three peaks of seasonal activity at store(1) in 2001; the first one appeared in late June, the second one early August and the last one late September. However, *P.interpunctella* adult populations peaked in late June and late July at the same store in 2002.

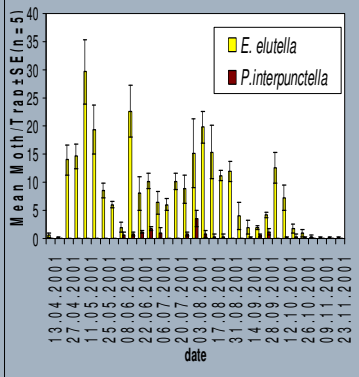


Fig3. Adult population fluctuations of *E. elutella* and *Plodia interpunctella* in Store(1) in 2001

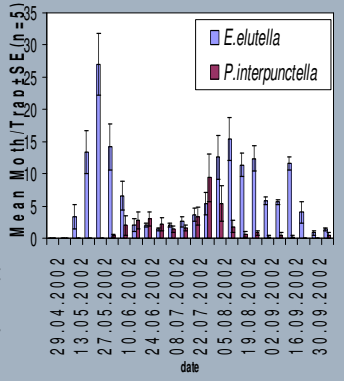


Fig4. Adult population fluctuations of *E. elutella* and *Plodia interpunctella* in Store(1) in 2002 .

On the other hand, the adults of *P. interpunctella* were also captured occasionally and at low rates in Osmangazi tobacco stores during 2001-2002 (Fig5 and 6). The result of our experiment do not agree with the findings of Buchelos and Trematerra (1998) who found that *P. interpunctella* had 5 generations in the tobacco stores in Greece.

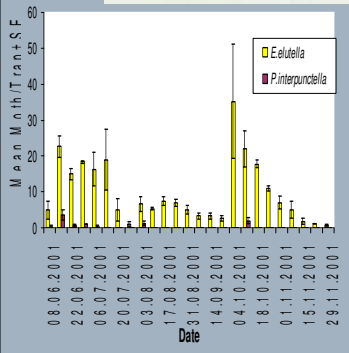


Fig 5. Adult population fluctuations of *Ephestia elutella* and *Plodia interpunctella* in Store(2)and (3) in 2001

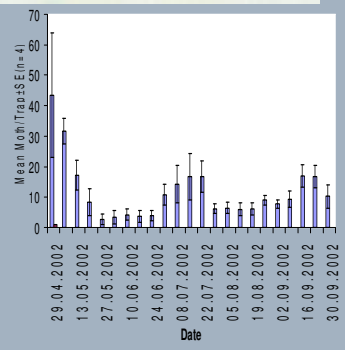


Fig 6. Adult population fluctuations of *Ephestia elutella* and *Plodia interpunctella* in Store(3) in 2002.

CONCLUSIONS:

1. The most important economic pest was *E. elutella* in stored tobacco. In addition adult flights of *P. interpunctella* were also observed in these stores but *P. interpunctella* populations didn't reach to critical levels.
2. The first adults of *E. elutella* were observed between April-May, whereas adult *P.interpunctella* first appeared in early June in the tobacco stores during 2001-2002.
3. *E. elutella* had 2-3 in tobacco warehouses in Osmangazi, whereas 3-4 peak flights were observed in Mudanya.
4. Pheromone traps were effective in determining the adult emergence and pest densities in storage houses. Thus they could be an important aid in appropriate timing of insecticide applications.

ACKNOWLEDGMENTS:
We are thankful to The Section of Scientific Res. Projects Grant No. 2000/ 43, Uludag University, Bursa, Turkey, for financial support.

Effect of Gamma Rays on Adult Flight Agility and Distribution of OWS, Chrysomya bezziana irradiated at the pupal stage

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Pupa stage of Old World Screwworm Fly, *Chrysomya bezziana* was used to study the effect of gamma rays from Gamma Cell 220 Unite and monitor the activity of the sterile insects released in the field.
Pupae at 2-5 days were irradiated by gamma cell 220 to find its effect the development of pupae and adult emergence (Table 1).

Table 1. Effect of gamma rays on adult emergence of OWS pupae irradiated at different ages

Gamma dose, Gray	Pupae age (days)							
	2		3		4		6	
	N	M	N	M	N	M	'H	M
0	81	0	81	0	82	0	82	0
15	77	5	37	3	59	2	60	0
30	67	7	33	8	42	4	69	0
45	60	10	25	1	67	0	61	0
60	28	6	10	2	60	2	57	0
75	14	3	4	1	44	5	62	0
90	3	0	12	3	29	1	22	0

N=Normal, M=Malformed

Pupae development to adults was decreased with increasing the rate of gamma dose. The regular development of unirradiated pupae was 81% – 82%, this percentage was decreased continuously with increasing gamma dose from 15 to 90 Gray. The percentage of adults emerged from pupae irradiated by 90 Gray at 2, 3, 4 and 6 days age were 3%, 12%, 29% and 22%. The appropriate age of pupae for gamma irradiation was four to six days, we selected five days old pupae for irradiation treatments. The lifespan of adults emerged from irradiated pupae were decreased with increasing the gamma dose (Table 2).

Table 2. Effect of gamma rays on lifespan of OWS adults Emerged from pupae irradiated at 5 days age *.

Gamma dose (Gray)	Number of adults live more than 5 days	
	Female	Male
0	88	96
30	72	68
45	56	64
60	60	56
75	40	44
90	28	32

* Four replicate each have 25 adults

Sixty Gray or less would not have an effect on the ovarian development, ovarian maturation occurred within 5 days of adult lifespan. Adults emerged from pupae irradiated by 75 and 90 Gray were not live more than 5 days, for this reason these doses were not used in the field release studies.
Female fertility and egg hatch was measured in crosses between normal adults or normal with irradiated adults (Table 3). Egg hatch was occurred only in crosses of normal adult or in normal females mated with males emerged from 45 Gray irradiated pupae, no egg hatch in any other crosses of 60 to 90 Gray treatments.

Sticky trap baited with swormlure was effectively catch *Ch. bezziana* and used to capture the adult flies under field conditions. This trap was used in Iraq for surveillance, prediction and release / recapture studies. The captured flies were:

Family Calliphoridae:
- *Calliphora* sp., Blow Fly
- *Lucilia* sp., Sheep blowfly
- *Chrysomya bezziana* , Old World Screw-Worm Fly.
- *Ch. megacaphala* , Oriental Latrine Fly.
- *Ch. albiceps* , Banded blowfly .

Family Sarcophagidae
- *Sarcophaga* sp.
- *Wholfahrtia magnifica* , Wohlfahrt's Wound Myiasis Fly

Family Muscidae
- *Musca domestica* , House Fly

Table 3. Effect of Gamma Rays on Oviposition and Egg Hatchability of different crosses of adults (normal or emerged from Irradiated Pupae).

Gamma dose (Gray)	Number of Females	Number of fertilized females	Number of females laid eggs	Egg hatch, %
Crosses of Normal Males X Normal Females				
0	60	56	55	84.6 a
0	40	38	37	86.4 a
0	40	37	36	88.6 a
Crosses of Irradiated Males X Normal Females				
45	40	36	35	3.6 a
60	60	53	50	0 b
75	60	49	47	0 b
90	60	49	48	0 b
Crosses of Normal Males X Irradiated Females				
45	60	56	4	0
60	40	36	1	0
75	60	55	0	0
90	60	54	0	0
Crosses of Irradiated Males X Irradiated Females				
45	40	36	1	0
60	40	35	0	0
75	60	50	0	0
90	60	49	0	0

Flight Ability Test

The flight ability test was done on *Ch. bezziana* irradiated by Gamma Cell 220; the emerged adults were tested for flight ability before they were released in the field. Small cylinder made from transparency papers (10 Cm. Long X 10 Cm diameter) lined by a baby powder to slip the climb adults. A group of adults (newly emerged from Irradiated pupae) were left inside each cylinder and left in the rearing room under appropriate temperature and humidity. Adults who able to flight and escaped from the cage would be flight inside the confined environment of the room, they were caught by sticky papers hung inside the room. The Flight Ability Index (F.A.I.) would be calculated by using the following equation:

$$F.A.I. = \frac{\text{Fully emerged flies} - (\text{Residual flies} + \text{Deformed flies})}{\text{Fully emerged flies}}$$

Where:
Fully emerged flies: adults released completely from the Cocoon.
Deformed: malformed Adults.
Residual: adults are unable to flight.
Partially emerged: adults released partially from pupae.
Unemerged pupae were calculated to find the F. A. I.

Adult flight ability under field conditions were measured by ground release of the flies emerged from irradiated pupae in livestock farms (Table 4).

Table 4. Effect of gamma rays on the development of adults and its Flight Ability after irradiation of 5 – 6 days OWS pupae of Iraqi strain.

Number of Pupae	No. of Emerged Adults	Malformed adults	Adult Emerged (%)	Residual Adult	Deformed Adults	Flight Ability Index (F.A.I.)
082 a	68	-	82.9	4	-	94.1
080 a	68	-	85.0	3	1	94.1
100 a	87	2	89.0	4	-	95.4
098 a	67	4	72.5	10	1	83.6
101 a	66	4	71.3	7	-	89.4
Avg.	-	-	80.14	-	-	91.3
080 b	57	1	72.5	7	-	87.7
081 b	63	6	85.2	13	-	79.4
079 b	63	2	82.3	3	1	93.4
100 b	45	16	61.0	8	4	73.3
099 b	60	3	63.6	5	5	83.3
100 b	55	11	66.0	13	3	70.9
080 b	51	7	72.5	9	3	76.5
Avg.	-	-	71.87	-	-	80.6

A=Non-irradiated pupae. B= Pupae irradiated at age 5 – 6 days with 9 Krad

Local Distribution of Released Laboratory-Reared OWS Flies

Four series of field releases of marked laboratory-reared Old World Screw-Worm Flies were carried out in southeast Baghdad to determine the local distribution of the sterile flies. Six sticky traps were fixed in the field, 200 meter was used as a regular distances between each trap. The adults were marked with a phosphoric dye to differentiate the irradiated adults from the wild OWS Flies; the released adults were separated for each group. Four experiments of ground release of flies were done during the years 2000 to 2001 (Table 5).

Table 5. Number of Released/Recaptured and catch rate of sterile flies within five days of releasing date.

Release date	Number of sterile flies										Catch rate	Wild adults	
	Released	Recaptured within five days at the following distances (meters)											
		200		400		600		800		1000			
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
19/9/2000	514	-	-	10	1	-	2	-	-	1	-	2.72	1	-
14/10/2000	309	1	2	4	1	-	1	-	-	1	1	2.34	1	1
23/11/2000	348	-	-	4	10	1	4	-	-	-	-	6.03	3	-
13/5/2001	400	-	-	6	2	1	-	-	-	-	-	2.25	1	-

It seems that the sterile flies flying to a distance ranged from 200 to 1000 meters, the number of flies caught in sticky traps were low compared with the total number of the flies released in the field. Few wild OWS flies caught under the field conditions during 2000 and 2001 compared with fly catch in 1999 in different areas in Baghdad (Table 6), this decrease in flies might be due to the heavy use of insecticides in the releasing area.

Table 6. Number of OWS, *Chrysomya bezziana* and other Flies Caught by Sticky Traps used weekly in 1999 in south, east and North of Baghdad.

Month	No. Traps	Total flies Per Trap	*Ch. bezziana*		*Ch. megacephala*		*Ch. Albecips*	
			♂	♀	♂	♀	♂	♀
Febr.	08	6938	10	14	48	54	28	113
March	24	22737	32	37	76	138	104	314
April *	14	3536	6	11	11	8	93	60
May	18	693	3	5	12	15	46	16
June	30	2213	5	8	36	46	14	12
July **	12	63	-	1	-	-	1	3
Aug.**	12	66	-	-	4	4	1	-

* Insects were decreased due to spraying insecticide for Dubas bug control.
** Insect caught were decreased due to hot temperature (> 40 °C) in July and August.

Nuclear DNA markers for identification of cryptic fruit fly species

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Introduction

Molecular approaches based on the analysis of DNA for the purpose of identification of pest species offer very promising alternatives to traditional taxonomic methods.

Among these advantages are the facts that:

1. **DNA is constant throughout the life cycle.** This means that DNA based identifications may be made using material available from any stage of development.

2. **DNA markers tend to be highly variable**. Noncoding regions of the genome can provide a wide range of character states for taxonomic identification purposes. This is especially important for the case of cryptic species which are very closely related.

3. **DNA based markers may be digitized for automated analysis**. Digital images of DNA results may be more readily analyzed using automated methods.

The DNA based markers we are using for taxonomic identification purposes are derived from introns of specific genes. Intron sequences are noncoding regions found in many genes. They are most useful for comparisons of Individual variation within populations and between closely related species and populations.

The variable intron sequences we have identified are first use to define the allelic variation present within a species and/or population. The allelic forms are then displayed on a DNA array to make species identifications through direct determination of the genotype of a specimen.

Materials and Methods

1) DNA is extracted from identified specimens.
2) EPIC-PCR is used (*Fig. 1*) to amplify intron regions.
3) EPIC-PCR products are cloned and sequenced.
4) DNA sequences are aligned. Allelic variants are identified.
5) Oligonucleotide sequences corresponding to each alllele are synthesized and placed on a DNA array (*Fig. 2a*)
6) Arrays are probed with genetic material from an individual (*Fig. 2a*)
7) Positive hybridization signals identify the alleles present in an individual (genotype) (*Fig. 2b*). Establishment of the genotype also identifies the species of the individual.
8) Initial experiments are carried out using individuals of known genotype to verify array based approach.

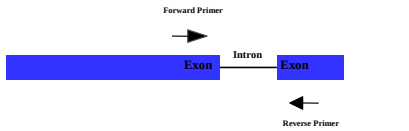

Fig. 1. Oriental fruit fly actin gene structure and exon-primed-intron-crossing polymerase chain reaction strategy

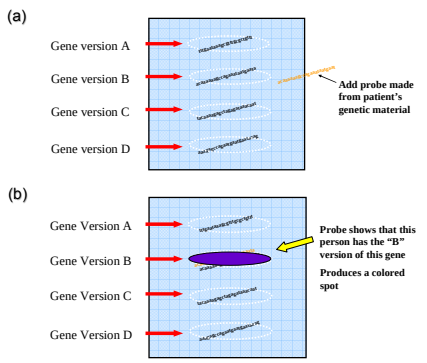

Fig. 2(a-b). DNA array schematic

Results

1) Alleles identified (*Table 1*)
2) Oligonucleotide sequences corresponding to allelic forms used to construct array (*Table 1* and *Fig. 3a*)
3) Probes made from individuals of known species and genotype validates array based approach (*Table 2* and *Fig. 3b-d*).

Table 1. Alleles identified from oriental fruit fly specimens

Alleles (by species)	Allele specific oligonucleotide (50-mer) derived from intron sequences
B. dorsalis	* * *
BdorA1	ACAAAAAAGCCAGAAATAATGAATAAAGCCTTTGCGCTGTTTTCGATGTA
BdorA2	ACAAAAAAGCCAGAAATAATGAATAAAGCGTTTACGCTGTTTTCGATCTA
BdorA3	ACAAAAAAGCCAGAAATAATGAATAAAGCGTTTACGCTGTTTTCGATGTA
B. dorsalis Control Areas	
BdorAICA	CCAGGTAATTTAATTGTTTCCACAAAAAAGCCAGAAATAATGAATAAAGC
BdorA2CA	CCAGGTAATTTAATTGTTTCTACAAAAAAGCCAGAAATAATGAATAAAGC
B. papayae	* * * * * * *
BpapA1	CTACAAAAAAGCCAGAAATAGTCAATAAAGCGTTTACGCTGTTTTCGATG- -
BpapA2	- -ACAAAAAAGCCAGAAATAATGAATAAAGCGTTTGCGCTGTTTTCGATGTA
BpapA3	- -ACAAAAGAGCCAGAGAATAATGAATAAAGCCTTTGCGCTGTTTTCGATGTA
B. carambolae	
BcarA1	TTTGAATAAAGCGTTTGCGCTGTTTTCGATGTATTCAATGCATTTTTTTA
BcarA2	ACAAAAGAGCCAGAAATAATGAATAAAGCGTTTGCGCTGTTTTCGATGTA
BcarA3	TTTGTATAAAGCGTTTGTGCTGTTTTCGATGAATTCAATCGAATTCTTTT
BcarA4	ACAAAAAAGCCAGAAATAATGAATAAAGCGTTTACGCTGTTTTCGATGTA
BcarA5	ACAAAAGAGCCAGAAATAGTCAATAAAGCGTTTACGCTGTTTTCGATGTA
B. latifrons	
BlatA1	TACAAAAGAGCTAGAGATAAACAATAAAGCCTTTGCGCTGTTTTCAATTC
B. tryoni	
BtryA1	AAAGAGCCAGAATGAATGAATAAAGCGTTTGCGCTGTTTTCGATTTATTC

Table 2. Verification of array based approach using known individuals

Individual	Genotype
#1	BpapA3/BpapA1
#2	BpapA3/BpapA3
#3	BdorA1/BdorA1

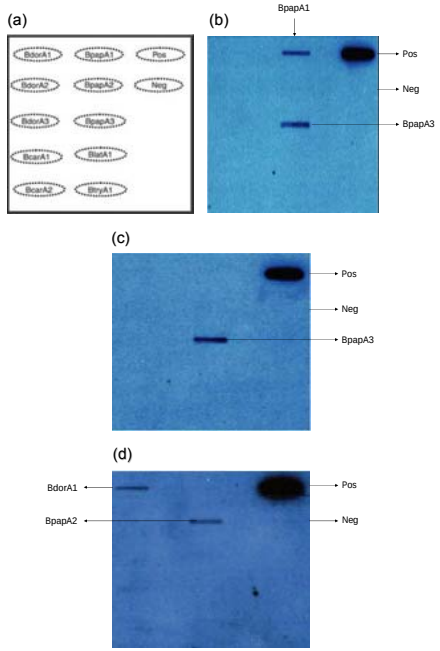

Fig. 3(a-d). DNA array validation
(a) DNA array layout
(b) Probe from individual #1 probe identifies the allele specific oligos corresponding to the known genotype of this specimen.
(c,d) Probes made from individuals #2 and #3 identify the correct alleles corresponding to the genotype of these specimens.
(positive and negative controls are shown as well for all blots)

Conclusions

1) Genotype and species of individual specimens can be rapidly and reliably determined.

2) Use of an array based approach for genotyping and species identification is validated.

Future Work

1) Survey additional individuals representing different populations and species.

2) Identify variable intron sequences from other genes

3) Switch to higher density microarray format for genotyping

4) Develop similar sets of markers for *Anastrepha* and *Rhagoletis* species and populations to demonstrated broad utility of this approach.

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Defining critical partnerships in area-wide pest management: the Hawaii experience

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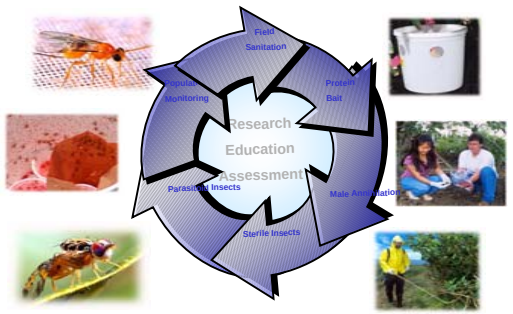
HAW-FLYPM
Area-Wide Fruit Fly Control



Aside from the technical issues that form the basis of any successful area-wide program, significant attention must be made to program organization and development of partnerships that facilitate the large numbers of non-technical issues that must be addressed in a successful area-wide program. The recent experience with the Hawaii area-wide fruit fly integrated pest management program (HAW-FLYPM) is a recent example of the trials and tribulations that occur when one attempts to set up such a program.

In our example, USDA-ARS researchers (and their predecessors) from the US Pacific Basin Ag Research Center had developed much of overarching strategies that are used today for the detection, control and eradication of many tephritid fruit fly species, especially Mediterranean fruit fly, oriental fruit fly and melon fly, all species that have become established in Hawaii over the last 100 years. Early researchers were responsible for such seminal technologies as the development of low cost diets for mass-rearing, attractants for several fruit fly species, early demonstrations of SIT against fruit flies and more recently development of augmentative biological control strategies against fruit flies. These early discoveries have been refined and improved by many USDA and non-USDA researchers over the subsequent decades but the basic technologies have remained the same.

The HAW-FLYPM program was launched in 1999 with the hope that improved fruit fly control technologies could be applied to help suppress fruit flies in the state and spur increased sensitivity for the need of statewide suppression or eradication programs if the HAW-FLYPM program was successful. In assessing the opportunities for success (or failure) of a program of such magnitude, and mindful of previous pilot test that had failed to move the fruit fly programs in Hawaii forward, the scientists knew that this program would have to develop a broad-based coalition of stakeholders from diverse but interdependent group associated with agricultural issues in Hawaii in order to launch a successful program.

- Developing strong alliances within agriculture is the key to successful implementation
  - Grower- retailer- export markets
    - Consistent markets, high quality, price, name recognition
  - Grower- researcher-extension
    - Current and best practices, cost effective, environmentally sustainable, Evidence
  - Consumer-retailer-grower
    - Quality, food safety, price

The resulting success of the HAW-FLYPM program has been better than expected and acknowledgement of the program coordination culminated in the receipt of the Federal Laboratory Consortium technology transfer award for team effort, the USDA Secretary Honor's Award and recently the IPM team award from the Entomological Society of America Foundation. The programs success has also spawned renewed interest in fruit fly programs in Hawaii that hopefully will translate into continued opportunities for additional partnerships to be formed and successes to be fulfilled.

Thus followed a series of humbling acknowledgements that the fruit fly control technology was only the start of a long journey that would only stop with the sustainable implementation of scientifically sound, environmentally compatible and economically feasible control practiced by the growers. The first challenge was to gather a "team" of individuals who could share a common goal and vision of a successful fruit fly program in Hawaii. This was accomplished through a series on frank discussions with individuals who represented key components of the program such as grower training, cooperative extension and community-based education on fruit fly issues (University of Hawaii), regulatory issues related to the implementation of this new program and the subsequent registration of any new technology developed (Hawaii Dept. of Ag) as well as public entities that could provide products already on the market for use by the growers (Dow Agrosciences, Better World Manufacturing, United Agricultural products, etc). The importance of setting up such alliances are frequently underestimated and like any large program of this type the group had successes as well as failures. Finally in addition to the scientists, an advisory group was set up to help guide the program along. The group met frequently and although not often in complete agreement, understood the need for consensus decision making as a key to the program's success.

Tunisian Rearing Facility a First Year Production Constraints and Prospects

IAEA-CN-131/171P

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Introduction

The Tunisian Medfly rearing facility is located in the north of the country in a small city named Sidi Thabet, near the capital. This facility was designed for rearing the Medfly Genetic Sexing Strain (GSS). The facility was established in 2001-2002 and has been started operations in February 2003.

The facility production capacity is 12 million sterile Medfly male pupae per week.

The facility was constructed to produce the sterile insects to establish a Sterile Insect Technique pilot program in 6,000 Ha of *citrus* orchards in Cap Bon Peninsula in north east Of Tunisia.

Facility Description

Egging rooms:

Three rearing rooms contain the cages for amplification colonies and filter system. The rearing cages designed locally are cubic and adapted for the tsl strain. Twenty-four big cages are producing eggs for the three amplification steps and 300 ml eggs are produced daily.

Mixing room:

The capacity of the mixer is almost 500 Kg of diet. The larvae diet that we are using is the diet based on the bran as a bulking ingredient, the torula yeast as a protein source and the sugar as a source of carbohydrates.

Larval initiation and maturation rooms:

The larval initiation room for the colony is kept at 25°C and 75% of RH, while the initiation for male only is kept at 30°C and 80% of RH. The facility has only one maturation room for the two colonies with 80 to 85% of RH and 22°C.

Larval collection room:

Larvae are collected in sawdust and the environmental conditions at the room are 18°C and 85%RH.

Pupae maturation rooms:

Pupation medium separation is done 24 hours after pupation and then 2 litters of pupae are on to the pupae maturation tray. The temperature of the room is 20°C and 70% RH. Before irradiation pupae is dyed, bagged then exposed to irradiation source and a doses of 145 Gray is given.

The auxiliary areas of the facility

Washing area, pupae synchronization room, sifting area and quality control rooms.

Strain

The strain of the Mediterranean Fruit Fly used in this facility is the Vienna-8 GSS based on the lethal sensitivity to the temperature (tsl).

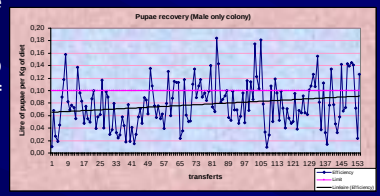

Fig 1

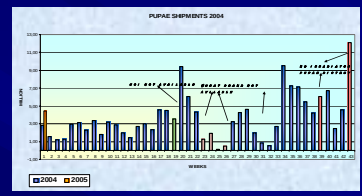

Fig 2

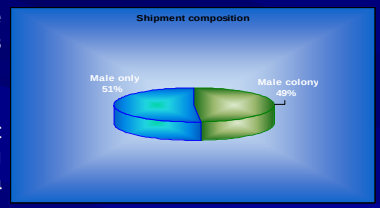

Fig 3

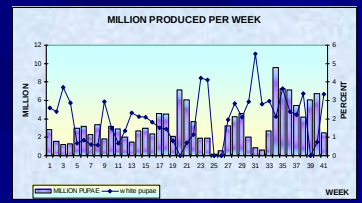

Fig 4

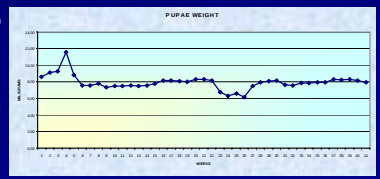

Fig 5

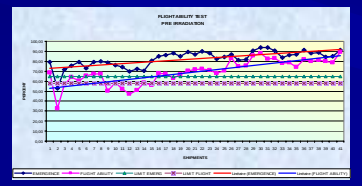

Fig 6

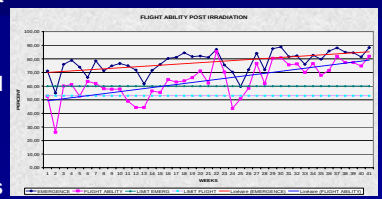

Fig 7

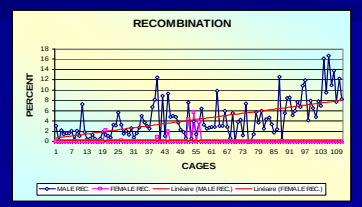

Fig 8

Results

Male only and colonies pupae recovery was fluctuating for the first year of mass production. The deviation from the standard limit was so significant (Fig.1). The breakpoint between batches 25 and 50 is coinciding with a lack of diet ingredients. In spite of this the tendency curve is indicating that there is an improvement.

The sterile males shipments profile (Fig 2), which starts at the end of February 2004, was not increasing for the first weeks due to a weak fecundity and air conditioning problems. But when the production starts to increase exponentially since June, two major breakpoints happened at the weeks 23 and 32, which were the lack of ingredients and breakdown of the mixer. However a kind of stabilisation occurs at the end of release season with an average of 8 millions sterile male per week.

Shipments contain males from male only colony and from injection and release colonies (Fig 3), the average of white pupae shipped coming from males colony is more or less 2% (Fig 4).

Nevertheless the summaries of the results of one year production for quality control tests are satisfactory and have a kind of stability like for pupae weight and flight ability (Fig 5,6 and 7).

The recombination percent is one of the most important indicators, we are trying to separate physically the colonies in absence of enough material from filter for injection, the average for all the colonies is 4% of males recombinants, but the tendency is to higher values reaching 8% (Fig 8).

Discussion

Instability production was characterising the first year of production. Mass rearing insects is a manufacturing operation that needs many successful conditions.

The availability of diet ingredients is one of the most important components to lead mass rearing. The rearing diet has a direct impact on yield and quality.

Conclusion

Variation in mass rearing yields has often been caused by such factors as lack of ingredients or diet contaminations, inadequate rearing space, breakdown of equipments and environmental conditions change. The majority of these problems can be improved but some of them need us to lead researches like to identify a bulking ingredient.

An attention should be given also to find solutions for disposal of factory waste products, to put in place a special administrative team for the facility to avoid delay of supplies and to think about personnel motivation.

Kairomones for trapping shot hole borer infesting tea

173 P

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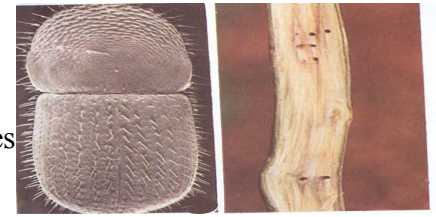
Shot hole borer (SHB), *Euwallacea fornicatus* (Scolytidae: Coleoptera) is an important perennial pest of tea in south India and Sri Lanka. Volatile odorous chemicals released by the partly dried cut stems of a jungle plant, *Montanoa bipinnatifida* (Compositae) attracted large number of SHB beetles in the field. A study was undertaken to find out the volatile profile of the partly dried stems to develop an attractant kairomone trapping system for shot hole borer management.

Research Objectives

- Isolation and identification of volatile compounds
- Laboratory screening of compounds and their mixtures
- Studies on suitability of different traps in the field
- Field bioassay and mass trapping of beetles



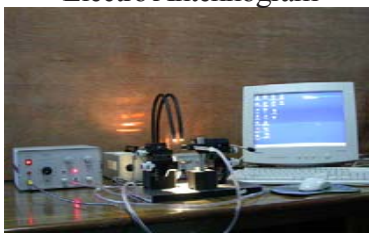
Shot hole borer SHB infested tea stem

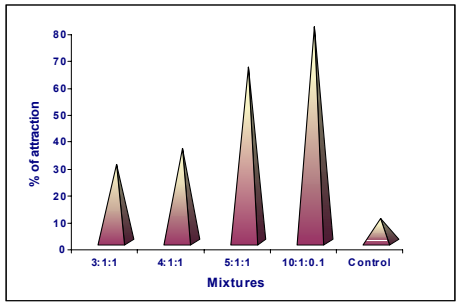
Results

Isolation and identification of volatile chemicals in GC-MS showed the presence of seven important compounds

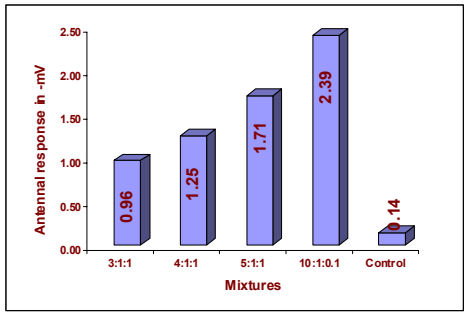
1. β- pinene
2. Germacrene- D
3. α-Pinene
4. Trans-caryophyllene
5. D- limonene
6. β- phellandrene
7. Iso-caryophyllene

Electro Antennogram





Response of beetles to different mixtures under wind tunnel

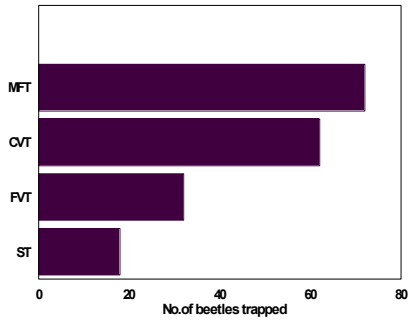


Response of beetles to different mixtures in EAG

Beetles showed varied levels of attraction towards individual compound and their mixtures in wind tunnel studies. Olfactory chemo sensilla situated in the antennae of the beetles showed different responses to different compounds and mixtures in Electro Antennogram (EAG) studies.

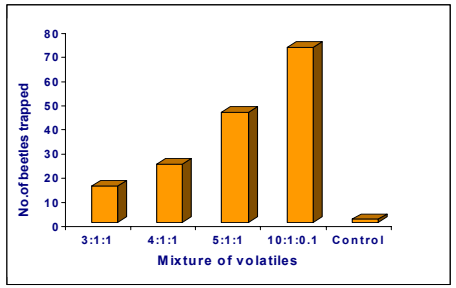
Among the mixtures screened, combinations of α-pinene, β-phellandrene and trans-caryophyllene (10: 1: 0.1, v/v) evoked appreciable level of response among the beetles in the lab. This mixture attracted sizable number of beetles in the field also. Synthesis and addition of germacrene-D, an important volatile compound may lead to increased attraction of beetles in the field.

Different types of traps were used in the field for mass trapping of beetles. Among them, multiple funnel trap was found more suitable.



MFT: Multiple funnel trap; CVT: Cross vane trap; FVT: Funnel vane trap; SBT: Sticky board trap

Level of attraction of beetles to different traps



Response of beetles to different mixtures in the field

Conclusion

1. Volatile odorous compounds from the partly dried stems of M.bipinnatifida, responsible for the attraction of shot hole borer have been identified.

2. Mixtures of few important compounds elicited larger response among the beetles in the lab and field.

3. Multiple funnel trap was found more suitable for trapping the SHB beetles.

Development of re-arrangeable gene transfer systems to create balancer chromosomes and increase transgene stability

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1. Synopsis

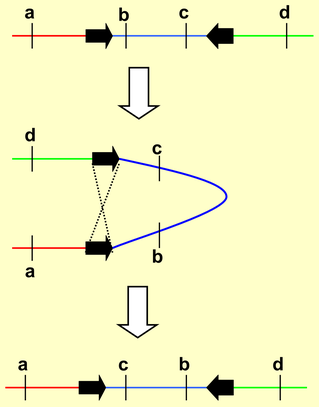
Transposon-based vectors provide the most suitable gene transfer systems for insect germ line transformation and therefore for the molecular improvement of the Sterile Insect Technique (SIT). However, the long time stability of genome integrated transposon constructs depends on the absence of transpsoson activity that could remobilize the transposon-integrated transgene. To achieve transgene stability, transposon vectors are usually non-autonomous and chosen so that no endogenous or related transposon activities are present. Nevertheless, the non-autonomous transposon-integrated transgene might become instable, when by horizontal gene transfer an active transposon of its own type enters the species. Due to the large numbers of progeny and the high mobility, transgenic insects present great environmental concerns. Therefore, it will be important that transgene constructs are as stably integrated as possible, so that the vector used for gene transfer cannot become unstable and unintentionally escape or even integrate into the genome of another organism. In this respect, we have developed transposon vectors whose terminal inverted repeats can be rearranged after integration, which prevents mobilization of the integrated transgene even in the presence of the corresponding transposase. Such re-arrangable gene transfer systems should help to avoid the instability of integrated transgenes and thus increase the stability of transgenic strains. Moreover, the thereby induced chromosomal rearrangements serve as partial balancer chromosomes to stably inherit a combination of transgene insertions during genetic crosses, which will facilitate the establishment and keeping of transgenic insect strain.

In order to develop re-arrangable gene transfer systems we made use of the yeast-derived *FLP* recombinase and its recombination target sites (*FRT*s), which have been shown functional in the vinegar fly *Drosophila melanogaster*. We have started to create transposon vectors based on the transposable elements *piggyBac* and *Hermes* that carry *FRT* sites. The vectors contain fluorescent protein-based transformation markers, whereby the *FRT*s are placed in the 5'UTR region and thus separate the transcriptional promoters from the coding region of the fluorescent marker. As original transformation markers, we have used the eye-specific yellowish-green fluorescent marker 3xP3-EYFP, the eye-specific cyan-fluorescent marker 3xP3-ECFP and the constitutive red fluorescent marker PUb-DsRed1. These vectors have been used to generate transgenic *Drosophila melanogaster* strains. In the different strains the genomic localization and orientation of the integrated transgenes has been determined by inverse PCR and comparison to the *Drosophila* full genome sequence. Suitable *piggyBac* and *Hermes* integrations have be recombined to establish strains that contain both types of transgenes on the same chromosome. In these strains, we induced the *FRT*-based recombination by expression of the *FLP* recombinase and isolated recombination events due to the change of fluorescent marker expression. After recombination, DsRed has become eye-specifically, and ECFP (or EYFP, respectively) has become constitutively expressed.

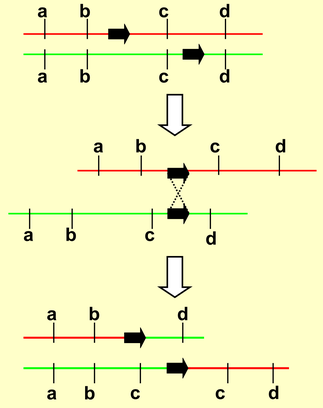
The *FRT*-mediated recombination generates localized inversions, which we have tested for local balancing effects that make stable co-inheritance of two transgene constructs possible at the same time. Moreover, the rearrangement destroy the regular alignment of both the *piggyBac* as well as the *Hermes* terminal inverted repeats. When combining pBac{3xP3-FRT-ECFPaf} with pBac{PUb-FRT-DsRedaf}, the rearranged situation consists of *piggyBac* constructs with two right (R) or two left (L) ends, respectively. To test if such a rearrangement will immobilize the transgene insertions we compared the mobilization rates of the original integrated transgenes and the rearranged transgenes, which confirm that the rearranged situation is immobile. Currently, we are also finishing tests on generating deficiencies and duplications based on this system.

2. Site specific recombination by FLP/*FRT*

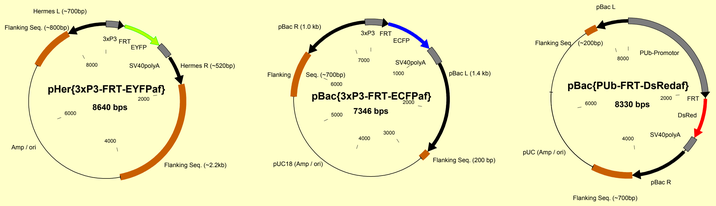
Inversion

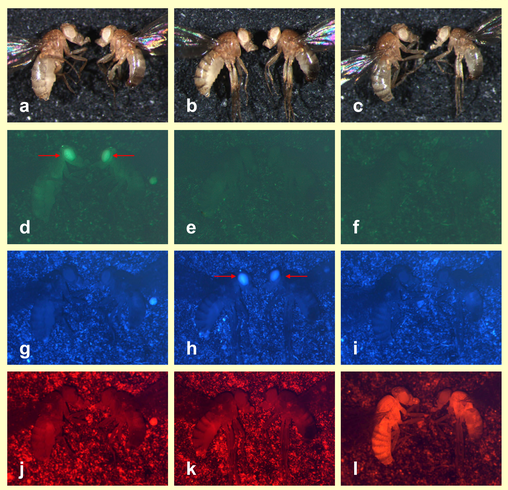


Deletion & Duplication

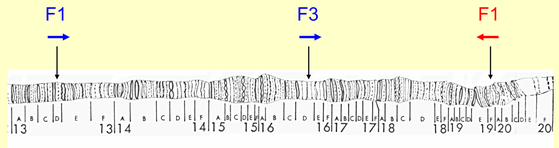


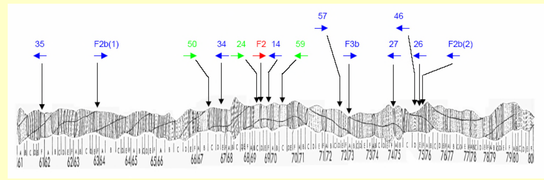
3. Site specific recombination constructs

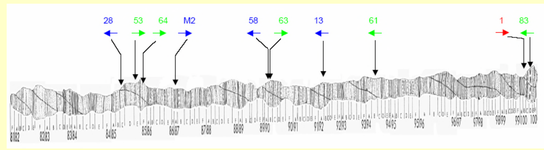




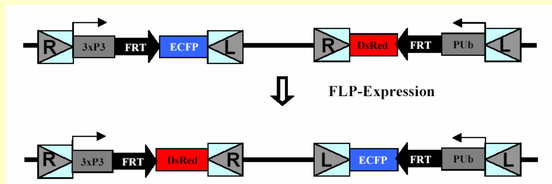
4. Distribution of inserted constructs on X and 3rd chromosome

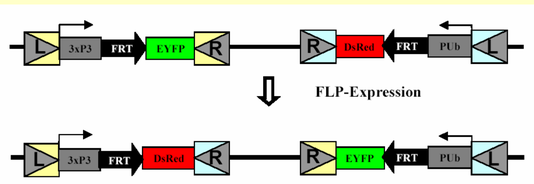


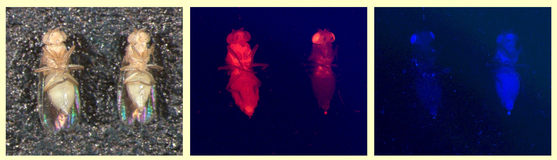


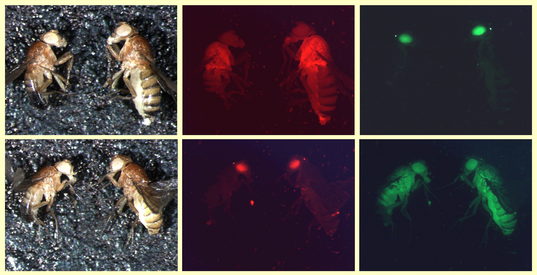


5. Obtained inversions act as balancers and immobilize transgenes









Assay to test X-chromosomal inversion as partial balancer

	total flies	flies without recombination 1) 3xP3-DsRed + PUb-ECFP 2) no fluorescence	flies with recombination 1) 3xP3-DsRed 2) PUb-ECFP	frequency of recombination [%]
Inversion-stock #1	1091	1091	0	0
Inversion-stock #2	1146	1146	0	0
Inversion-stock #3	1124	1124	0	0
Inversion-stock #4	1217	1217	0	0
Control 1	**total flies**	**flies without recombination 1) 3xP3-ECFP + PUb-DsRed 2) no fluorescence**	**flies with recombination 1) 3xP3-ECFP 2) PUb-DsRed**	**frequency of recombination [%]**
Recombination-stock #1	1086	857	229	21,1
Recombination-stock #2	1137	929	208	18,3
Recombination-stock #3	1024	818	206	20,1
Recombination-stock #4	1179	982	197	16,7
Control 2	**total flies**	**flies without recombination 1) 3xP3-ECFP + PUb-DsRed 2) 3xP3-DsRed + PUb-ECFP**	**flies with recombination 1) 3xP3-ECFP +PUb-ECFP 2) 3xP3-DsRed + PUb-DsRed**	**frequency of recombination [%]**
Recombination over Inversion #1	1910	1910	0	0
Recombination over Inversion #2	1832	1832	0	0
Recombination over Inversion #3	1727	1727	0	0
Recombination over Inversion #4	1110	1110	0	0

Test of X-chromosomal inversion-stock(s) for re-immobilisation of their *piggyBac* -elements

Crossing	total flies	females with 3xP3-DsRed + PUb-ECFP	females without fluorescence	males with PUb-ECFP	males without PUb-ECFP
Inversion-stock #1 x Hs-Jumpstarter	1895	954	0	0	941
Inversion-stock #2 x Hs-Jumpstarter	1835	818	0	0	817
Inversion-stock #3 x Hs-Jumpstarter	2029	1043	0	0	986
Inversion-stock #4 x Hs-Jumpstarter	2028	1092	0	0	936
Crossing					
Control	**total flies**	**females with 3xP3-ECFP + PUb-DsRed**	**females without 3xP3-ECFP**	**males with PUb-ECFP**	**males without PUb-ECFP**
Recombination-stock #1 x Hs-Jumpstarter	3128	1430	**199**	**93**	1406
Recombination-stock #2 x Hs-Jumpstarter	2736	1282	**167**	**85**	1204
Recombination-stock #3 x Hs-Jumpstarter	2668	1198	**177**	**93**	1210
Recombination-stock #4 x Hs-Jumpstarter	3110	1440	**213**	**100**	1357

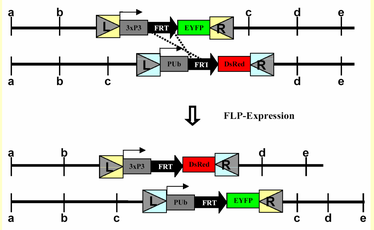
Analyzed inversions on III. chromosome

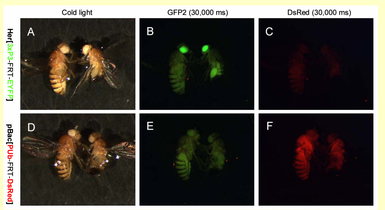
Inversion	Chromosome	Chromosomal insertion (cytobands)	Character of analyzed inversion
F2/14	III. (3L)	69C1/95F3	paracentric
F2/26	III. (3L)	69C1/74E5	paracentric
13/1	III. (3R)	92A13/100B8	paracentric
14/1	III. (3L;3R)	69F5/100B9	pericentric
26/1	III. (3L;3R)	76E5/100B9	pericentric
F2/59	III. (3L)	69C7/76C8	paracentric
1/83	III. (3R)	100B9/100D1	paracentric

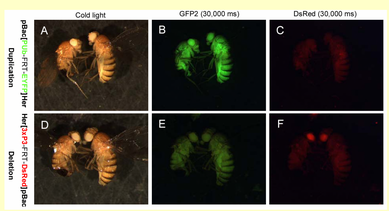
Jumping and excision rates

Transposase source	Recombination	Jumping rate (%)	Excision rate (%)
α-tub-piggyBac	F2/59	0	42.9 (6/14)
	1/83	0	5 (1/20)
hsp70-Hermes	F2/59	46.6 (7/15)	53.3 (8/15)
	1/83	79 (15/19)	26.3 (5/19)

6. Obtained deletions and duplications







Germline transformation of the olive fly *Bactrocera oleae* using a versatile transgenesis marker

Martha Koukidou*, Apostolos Klinakis*, Chronis Reboulakis, Laskaro Zagoraiou, Nektarios Tavernarakis, Ioannis Livadaras, Aristidis Economopoulos and Charalambos (Babis) Savakis

* These authors have contributed equally to this work

Introduction

Bactrocera oleae **is the main pest of olives in the Mediterranean region and in California, where it has been introduced recently. Currently, control of *B. oleae* is based on the use of insecticides either in bait or in cover sprays, resulting in adverse effects on the environment (Ferreira & Tainha, 1983). Availability of a method for olivefly transgenesis is a prerequisite for genetic manipulation of this species for development of alternative control strategies, such as SIT.**

The technologies for insect transgenesis developed so far rely on transposable elements. The transposon *Minos* from D. hydei belongs to the *Tc1*/Mariner family of transposable elements (Franz & Savakis, 1991) which, in addition to insects, has been shown to mediate transgenesis in human cells (Klinakis *et al*., 2000), in mouse somatic and germ cells (Zagoraiou *et al*., 2003; Drabek *et al*., 2003) and in the ascidian Ciona intestinalis (Sasakura *et al*., 2003). Encouraged by this broad host range, we attempted germ-line transformation of *B. oleae* with a *Minos* vector carrying a self-activating cassette which overexpresses EGFP. The cassette has been tested in transgenic *D. melanogaster* and *C. capitata*. The versatility of this novel EGFP based marker was further explored in a transgenic nematode (*C. elegans*) and in somatic cells of animals and plants.

Figure 1. Top: the tTA/EGFP self-stimulating expression cassette. Bottom: EGFP expression

a-d, transformed *D. melanogaster* adult, embryos, 3rd instar larva and pupae;

e-f, transformed medfly pupa and adult;

g-h, stably transfected human HeLa cells;

i-j, transiently transfected *Nicotiana tabacum* leaf;

k, transgenic C.elegans

Red arrowheads point to non-transformed individuals.

Figure 2. Schematic representation of *B.oleae* transformation experiment.

3833 preblastoderm dacus eggs were injected

↓

641 larvae hatched

↓

373 pupae

151 males — **174 females**

Back-crossed to non-injected flies

↓

8824 3rd instar larvae were screened

↓

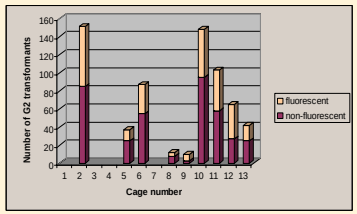
Recovered

67 fluorescent G1 larvae

13 fluorescent G1 adults

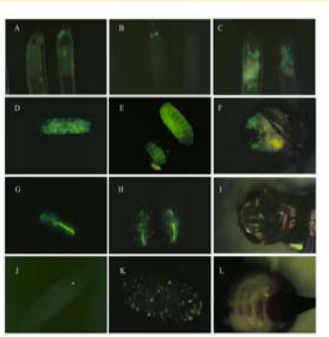
8 phenotypes

Figure 3. Frequencies of transformants among the olivefly G2 progeny.



EGFP expressing G1 progeny were individually backcrossed to wild-type flies. The G2 progeny was screened for EGFP expression. Bars indicate the total number of G2 flies from each G1 parent. The proportion of G2 progeny expressing the EGFP marker is also indicated. No G2 progeny was obtained from cages number 1, 3, 4 and 7.

Figure 4. Patterns of EGFP expression in injected (G0) embryos and in transformed *B. oleae* individuals.



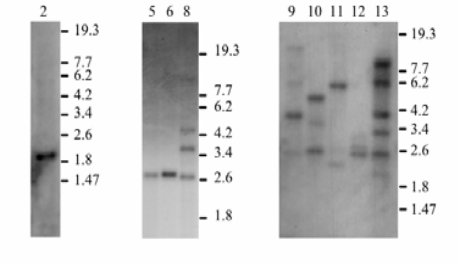
A-C, injected eggs

D-F, EGFP expression in larva, pupa and adult G2 progeny from line 12

G-J, EGFP expression in larva, pupa and adult G2 progeny from line 10

I-L, EGFP expression in larva, pupa and adult G2 progeny from line 9

Figure 5. Southern blot analysis of transformed olivefly lines.



Southern blot analysis of the *Eco*RI digested genomic DNA from individual EGFP expressing G2 progeny, using an EGFP-specific DNA probe. Line numbers are indicated at the top of the panel. Marker size is in kilobases.

Figure 6. Sequences flanking *Minos* insertions.

Line 9
Minos..GGGGCTCGTAATACTCTAGAATATCAAGATATAACTATCC*GATC*

Line 11
Minos..GGGGCTCGTATTCGGATTTTGATTCG*GATC*

Line 12
Minos..GGGGCTCGTATGAGTGTATATTTGTTTAGGTGTTTGCTTATGTATGTATGTATGGCTGTTAAATATA
TTTGAGCATACATATTTATGATTAGTGGACTGTATATTCCAACATGTTTCTTATCTTAACATGTAAAATAATA
AACCTAAATTTATTGCTGCACGCCGTCTAAGTAGATAAT*GATC*

Line 10
Minos..GGGGCTCGTAGAAGCTGGCTAACGTGCTTGGCATAATTTATAGAAGCATTTGTGTAGGCTTGTCTTA
ATGGCGAAAAATTGAAAACATTTAGCATTTACAAGCACTTGGTACCGGCCTAGGAGCCA*GATC*

Flanking sequences were determined by inverse-PCR. The end of the transposon sequence is in bold face. The *Mbo*I restriction site used in inverse PCR is underlined. The TA target dinucleotide is indicated.

Concluding remarks

We describe a self-stimulating transcription system for high levels of regulatable expression of a marker gene, which can be used in diverse species. Using this system in combination with a *Minos* transposon, we demonstrate stable and efficient transgenesis of the olive fly *Bactrocera oleae*.

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Minos vectors carrying the versatile tTA/EGFP marker cassette are available upon request

SEX PHEROMONE OF THE NEW WORLD SCREWWORM FLY: Identification, Bioassay & Analysis of Natural compounds

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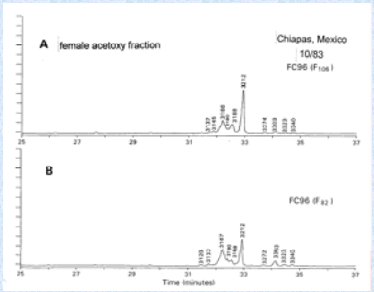
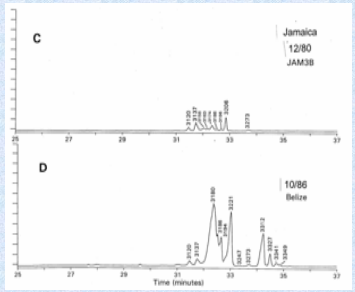
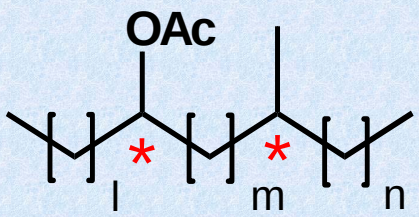
Male responding to synthetic pheromone Compound 1 treated onto dead sibling male at 5 ug

INTRODUCTION: Sex pheromones of *Cochliomyia hominivorax* (NWS) been studied since 1983 when its presence was demonstrated as a female-produced sex stimulant. Pomonis et al. (1993) determined chemical structures for 13 secondary alcohol acetates that were 29 carbon chain hydrocarbon compounds, half of which had one methyl branch near the other end of the chain. Isolation of individual components for bioassay failed (Pomonis, unpublished data) and none were synthesized until recently.



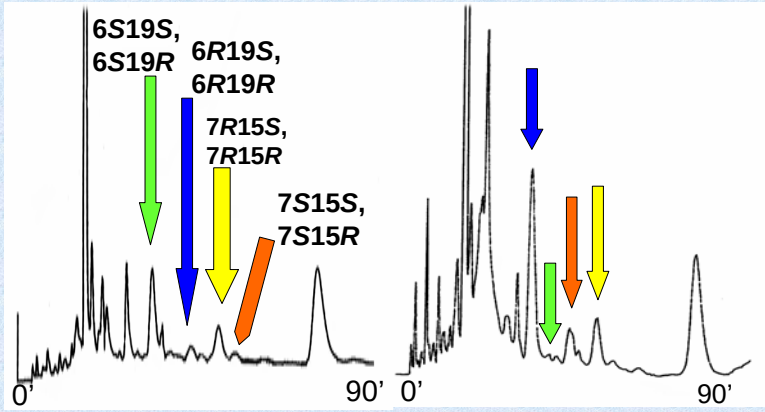




(1): l = 4, m = 12, n = 9 are most active;
(4): l = 5, m = 7, n = 13 less active;
Thus each methyl-branched structure has 2 assymetric centers (*) and 4 enantiomers

(1*R*,2*R*)-2ACycloH-COO derivative of a natural sample

(1*S*,2*S*)-2ACycloH-COO derivative of a natural sample

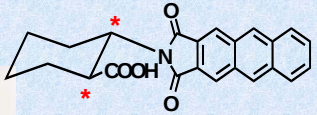


Column: Develosil ODS-A-3 (4.6 mm id x 150 mm) at 0 Mobile phase: MeCN:THF (2:1) for HPLC chromatograms of 2ACyclo-COO derivatives & stds.

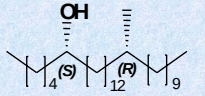
Pomonis et al. (unpublished data) analyzed many different strains of NWS females by GC. Female flies from long-term colonies produced little sex pheromone materials. Old colony females from Chiapas (A, B) & Jamaica (C) produce much less pheromone than wild females from Belize (D) and Libya (ca 200 X). When little of these pheromones are present, wild and colony males will not attempt to actively mate with these "pheromone-depleted" females.

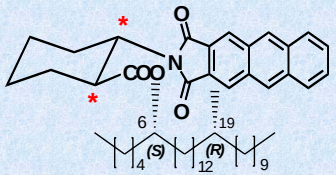
REFS: J. G. Pomonis, L. Hammack, H. Hakk, *J. Chem. Ecol. 19*, 985-1007. **1993**
A. Furukawa, C. Shibata, K. Mori, *Biosci. Biotechnol. Biochem. 66,* 1164-1169. **2002**
K. Mori, *Biosci. Biotechnol. Biochem.*, *67,* 2224-2231. **2003**
D. A. Carlson, D. Berkebile, K. Mori *Naturwissenschaften* (submitted)

For HPLC, these highly fluorescent derivatives are visible at 1-100 fm femtomoles)

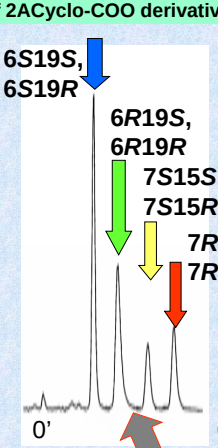


(*rac*) -, (1*R*, 2*R*) - or (1*S*, 2*S*) - **2ACyclo-COOH**

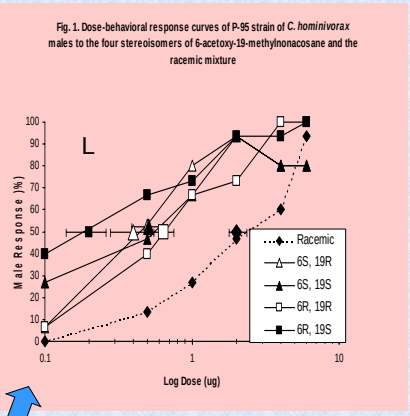


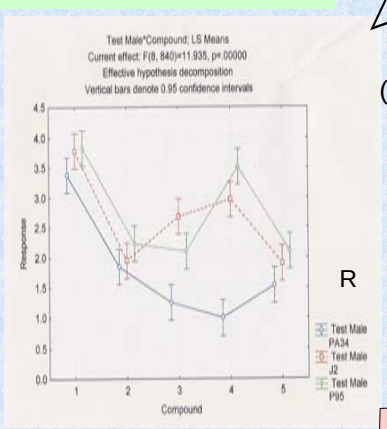


(1*R*, 2*R*) – (6*S*, 19*R*)
(1*S*, 2*S*) – (6*S*, 19*R*) = (1*R*, 2*R*) – (6*R*, 19*S*)



(6*R*,19*R*)-6-acetoxy-19-methylnonanocosane, and (7R,15R) & (7R,15S) -7-acetoxy-15-methylnonanocosane were detected by using both enantiomers of the reagent & are present in the natural sample. These are the only 2 isomers for which all 4 enantiomers were synthesized.

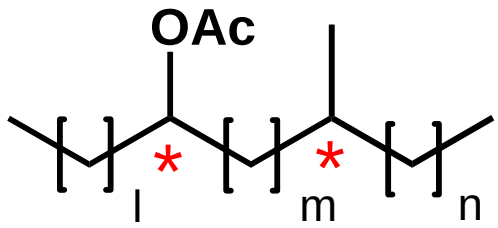




L: Bioactivity of the of all 4 synthesized enantiomers of the most active compound (1), 6-acetoxy-19-methylnonacosane: 6R,19S- was most active, giving 50% sexual responses at 0.2 ug, while others needed 0.5 ug. **R:** Bioassays of 5 racemic isomers showed activity as Compound **1>4>3=5=2**. Note the oldest strain Panama 34 responded poorly to **4**.

Questions arise: This pheromone is a complicated mixture of optically-active enantiomers.
1. **Do all colonized females Time-change and lose the ability to synthesize sex pheromone?**
2. **Are females from exotic locations alike or different from old colonized females? (Yes)**
3. **Are females from Jamaica like or not like Tuxtla-produced females? (Probably No)**
4. **Do wild J males mate well with factory females? (Recent study: No.)**
5. **Do factory males mate well with wild J females? (Recent study: Yes, good for SIT).**
6. **Has this Time-change affected the Jamaica program?**
7. **Is this Time-change a problem for other SIT programs in the Caribbean and South America?**

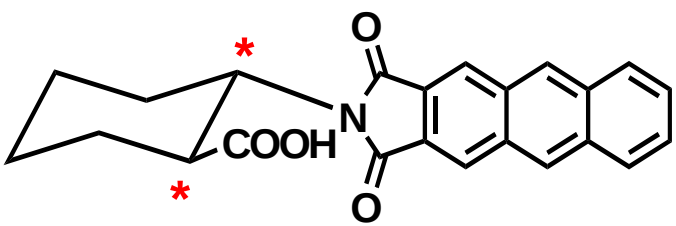
HPLC of (1*R*,2*R*)-2ACycloH-COO derivatives of standard compounds. (Match vertically with trace above)



l = 4, m = 12, n = 9 very active
l = 5, m = 7, n = 13 active

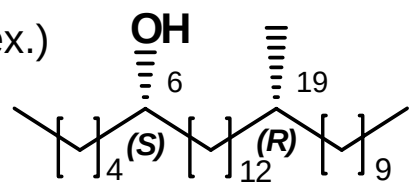
Structures of the pheromone components of the Screwworm Fly, *Cochliomyia hominivorax*.

J. G. Pomonis, L. Hammack, H. Hakk, *J. Chem. Ecol.* **1993**, *19*, 985-1007.
A. Furukawa, C. Shibata, K. Mori, *Biosci. Biotechnol. Biochem.*, **2002**, *66,* 1164-1169.
K. Mori, *Biosci. Biotechnol. Biochem.*, **2003**, *67,* 2224-2231.

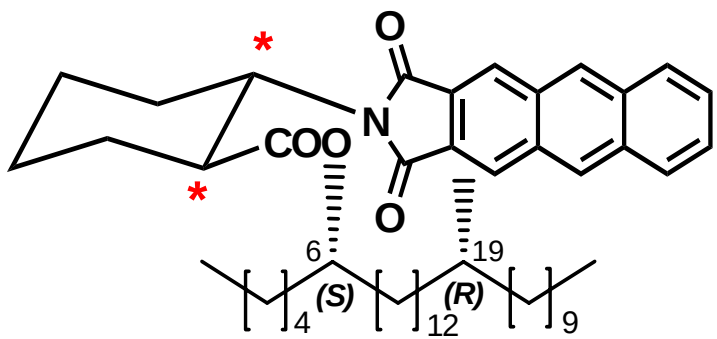


(*rac*) -, (1*R*, 2*R*) - or (1*S*, 2*S*) -
2ACyclo-COOH

ex.)



EDC, DMAP
toluene, CH_3CN



(1*R*, 2*R*) – (6*S*, 19*R*)

(1*S*, 2*S*) – (6*S*, 19*R*) = (1*R*, 2*R*) – (6*R*, 19*S*)

Figure . Derivatization procedure of branched secondary alcohols.

IAEA-CN-131/179 P

DEVELOPMENT OF THE NEW HIGH TEMPERATURE SHORT TIME VERTEBRATE BLOOD PASTEURIZATION EQUIPMENT FOR THE TSETSE FLIES DIET

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INTRODUCTION

Tsetse flies feed only on vertebrate blood, but the collection and processing of blood is expensive, it must be stored at – 20°C requiring costly storage rooms and reliable electricity, and it must be irradiated to reduce bacterial contaminations. For smaller colonies this is tolerable, but as colony sizes increase to service large-scale programmes, the supply and processing of blood is going to become critical. Blood is normally collected from cattle at slaughter. This process is necessarily not aceptic, and large-scale collection is only possible where the animals are suspended for bleeding. Large slaughter facilities with the necessary throuhput of animals for slaughter and appropriate equipment for suspension are not common in Africa. The blood must be prevented from coagulating, which is normally achieved by mechanical defibrination. This is laborious and potentially removes valuable nutrients from blood. Heparin has been used as an alternative, but it is both expensive and thermolabile, making is extremely difficult to deliver to destinations in Africa. Because collection is not aseptic, bacterial contamination must be reduced, which is currently achieved by gamma irradiation. Large irradiators are available in very few locations in Africa, and the lack of suitable legislative control prohibits the installation and use any for of irradiators in several countries. The collected, defibrinated blood must be stored frozen. The lifespan of frozen blood may be limited to a few years. Commercial cold storage is often not available, and the installation and running of dedicated storage is expensive. To address some of these issue one of the alternatives is use the High Temperature Short Time Pasreurization (HTST) method. The food processing industry uses pasteurization to reduce bacterial load in a wide range of products. The equipment required for pasteurization is simple in concept, and available to process quantities from a few litres for experimental work, a few hundred litres as used on individual farms, to very large/scale plants for the commercial processing of dairy and other products. The main idea of our proposal is to develop the adequate bech top operation pasteuriser HTST unit which allows use the effects of heat processing on blood product quickly and economically. The HTST pasteurization process can be undertaken using very small quantities of blood in a relatively short time, so the production is both quick and economical.

Pasteurization device MP 200

- up to 1 500 l/day
- 200 l/hour
- temperature 50-90°C
- plate heat exchanger
- holding time 25 s
- good cleaning possibility

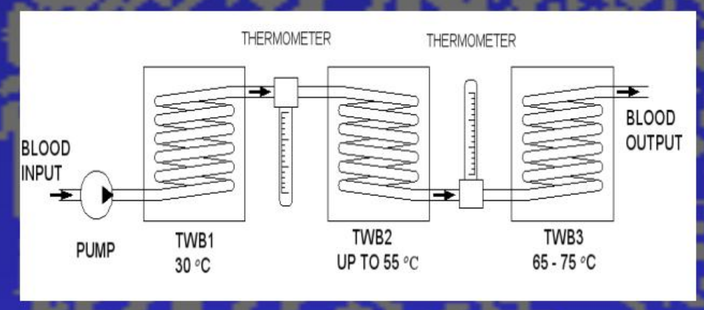
MP 200 – hplding time 12 s

- inner diameter 25 mm
- lenght 1360 mm

MP 200 – holding time 3 s

- inner diameter 19 mm
- lenght 540 mm

Laboratory simulation



TWB1 – Thermal water bath 1
TWB2 – Thermal water bath 2
TWB3 – Thermal water bath 3

Laboratory experiments

- temp. of the room 24°C
- blood circulation
- lenght of the tube in TWB3 – 2000 mm
- output temp. 58-62°C
- output temp.in the glass 35.2°C and rise up
- blood coagulation

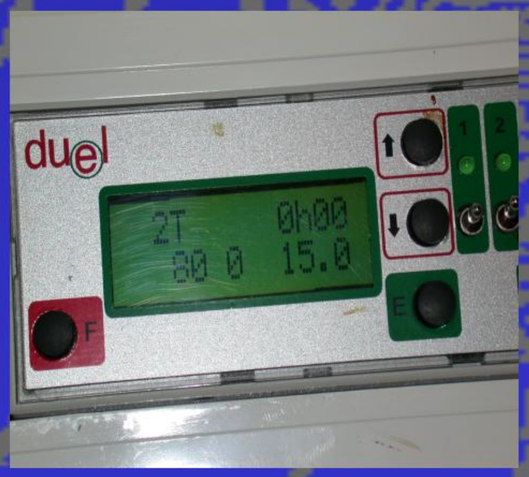
Thermal water bath 1 –30 °C

- preheating the blood to 30°C

Cooling system

- ice cold water
- plastic tube was rolled up on the cylinder
- water temp. 13°C
- good blood quality

External thermometer 80°C



CONCLUSIONS

- determine the highest temperature of the blood coagulation
- change the sodium citrate concentration
- determine outlet time – temperature
- design new industrial pasteurizator

IAEA-CN-131/180P

Hormone in the Uterus from the Flesh Fly *Sarcophaga bullata* Stimulates the Parturition and Abortion of the Tsetse Fly *Glossina morsitans centralis*

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Introduction

Tsetse is one of the few insects that have evolved a mammalian-type of reproductive strategy of producing only a single offspring at a time. At the culmination of a 9-10 day pregnancy cycle the female gives birth to a fully grown larva. The expulsion of the larva is regulated by a hormone present in rich abundance within the female's uterus. The hormone elicits parturition when injected into a pregnant neckligated female. It also stimulates abortion in earlier stages of pregnancy. Parturition hormone (PH) activity is present also in other species, not only in tsetse *Glossina morsitans* but also in the oviduct of *Bombyx mori*, in the oviduct and ejaculatory duct of *Schistocerca gregaria*, as well as in the uterus of the flesh fly *Sarcophaga bullata*.

Methods

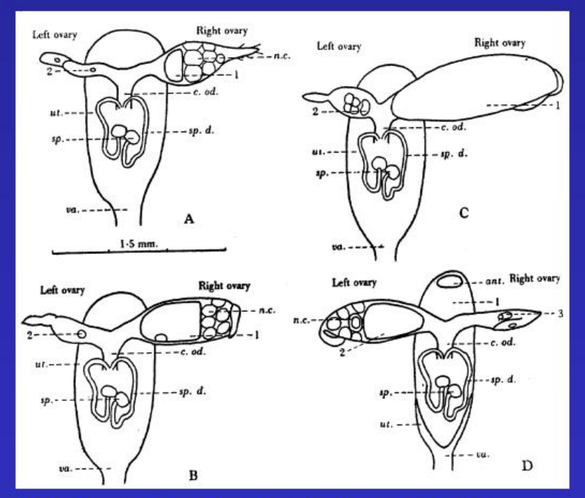
The uteri from *S. bullata* were dissected from the pregnant females on the expected day of parturition (11 days after adult eclosion at 25°C) in cold saline. Samples were homogenized, centrifuged (10.000G, 10 min) and injected. Pregnant tsetse females used in the bioassay were neckligated in the morning of the expected day of parturition and injected with either Locke's buffered saline or saline tissue extract in the afternoon.

Results

We observed a low incidence of parturition in *G. morsitans* in response to saline injection, while the injection of uterine extract from *S. bullata* containing 0,2 uterus equivalents (UE) elicited a high incidence of parturition, over 67%. When injected uterine extract containing 0,4 UE it also elicited a high incidence of parturition, 80%.

In our bioassay we used also uterine extracts of *G. morsitans*. Uterine extracts containing 0,4 UE elicited parturition in 70% of the ligated females and the extract containing 1 UE elicited parturition in over 90% of the ligated females. As we can see, uterine extracts of both, tsetse and the flesh fly, elicited a high incidence of parturition, in over 60% of the ligated females. In most cases, the response was immediate, within 10 minutes after injection.

We compared the effect of the uterine extract with and without the proteases inhibitor. Using the extracts of tsetse uterus, there was no difference, both elicited a high incidence of parturition, 70%. However, not in the case of the flesh fly's uterine extracts. The uterine extracts with inhibitor elicited parturition in 42% of ligated females, while the uterine extract without the inhibitor elicited parturition only in 10% of ligated females.



The ovaries alternate in producing the egg

Nulliparous female, no ovulation

Female with one ovulation egg *in utero*

Injecting the neckligated female

The neckligated female is giving birth to larva after being injected

Tanning – the colour changes several minutes after the puparium is formed

Conclusions

The tsetse uterus is a rich source of a hormone that elicits parturition. When injected into a neckligated female on the expected day of parturition, PH prompts the female to quickly give birth, and when injected into females in earlier stages of pregnancy it acts as an abortifacient, causing the female to expel the contents of her uterus. However, the PH does not act alone in regulating parturition. The response can only be elicited in females that have been neckligated. Intact females do not respond to PH by expelling their larvae. Presumably the natural coordination of parturition involves both neural and humoral input, and only when a nervous inhibition from the mother's head is experimentally removed by neckligation can the fly respond to PH. The discovery that an extract from the uterus of *S. bullata* and genital ducts of several other insect species also elicits parturition in tsetse suggests that this hormone may be widely distributed in insects and should have common genetic origin with possible oviposition/larviposition stimulating peptides of other insects.

IAEA-CN-131/181P

Examination of the Flight Muscle Development in Male *Glossina pallidipes*

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Introduction

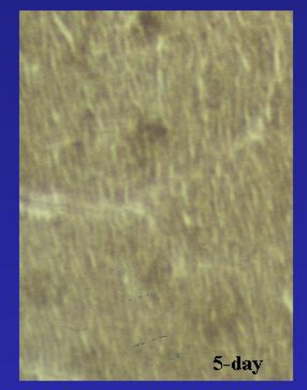
Flight muscle growth in adult tsetse flies has become important in recent years because of the interest in the sterile male release control technique. The flight muscles of tsetse flies reared in the laboratory grow much more slowly than those of wild tsetse flies and may not always attain full size. If fully viable males are to be reared in large numbers this difficulty will have to be overcome. Forced flight may be a solution but there are great practical difficulties in forcing these flies to take flight. The main idea of our presentation is also some information about the development of the thoracic muscle and flight behaviour of *Glossina pallidipes*.

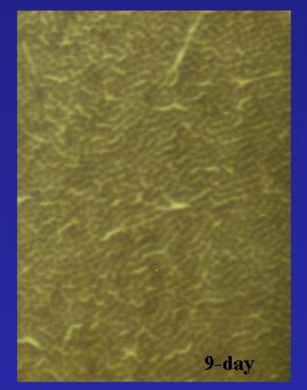
Methods

Thorax muscles of tsetse flies of various age were investigated using some methods. Succinic dehydrogenase activity (SDH) to monitor thoracic muscle energy metabolism. ATP-ases (myosin and mitochondrial) to demonstrate activity of actomyosin. The relation between size of the thoracic surface and the residual (= not fatty) dry weight (RDW) was determined for teneral and non-teneral males. Ultrastructural changes in the flight muscles during growth of the tsetse flies demonstrates transmission electron microscopy examination.

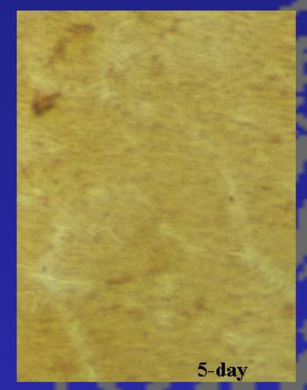
Results

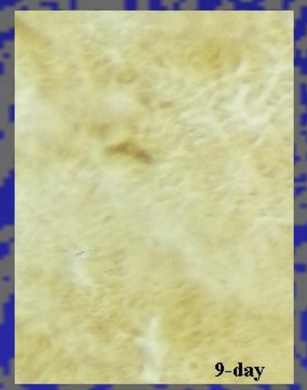
The results showed two types of thorax muscles – dorsolongitudinal and tergosternal muscles. Dorsolongitudinal and tergosternal muscles differ in SDH activity. Dorsolongitudinal muscles exhibited high, while tergosternal muscles very low enzyme activity, but there were no apparent differences among age groups. Marked myofibrillar actomyosin ATP-ase activity was observed in both types of muscles, and it gradually increased with the age of flies, while mitochondrial ATP-ase has opposite course. RDW of the non-teneral tsetse flies is much greater than of the teneral. Electron microscopy results show age-related increase in the volume of myofibrils in muscle fibres, while sarcoplasmic volume correspondingly declined.



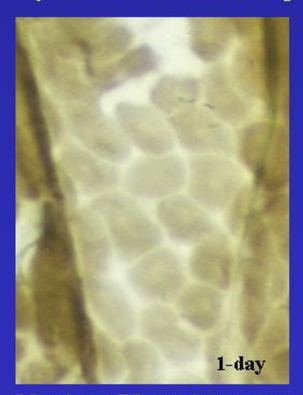


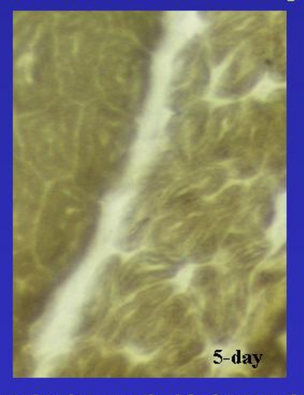
Myosin ATP-ase in dorsolongitudinal muscles increased with the age of flies

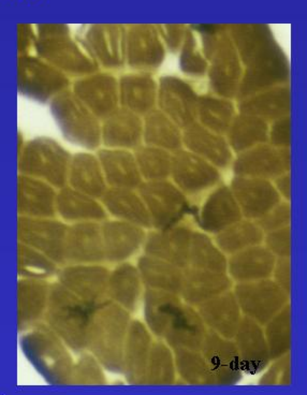




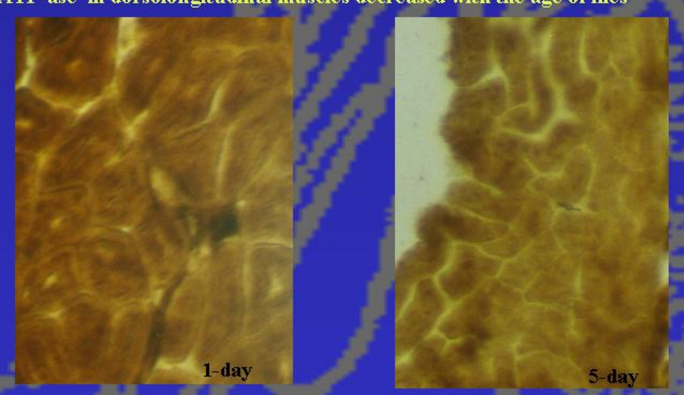
Mitochondrial ATP-ase in dorsolongitudinal muscles decreased with the age of flies



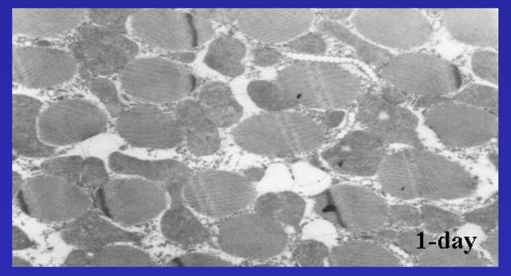


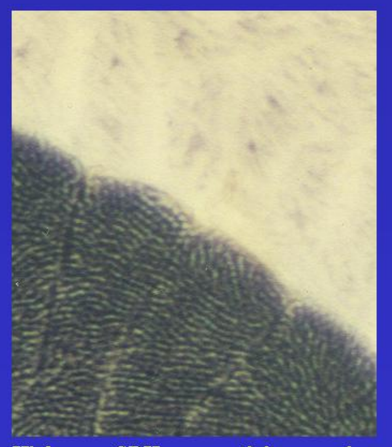


Myosim ATP-ase in tergosternal muscles increased with the age of flies

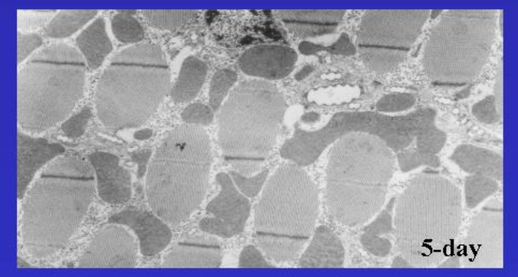


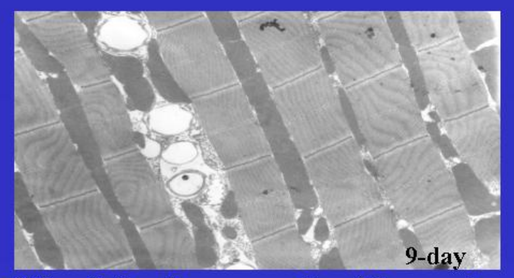
Mitochondrial ATP-ase in tergosternal muscles decreased with the age of flies



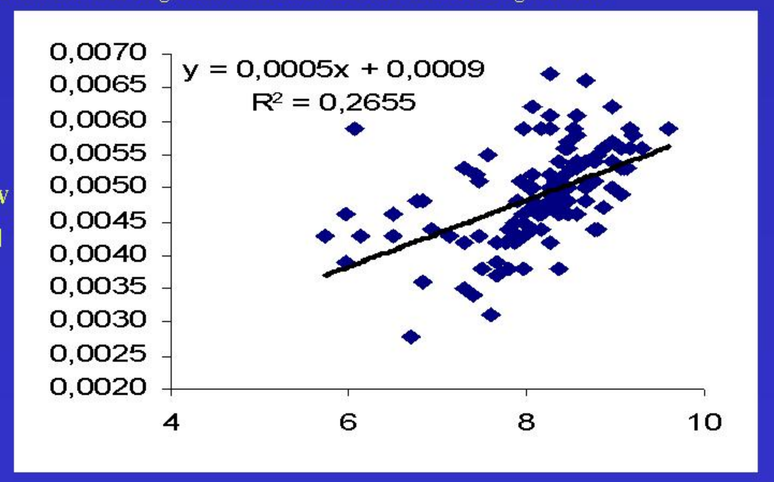


High SDH activity in dorsolongitudinal (dark) and low in tergosternal muscles (light) in all age groups

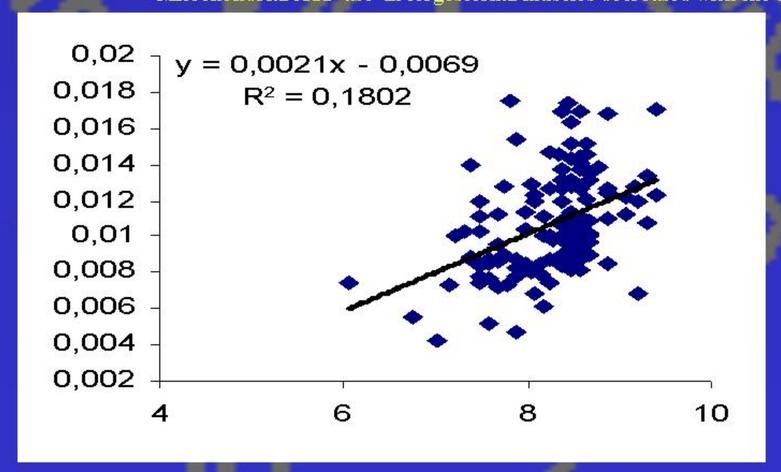




Differentiation of sarcomere units and decreases in sarcoplasmic volume in respect to the age of flies



RDW of the non-teneral tsetse flies is much greater than of the teneral



Conclusions

Preliminary results also suggest, that high activity of enzyme involved in energetic metabolism is in the most developed contractile filaments of flight muscles. It is interesting that ATP-ase activity localized in mitochondria exhibited higher activity in muscles of younger flies than muscles of older ones, and so has opposite course than ATP-ase localized in myofibrils. This is probably due to different activity and energy metabolism of mitochondria during the development of the fly. Studies noted that the RDW of the non-teneral tsetse flies is much greater than of the teneral and it is thought that the post-teneral development of thoracic musculature might account for the increased firmness of the thorax. Electron microscopy examination show progressive, age-related increase in the volume of myofibrils in muscle fibres, while sarcoplasmic volume correspondingly declined. Development and differentiation of sarcomere units was clearly visible in connection with increasing age of the fly studied.

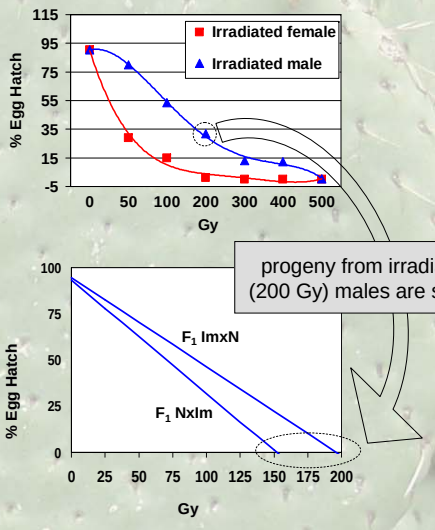
Developing the sterile insect technique for area-wide management of the invasive cactus moth, *Cactoblastis cactorum*

J. Carpenter, USDA-ARS-CPMRU, USA
S. Hight, USDA-ARS-CMAVE, USA
S. Bloem, CBC-FAMU, USA
K. Bloem, USDA-APHIS-CPHST, USA
C. Tate, USDA-ARS-CPMRU, USA

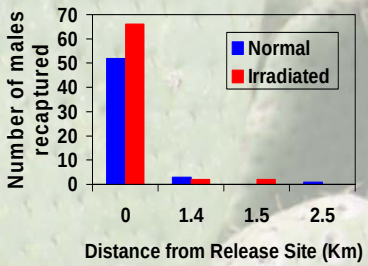
- Although well known as a highly effective biological control agent against invasive *Opuntia* spp., the cactus moth has become a serious threat to both native and cultivated *Opuntia* species throughout the world. Its presence in the Caribbean and rapidly expanding range in the southeastern USA are imminent threats to the biodiversity, and to the forage, vegetable and fruit production of the southwestern USA and Mexico.
- To address this threat, a strategic plan is under development which has a strong emphasis on the development of the sterile insect technique (SIT) to control, contain, or mitigate the geographical expansion of *C. cactorum*.
- We have conducted research on sterilization, mass-rearing on artificial diets, handling and transportation of irradiated moths, and field efficacy of mass-reared and irradiated moths.
- A dose of 200Gy was effective in completely sterilizing the adult females, reducing the reproductive rate of males and producing complete sterility in the progeny of irradiated males.
- Research on artificial diets and diet presentation systems have indicated that *C. cactorum* can be reared more efficiently on artificial diets than on host plants.
- To evaluate the efficacy of laboratory-reared moths, we released partially sterile males alone or in combination with fully sterile females at a 5:1 or 10:1 (treated:fertile) ratios inside large field-cages containing *O. stricta* host plants. Limited field release-recapture experiments to examine the dispersal ability of untreated and treated cactus moth males were also conducted. Results suggest that an overflooding ratio as low as 5:1 can effectively suppress *C. cactorum* in field-cages, and that releasing both genders together may be more effective than releasing males only. Finally, our results suggest that the dispersal ability of *C. cactorum* is not significantly affected by treating adults with 200Gy.
- An area-wide field trial currently is in progress to evaluate the ability of the SIT in combination with cultural controls to reduce *C. cactorum* populations near the leading edge of the expanding geographical range.

Mass-rearing

Sterilization



Release-recapture studies



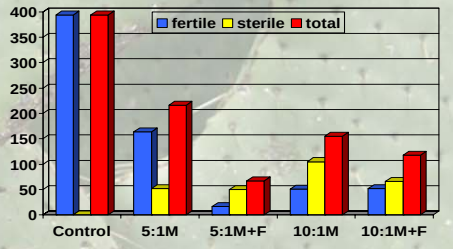
SIT field efficacy study

Dauphin Island
Sanitation + SIT

Pensacola Beach & Okaloosa Is.
Sanitation only

Field-cage studies

Number of F_1 larvae produced per treatment



- **4 test sites (control, sanitation, & SIT + sanitation) -** control location (St. George Is.) not shown on map
- **Population monitoring includes:**
  1. pheromone traps
  2. sentinel plants for egg/larval counts
  3. egg/larval/pupal counts from samples taken during sanitation activities
- **Treatment efficacy will be evaluated by analyzing the relationships between:**
  1. wild males captured in traps
  2. release/recapture of irradiated males
  3. virgin female mating tables
  4. overflooding ratio (irradiated : wild)
  5. percentage hatch of collected eggs

NEW INSECTICIDES FOR CONTROL OF THE INVASIVE CACTUS MOTH, *Cactoblastis cactorum*, IN FLORIDA

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Stephen Hight, USDA-ARS-CMAVE, FL, USA
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- The cactus moth is an invasive *Opuntia* feeding Pyralid moth first detected in Florida, USA in 1989.
- As it rapidly moves westward, it threatens the fragile *Opuntia*-rich desert ecosystems of the western USA and Mexico, which are the centers of *Opuntia* diversity.
- Furthermore, the cactus moth is a grave threat to the thousands of hectares of cultivated *Opuntia* in Mexico.
- In response to these concerns we are developing an area-wide program based on the sterile insect technique (SIT) and other complementary suppression tactics to prevent the westward spread of the cactus moth, and to treat new infestations.

- Nine insecticides were assayed in the laboratory as mortality agents for eggs and newly emerged larvae of the cactus moth.
- Freshly laid eggs, as well as eggs with late embryonic development, were tested by dipping egg-sticks in each product for one minute.
- Cactus pads were dipped into the different products and assayed after 24 hours or stored for 30 days before offering them as substrate to young larvae.
- Our results showed that several products were 100% effective at killing both eggs and neonates of the cactus moth under laboratory conditions.
- These products may be useful as an aid to clean-up operations in preparation for SIT or to protect *Opuntia* agricultural commodities and specimen plants within infested areas.

Product	Mean (± S.D.) % Survival			
	Eggs		Neonate Larvae	
	Treated 1-day old	Treated 28-days old	Exposed to pads 24 hrs post treatment	Exposed to pads 30 days post treatment
Control (H_2O)	79.5 ± 20.8 a	84.8 ± 11.0 a	64.4 ± 44.4 a	81.4 ± 9.4 a
Cypermethrin (Ammo)	0 c	0 c	0 c	0 c
Emamectin Benzoate (Proclaim)	5.8 ± 7.4 c	0.6 ± 1.9 c	0 c	7.9 ± 25.0 c
Abamectin (Avid)	4.3 ± 9.1 c	3.3 ± 8.4 c	0 c	85.6 ± 8.5 a
Spinosad (Spintor)	0 c	0 c	0 c	0 c
Azadirachtin (Azatin)	52.7 ± 35.1 b	85.5 ± 11.5 a	54.6 ± 30.2 ab	43.6 ± 40.5 b
Fenoxycarb (Distance)	8.57 ± 27.1 c	40.3 ± 35.4 b	64.0 ± 35.2 a	73.9 ± 13.4 a
Imidacloprid (Admire)	0 c	0.6 ± 1.9 c	0 c	3.6 ± 10.2 c
Acephate (Orthene)	38.9 ± 33.4 b	39.7 ± 37.0 b	35.4 ± 38.7 b	87.3 ± 10.2 a
Bacillus thuringiensis	-	-	0 c	0 c

Survey and detection strategies for area-wide control of the cactus moth, *Cactoblastis cactorum*

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S. Bloem, CBC at FAMU, FL, USA
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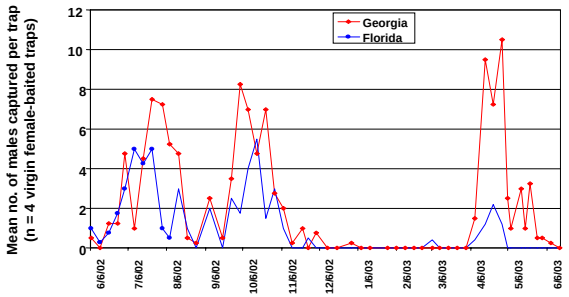
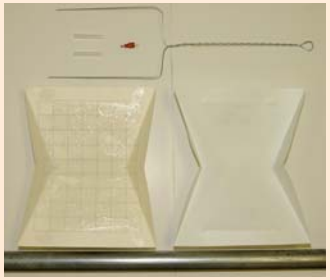
- The South American cactus moth is renowned for its ability to control invasive cacti (*Opuntia* spp.).
- However, the moth's accidental arrival into Florida, USA, in 1989 has turned this beneficial biological control agent into an invasive species.
- Its rapidly expanding range is threatening the *Opuntia*-rich ecosystems of the western USA and Mexico, which are the centers of *Opuntia* endemism.
- Our group has conducted research to develop basic survey and detection tools to study and track the cactus moth's rate of spread, identify new cactus moth outbreaks, and support control-suppression programs using the SIT.
- We have evaluated different trap types, trap placement heights and trap colors, as well as the number and age of virgin female "baits" for their ability to capture wild male cactus moths.
- We also examined the attractiveness of fertile and sterile (treated with 200Gy of gamma radiation) cactus moth females as bait in traps.
- In addition, studies are underway to identify the female sex pheromone to use as bait in the most effective trap design. Experimental synthetic lures have been identified and evaluated in laboratory bioassays and field trials. The data indicate that the lures are attractive to cactus moth males and may be useful as a survey tool in an expanded trapping and detection network for *C. cactorum*.
- Our work has documented that the cactus moth completes three generations per year in the USA.

The best survey and detection tool that we have thus far is a normal (factory white) color wing trap placed at a height of 2 meters above ground and baited with either 1-day-old (fertile or sterile) females or with a cactus moth experimental lure.

C. cactorum monitoring 2002-2003 three generations per year



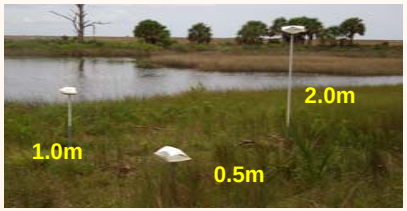
Components of experimental lure trap based on female sex pheromone.



TRAP TYPE

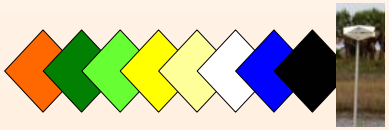
Trap captures were greatest with Wing > Delta > Bucket.
Wing traps caught 18% more moths than Delta traps and 75% more than Bucket traps.

TRAP HEIGHT



Trap captures were greatest at 2.0 m > 1.0 m > 0.5 m.
Traps placed at 2.0 m caught 13% more moths than traps at 1.0 m and 38% more than traps at 0.5 m.

TRAP COLOR



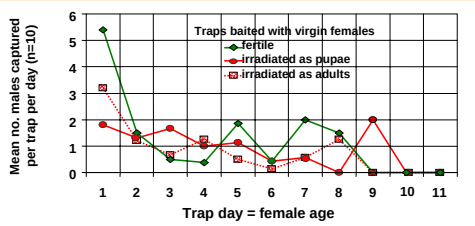
No significant differences in trap capture due to color.

YOUNG vs. OLD FEMALES

Traps with females that were 1-day-old caught 85% more males than traps with 4-day-old females.

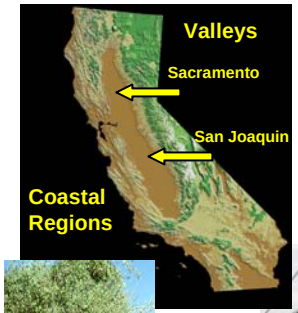
FERTILE vs. STERILE FEMALES

No significant differences in trap capture when traps were baited with fertile females vs. females that were irradiated as pupae or adults.



Systems Approach for Control of Olive Fruit Fly in California

Victoria Y. Yokoyama, Ph.D., and Gina T. Miller, M.S.
USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA, USA



CALIFORNIA OLIVE CROP VALUE:

$90 U.S. Million
Canning Olives
Olive Oil

***Bactrocera oleae* (Gmelin)**
Olive Fruit Fly (OLFF)

LIFE CYCLE IN LABORATORY:

Temp. 70°F, 60% RH

Eggs	5-6 d
1st Instar	7-8 d
2nd Instar	9-11 d
3rd Instar	12-15 d

Gail E. Sergent---Rearing OLFF on Olives

DETECTION & MONITORING:

Pherocon® AM

ChamP

Mature Orchard
Fresno, CA
Inland Valley

Greenhouse Test Cages

SURVIVAL IN LABORATORY TESTS:

ºC	% RH	Food, d	No Food, d
5	85	30-40	10-17
15	65	44-76	4-5
25	25	17-32	2-4
35	25	2-4	1

No. Adults/Day

	Pherocon	ChamP
Spring	16.0	11.8
Summer	2.0	0.8

FRUIT SUSCEPTIBILITY:

Size	No. Stings
<1 cm³	0-1
≥1 cm³	≥2

SURVIVAL IN GREENHOUSE:

Temp. 24ºC, 65% RH

	Food & Water	No Food or Water
OLFF	53-202 d	5-12 d
Parasitoid	17-69 d	4-15 d

Temp. 35ºC, 30% RH

OLFF	1-11 d	2-3 d
Parasitoid	3-7 d	1 d

ORCHARD SANITATION:

March 0.4 pupae/10 g - May 6.4 pupae/10g

BIOLOGICAL CONTROL:

***Psyttalia* cf. *concolor* (Szépligeti)**

Reared on Mediterranean Fruit Fly
USDA-APHIS PPQ
MOSCAMED, Guatemala

Shipping Containers
1% Mortality---
Optimum Conditions

Field Releases
10% Parasitism

Field Cages
100% Parasitism

This research was supported in part by the California Olive Committee, Fresno, CA, USA. The biological control studies were conducted in cooperation with Dr. John Sivinski, USDA-ARS, Gainesville, FL, and Dr. Pedro Rendon, USDA-APHIS, Guatemala.

Development & Evaluation of a Conditionally Lethal Transgenic Pink Bollworm

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Introduction

A new area-wide pest control strategy using the pink bollworm, *Pectinophora gossypiella* (Saunders), (Lepidoptera: Gelechiidae), genetically transformed with a conditionally lethal gene, is under development. Conditional lethality of several transgenic pink bollworm strains was demonstrated in a series of laboratory rearing experiments. Pink bollworm were transformed with genetic constructs using the RIDL technology (Release of Insects with a Dominant Lethal gene) for development of an autocidal biological control system for possible supplement or replacement of radiation based sterile insect release [1,2].

LA1124 is a lethal construct controlled by a tetracycline repressible transactivator protein (tTA), in which binding of tTA to its specific target sequence tetO drives production of more tTA. In the absence of tetracycline, this leads to lethality by high expression of tTA. When tetracycline is present, tTA does not bind tetO, and so the positive feedback cycle is not established and tTA remains at a low, non-lethal level (see [3] for more detail on the tetO-tTA system). Tetracycline (in the form of chlortetracycline or CTC) is a normal part of the pink bollworm artificial diet, so such a strain of pink bollworm could readily be incorporated into the current mass-rearing system..

Methods

The progeny of several independently transformed pink bollworm (PBW) lines with RIDL construct LA1124 were reared with and without tetracycline in the food to estimate mortality rates.

- Four PBW lines tested: 1124A, 1124B, 1124D & 1124E
- Four replicate crosses, one replicate = one ♂ 1124/+ X +/+ (wild type) ♀
- Eggs collected for 3 days and infested on TET-PLUS and TET-MINUS diets, half of the eggs from each collection date infested on the two diet types.
- Four treatments, 1124/+ reared on TET-PLUS, 1124/+ on TET-MINUS, +/+ on TET-Plus & +/+ on TET-MINUS
- Pupae screened at larval cut-out 20 d after infestation, 1124/+ genotypes determined by DsRed fluorescence
- Mortality scored at pupation and at adult eclosion
- Randomized complete blocks design, ANOVA followed by Tukeys mean separation
- Adult longevity for 1124/+ genotype raised as larvae with tetracycline (TET-PLUS), fed with and without tetracycline in adult diet.

Results & Discussion

The mean proportion of mortality for 1124/+ on TET-Minus diets ranged from 0.60 to 0.92 for the four lines and was statistically significantly higher than the mortality in the three control treatments which had mortality rates ranging from 0.01 to 0.17 (Fig 1.) ANOVA, effect of diet, F = 111.95, $P< 0.001$ df=1, Tukeys, $P< 0.05$.

Most mortality occurred at prepupal stage (Fig.2) though there was also mortality at adult eclosion and early larval mortality.

Adults of both 1124/+ and +/+ genotypes when fed adult diet with tetracycline lived longer than adults of both genotypes when fed without tetracycline (Fig. 4).

In tests of several independent lines, mortality of transgenic pink bollworm reared on artificial diet without tetracycline ranged from 60 to 90%. For current constructs, most of the mortality appears to occur at the prepupae stage. Future work will focus on developing lines that have higher rates of mortality at earlier stages and to quantify the fitness and performance of transgenic strains in small scale artificial rearing systems.

Figure 1. Mortality of progeny from LA1124 heterozygote males crossed with wild type females reared with and without tetracycline food. Mortality is scored at pupal and adult eclosion stages, and summed together. Each pair of bars consists of a paired experiment where the mortality of transgenic and wild type progeny reared on the same diet type are compared. Mean values for pairs of bars marked with a different letter are significantly different at P < 0.05 by ANOVA followed by Tukey's means separation. These are data from one experiment, for LA1124A, LA1124B, LA1124D and LA1124E strain pink bollworm crosses to wild type, the number of eggs reared on the two diet types were 620, 768, 540 and 976 respectively.

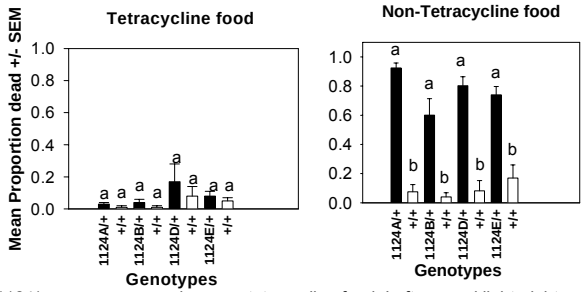


Figure 2. Dead 1124/+ prepupae reared on non-tetracycline food. Left, normal light, right same insects with excitation light showing DsRed marker showing the fluorescent phenotype of 1124/+ insects.

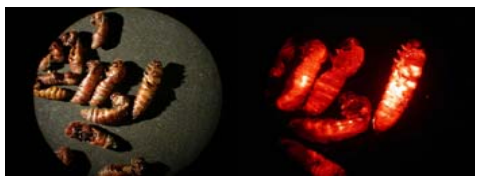


Figure 3. Healthy DsRed fluorescent 1124/+ and non-fluorescing +/+ pupae reared without tetracycline food shown under excitation light.

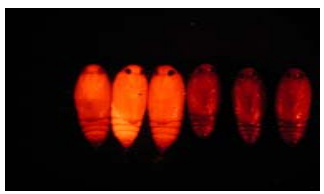
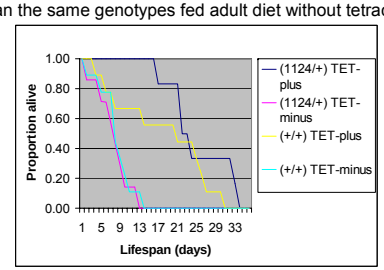


Figure 4. Adults of both 1124/+ and +/+ genotypes lived longer with tetracycline in adult diet than the same genotypes fed adult diet without tetracycline



Trabalho de Investigação realizado no âmbito do Projecto INTERFRUTA (MAC/3.1/A1), co-financiado pelo programa INTERREG III B

Applying GIS software to monitor adult *Ceratitis capitata* Wiedmann (Diptera: Tephritidae) behavior in Terceira Island, Azores

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Introduction

The capabilities of Geographic Information System (GIS) in our days are wide known and it has innumerous applications in many areas.

Monitoring *Ceratitis capitata* adult dispersion is very important because this pest is spreading on to many cultures causing severe losses on the orchards production.

Knowing the spread capabilities of *C. capitata* it's also important to evaluate the areas which are more affected by this pest, its population dynamics and its seasonal presence.

To monitor the *C. capitata* adult population, it was installed a network of traps in three fruit production areas of Terceira island. The installation of this network was integrated in the INTERFRUTA project since January 2004, and had the goal of a better knowing the evolution *C. capitata* adult dispersion using GIS, under ESRI software, ArcView 3.2.

Methods

The traps used on the network were Jackson and Tephri. In the first phase of work, it was necessary identify the possible spots where to install the traps. So, using ArcView 3.2, a grid with one squared km (fig. 1) was created over the three fruit production areas of Terceira island.

After that, the traps were installed (fig. 2) using a GPS in their spots previously determined.

The second phase of work was to analyse the trap counts records in ArcView. To perform spatial analysis regarding the data from the network traps, it was used spatial analysis extension and applied the Inverse Distance Weighting (IDW) method with a grid of 20m.

It was also used a digital model of terrain in order to cross information from the network traps and the topographic information.

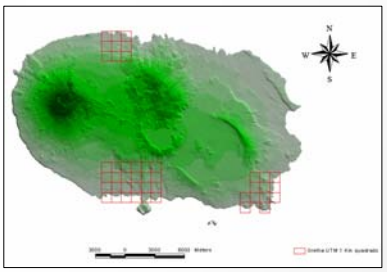

Figure 1 – UTM grid with one km square over each zone

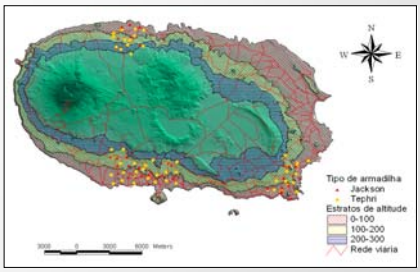

Figure 2 – Location of traps

Results

The results of spatial analysis in ArcView (fig. 3) show some dispersion over the three zones, with a remarkable concentration of this specie between 0 and 100 meters of altitude.

With the three-dimensional analysis (fig. 4), it is possible to see that the adults of *C. capitata* don't move a lot and prefer the areas located in some topographic depressions and areas with a higher vegetation density, which give these insect some climate protection, and conditions for building larger density populations.

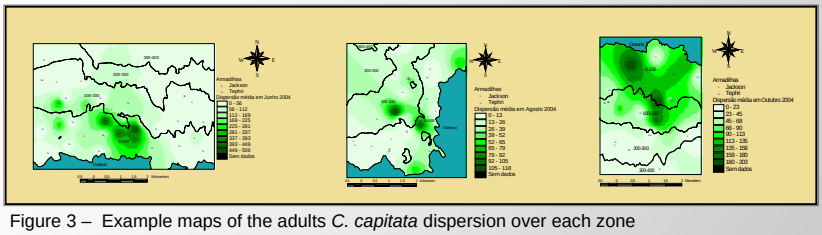

Figure 3 – Example maps of the adults *C. capitata* dispersion over each zone

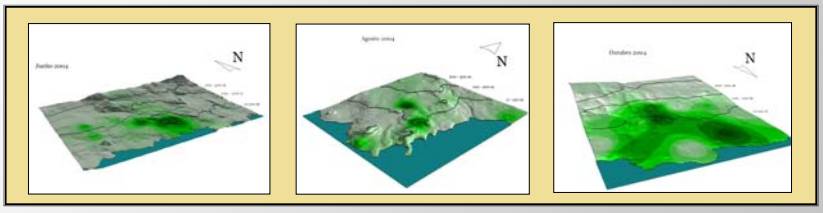

Figure 4 – Example three-dimensional analysis of the adults *C. capitata* dispersion over each zone

Conclusion

This software has demonstrated to be very useful to identify the problematic areas, as well as, in the study of the *C. capitata* dispersion behaviour.

The fact of this specie to be more concentrated in areas located in some topographic depressions and for areas with a higher vegetation density, this could be understood as a behaviour indicator for protection against the bad weather and adverse conditions for its development.

Attend to this specific behaviour, the host type, as well as the ripening period of fruit evolution, all these facts could explain the adult population dynamic registered in the studied areas.

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The INTERFRUTA project and the study of the Mediterranean fruit fly (*Ceratitis capitata* Wiedmann) (Diptera: Tephritidae) distribution in the fruit orchards of Terceira Island, Azores

Lopes, D.J.H. [1]; Pimentel, R. [1]; Costa, R. [1] ; Perez, C. R. [2] ; Dantas, L. [3]; Ornelas, L. [4]; Silva, D. [4]; Carvalho, F.C. [5]; Mumford, J. [6] & Mexia, A. [7]

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Trabalho de Investigação realizado no âmbito do PROJECTO INTERFRUTA (MAC/3.1/A1) co-financiado pelo PROGRAMA INTERREG III-B

Introduction

The INTERFRUTA is a European community project that as the goal of enlarges the fruit production (Lopes *et al*., 2004a).

It's a project of interregional cooperation between three regions and several Institutions that is financed by the EC (FEDER) by the program INTERRREG–III-B.

➢ **The Leading Institution of this project is the University of Azores (Department of Agricultural Sciences) and the other partners of this project are:**

- ✓ The Regional Government of Azores (Regional Secretary of Agriculture and Forests, Regional Direction of Agrarian Development;
- ✓ The University of La Laguna (UDI –Fitopatologia, Dept.º de Biologia Vegetal);
- ✓ FRUTER (Producers Association of fruits, horticulture products and flowers of Terceira Island);
- ✓ The University of Trás-os-Montes e Alto Douro (Plant Protection Department);
- ✓ The Imperial College of Science, Technology and Medicine (Department of Environmental Science and Technology).

In the field work of this project four fruit trees are being studied (orange, apple, peach and banana) in three different sites of Terceira island fruit production (Angra do Heroísmo, Porto Judeu/São Sebastião e Biscoitos) (Fig.1).

➢ **Some of the goals of this project are:**

✓ Achieve a better knowing of all the phytossanitary problems and natural enemies present in the fruit trees on the orchards and survey it's population evolution;

✓ Measure and calculate the economic impact and damage caused by the several pests that affect the fruit trees;

✓ Study and test different ways of control that may be use against the pests that can be easily used buy the fruit producer;

✓ Perform some sexual compatibility tests with sterile Mediterranean fruit flies from Madeira Biofabrica.

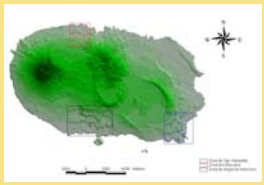

Figure 1- Location of the three production areas studied.

In this project we look in an integrated way to the fruit ecosystem as all, we also want to know the fungi and virus that cause problems in fruit trees, but above all the composition and the dynamics of all the insects present and in a special way the Mediterranean fruit fly (*Ceratitis capitata* Wiedman) (Diptera: Tephritidae) distribution and it's life cycle and impact, in terms of fruit infestation on the different orchards studied (Lopes *et al*., 2004b).

Material and Methods

➢ **Monitoring the Mediterranean fruit fly dispersion**

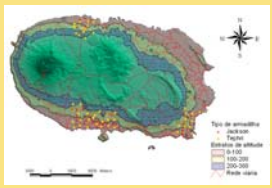

Figure 2 - The trap distribution in the three areas studied.

Monitoring *Ceratitis capitata* dispersion was very important because this pest is in to many cultures causing severe losses in the orchards fruit production.

To monitor the *C. capitata* population, was installed a network of 99 traps (Jackson and Tephri) in three fruit production areas of Terceira island (Fig. 2). In this net were followed the AEIA (2003) recommendations, using a ratio of four Tephri traps to one Jackson for each square kilometre. In Angra was monitored a area of 28,45 km2, in Biscoitos 10,20 km2 and in S. Sebastião 15,36 km2. The installation of this network was made using a portable GPS and the data collected were analysed with Arc View. All the traps were colleted every fifteen days, during a year and the lures were changed every 2,5 months (Nunes *et al*., 2004; Pimentel *et al*., 2005).

➢ **Estimating Mediterranean fruit fly damage in the fruits**

The estimate of Mediterranean fruit fly damage in the fruits was made using two methods: 1º marking the fruits in the trees trying to determinate the number of fruits damage by this pest (Fig.3) and 2º collecting the damage fruit near it's maturation period in the orchards and around the traps and bringing them to the laboratory (Fig.4). Here the fruits were put in small square plastic containers with a net top, in groups and individually to determinate the number of larvae per kilogram of fruit and the number of larvae found in each fruit, respectively, until all the larvae went out of the rotten fruits in the plastic recipient (from 1 to 3 months). In other hand using this fruits we tried to make a list of potential *C. capitata* fruit hosts during all year (Pimentel *et al*., 2005).

Figure 3 – Marking the fruits for estimating *Ceratitis capitata* (Wied.) damage.

Figure4 - Fruit in the recipients to register their larvae content

➢ **Traps testing**

Also were tested different ways of control that easily can be use by the fruit producer in monitoring and fighting against the Mediterranean fruit fly. For that purpose were tested six different kinds of traps: "Water Bottle" (Fig.5a); "American" (Fig.5b); "Easy Trap" (Fig.5c); "Jackson" (Fig.5d); "Tephri" (Fig.5e) and "Israeli" (Fig.5f) (Costa, 2005).

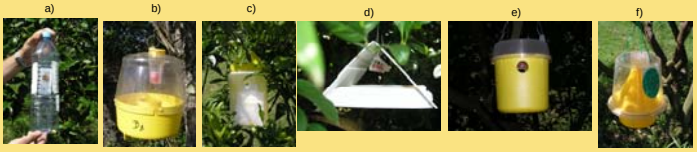

Figure 5 – The different types of traps tested in the capture of the Mediterranean fruit fly adults ("Water Bottle", "American", "Easy Trap", "Jackson", "Tephri", and "Israeli").

These traps were tested during four months, in two completely distinct orchards, one (S. Carlos) of homogeneous characteristics, in the distance of trees, and in terms of culture (citrus), and the other orchard (Quinta do Martelo) of heterogeneous characteristics, in distance between the fruit trees and with several types of fruit trees (Loquat, Citrus, Custard apple, and Strawberry guana).

In all these different traps were used two types of attractive with the same objective of the capture: for the males a sexual pheromone (trimedlure) and for the females a food-based synthetic lure with three component attractant (trimethylamine, amonium acetate and putrescine). Both the essays have been carried near Angra at the south part of Terceira Island, at an altitude of approximately 100m (Costa *et al*., 2005).

Results and Discussion

✓ In the **traps net** during 2004 were captured 91.118 adults of *Ceratitis capitata*. The results obtained pointed to the fact that the population level of the Mediterranean fruit fly reaches his peak near the ripe period of the fruit in the orchard (Fig.6), normally in mid summer. The peach was the fruit tree most affected in the three fruit areas studied.

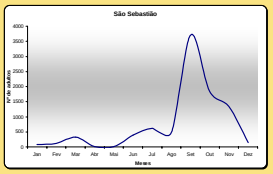

Figure 6 - The distribution of the adult *C.capitata* population in an S.Sebastião orchard.

✓ The results of **spatial analysis in GIS** using Arc View, show some dispersion over the three zones, with a remarkable concentration of *Ceratitis capitata* adults between 0 and 100 meters of altitude (Fig. 7). After using a three-dimensional analysis, it is possible to see that the *C. capitata* adults didn't move a lot and have a preference for areas located with some topographic depressions witch could give some climate protection to develop their populations (Pimentel *et al*., 2005).

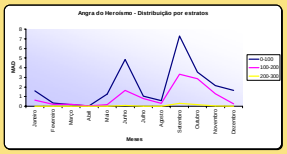

Figure 7 - The distribution of the adult *C.capitata* population regarding the altitude.

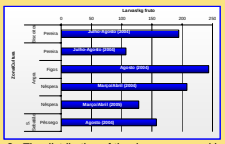

Figure 8 - The distribution of the damage caused by *C.capitata* in each type of fruit.

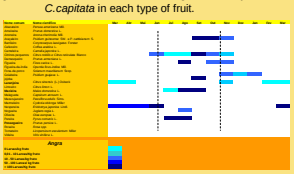

Figure 9 - The obtained list of *C. capitata* damage fruits hosts.

✓ From the analysis of the Mediterranean fruit fly **damage in the fruits** (Fig.8) in Biscoitos the most affected fruit was the pear (near180 larvae/kg of fruit). In Angra loquat were the fruits that showed the greater damage of all (near 250 and more than 200 larvae /kg of fruit, respectively) and in S. Sebastião the peach (150 larvae/kg of fruit) (Pimentel *et al*., 2005).

✓From the fruits collected around the traps was possible to make a **list of *C. capitata* damage fruits hosts** (Fig. 9), and the period between the middle June to the end of November seems critical in terms of damage caused in the fruits that ripe on that season of the year (Pimentel *et al*., 2005).

✓ In the four months **tests of different traps** against the adults of *Ceratitis capitata* were captured 17.789 adults, 2.062 in the S. Carlos orchard (of homogeneous characteristics) and in the Quinta do Martelo (the mixed orchard) 15.727 adults. According to the results obtained, the trap that showed greater efficiency in the capture of males of *C. capitata* (Weid.) was the "**Easy Trap" (Sorygar)** (Fig.5c), with 37% of the total captures registered in the S. Carlos orchard and 36% in the Quinta do Martelo orchard (Costa *et al*., 2005)

✓ All this investigation work as the goal of contribute to a better knowledge of the Mediterranean fruit fly population level, the study of its evolution, appearance and measure the impact of this pest that, in a sever way, limit the fruit production in some of the fruit trees studied.

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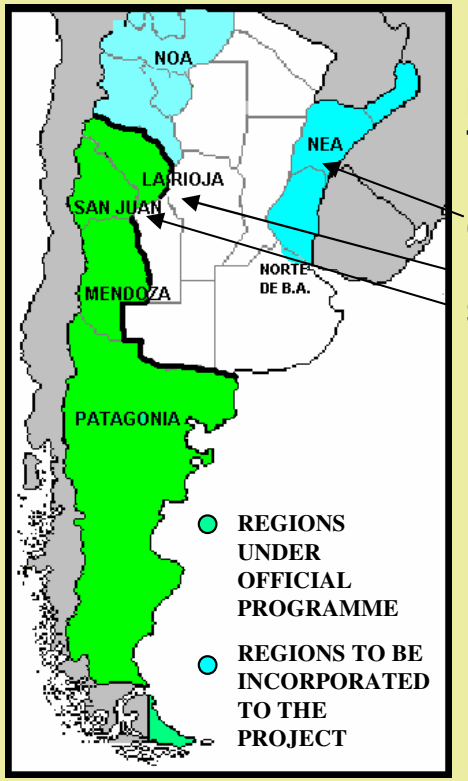
FRUIT FLY CONTROL AND ERADICATION NATIONAL PROJECT IN ARGENTINA

Diana Guillén, Ricardo Sánchez & others - SENASA



NEW AREAS TO BE INCORPORATED TO S.I.T.
Monte Caseros-Colón: 70.000 ha
La Rioja: 10.000 ha
S. Juan: 10.000 ha

PRESENT SITUATION				FUTURE SITUATION (with Project)			
SURVEILLANCE		SIT		SURVEILLANCE		SIT	
surface (ha)	traps	surface (ha)	pupae/week	surface (ha)	traps	surface (ha)	pupae/week
670.042	8.568	180.000	140 million	826.042	13.248	270.000	300 million

BENEFITS OF THE PROJECT IN NORTHERN EAST OF ARGENTINA (N.E.A.)

(56.000 hectares of citrus, 2.400 growers, 932.000 tons of fruit production)

1.- Increase in the Regional Income, estimated in 7 million dollars/year as from the 4th year.

		Year 1	Year 2	Year 3	Year 4
Fruit Damage Reduction	%	13,00%	5,00%	1,00%	0,50%
Commercialised Production	tons	811.311	896.414	922.918	927.709
Income Increase NEA	dollars	0	5.000.000	6.600.000	**7.000.000**

Equipment and materials to assist Emergency Plans, as well as important investments in the current Phytosanitary Protection System of Patagonia and Cuyo regions, are considered in the Project in order to maintain the Pest Free and Low Prevalence Areas

2.- Reduction in the costs of Control

		Present situation	4th year w/project	Benefits (reduction control costs)
Regional cost	dls/yr	5.700.000	3.000.000	2.700.000
cost / ha	dls/yr	101	53	48

3.- Reduction of the Environmental Impact due to the reduction of malathion application

		Present situation	4th year w/project	Yearly reduction
Malathion applied	liters/yr	83.900	16.000	67.900

SENASA

Integrated Management of Fruit Flies in Peru

Blgo. Rafael Guillen Encinas. Director del Programa Nacional de Moscas de la Fruta - SENASA

SENASA

National Detection System

Trapping
McPhail and Jackson traps geograhically referenced and established in quadrants of 20 and 180 ha according to the agricultural condition.

Directed Sampling
Activity additional to the trapping, wich consist in the picking of fruits with signs of fruit flies attack.

Farmers

Field Quarantine

Field Protection
Implementation of entry restriction and banning of risk products for the agricultural field.

Fruits Picking
Picking and Intensive destruction of infested fruits aimed to eliminate fruit flies inmature stages.

Terrestrial Release.

SENASA

Integrated Control

Chemical Control
Localized applications of toxic baits to the foliage to drastically decrease adult populations of the pest.

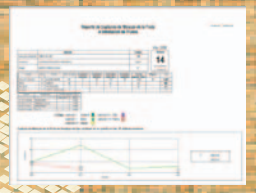
Systematic Sampling
Intensive sampling and tree marking for the timely detection of fruit flies inmature stages.

TIE Control
It is implemented in areas with minimun wild/native fruit flies MTD (MTDs lower than 0.02).

Integrated Information System

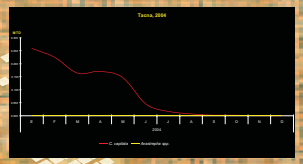
Report of Captures
Geographic information about captures and infestation.

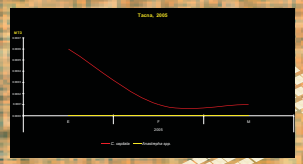
Report to Farmers
E-mail delivery of Report to Farmers.

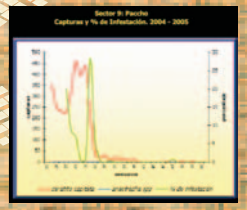


Epidemiological Reports
Fruit Flies Epidemiological graphic by Field.

Results







Populational Reports
Progressive reduction of MTD through control activities.

SENASA
Servicio Nacional de Sanidad Agraria
PERÚ
Programa Nacional de Moscas de la Fruta

JOIN NOW!
SEE HAND OUT

Tephritid Workers Database

www.tephritid.org

Bakri Abdeljelil[1], Jorge Hendrichs[2], and Alan Robinson[3]

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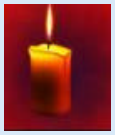
[3]Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria

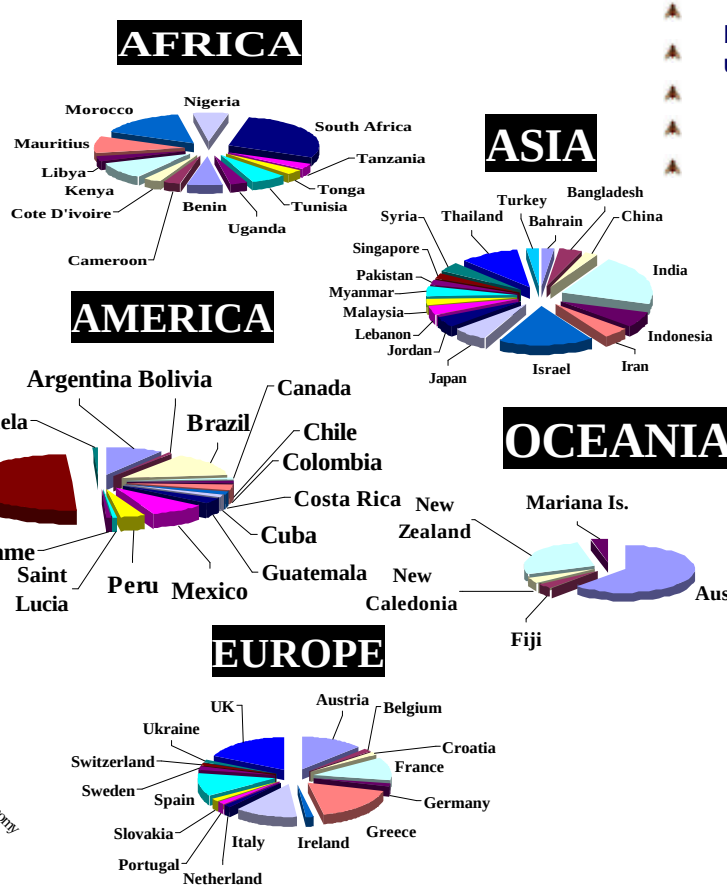
GOALS of TWD are to provide:

- ✓ Directory of tephritid fruit fly workers
- ✓ Worldwide activities on tephritid fruit flies
- ✓ Updated literature on tephritid fruit flies

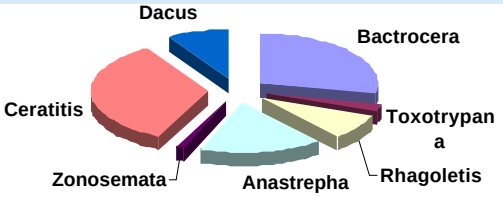


Launched one year ago on
May 7th, 2004,
TWD has already reached 310 members.

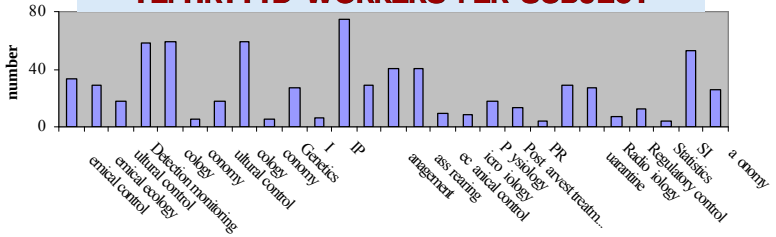
TEPHRITID WORKERS PER CONTINENT



TEPHRITID WORKERS PER GENERA

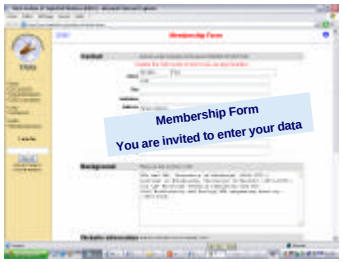


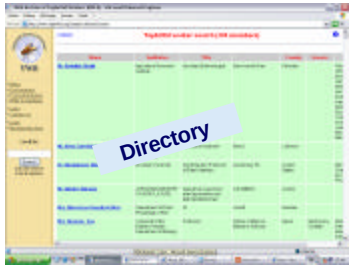
TEPHRITID WORKERS PER SUBJECT



DATABASE-DATA ENTRY

TWD data are entered and protected by the members with their chosen username and password.





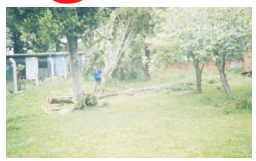
Pulling up the evil by the root:

Erradication Program of the Codling Moth in Brazil

Your apple tastes much better without this filling!

Adalecio Kovaleski
Embrapa Uva e Vinho
Vacaria, RS, Brazil
adalecio@cnpuv.embrapa.br

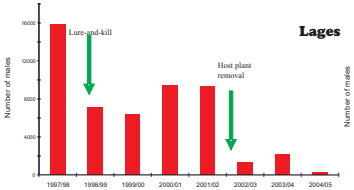


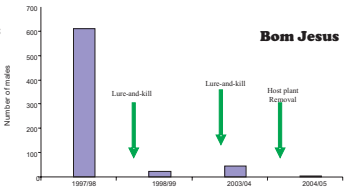



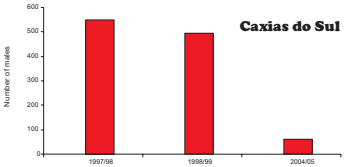

This poster brings updated information on the codling moth status in Brazil and results obtained through the combination of lure-and-kill and host plant removal from urban areas. It is estimated that 60% of host plants have already been replaced for non-host ones in the four affected municipalities:

Municipality	Estimated number	Replaced trees	% removed
Lages	35,000	34,500	97%
Vacaria	15,000	12,500	83%
Bom Jesus	2,000	500	25%
C. do Sul	60,000	20,000	33%
Total	112,000	67,500	60%

Engagement of apple growers who provided personel and equipment for host plant removal and of the population who allowed the removal of their plants was crucial for the outcome of the campaign. Host plant removal is underway and it is expected to be accomplished by 2006.





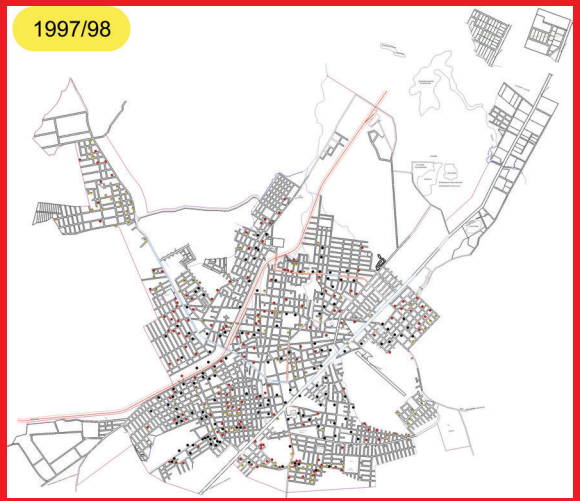


At the end of the program, the application of the SIT may come to play an important role in suppressing residual populations in defined spots.

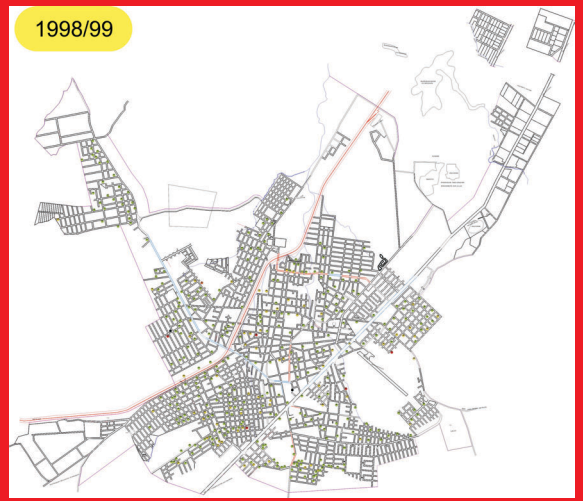
The delay in dispersal of the codling moth from the core area of the city into commercial apple orchards represent a real benefit to growers. If no actions had been undertaken, probably, the pest would have already reached commercial orchards and brought direct and indirect damage.

Trap Catches in the urban area of Vacaria

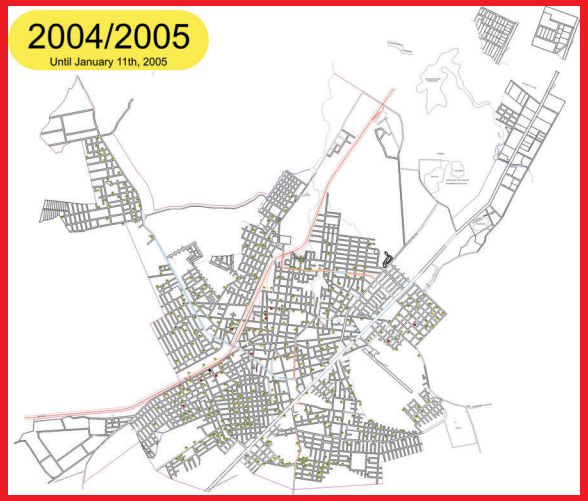
In the maps, green dots represent traps with no catches along the season. Yellow points represent traps with 1-3 catches, red represent traps with 4-13 catches and black, traps with more than 14 males along the season.



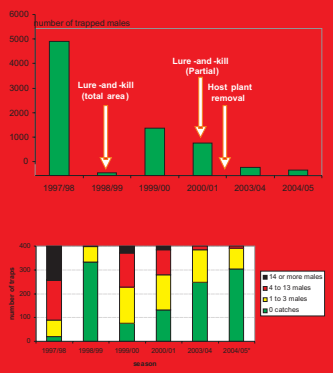
First year of monitoring



Lure and kill



Current situation



Thanks are due to several colleagues for fruitful discussion and to the International Atomic Energy Agency for finnancial support. For further information, please contact: adalecio@cnpuv.embrapa.br.

NATIONAL FRUIT FLY CONTROL AND ERADICATION PROGRAM IN ARGENTINA - PROCEM -

INTRODUCTION

Anastrepha fraterculus (Wiedemann)
South American Fruit Fly

The National Fruit Fly Control and Eradication Program started in Argentina in 1994. It is coordinated by the Agroalimentary Health and Quality National Service - SENASA.
From the fruit fly complex , the two species present in this country are *Ceratitis capitata* (Wiedemann) and Anastrepha fraterculus (Wiedemann).
The problem produced by these species is that some countries have regulations that prohibit imports of farm products from fruit flies infested areas. On the other hand, costly quarantine treatments must be made.

Ceratitis capitata (Wiedemann)
Mediterranean Fruit Fly, Med Fly

OBJECTIVE

The General Objectives of this National Program are:
-To obtain Fruit Fly Free Areas, recognized by National and International Authorities
-To reach new markets, which will benefit our country By SENASA Norm Nº 515 / 01, Procem has organized the areas considering the status of the plague and the Quarantine Protection System such as:

- Free Areas: Andean Patagonian Valleys, Malargüe -Sosneado and Uco Valley in Mendoza. First reconocing Area oficial free for Senasa where it was eradicated the Mediterranean Fruit Fly with SIT.
- Low Prevalence: Provinces of Mendoza, Patagonian Region and Bermejo Valley in Provinces of La Rioja.
- Under Control: Provinces of La Rioja and San Juan.
- Determination of presence, distribution / Diagnosis: Misiones.
- Cultivated Oasis

MATERIALS AND METHODS

The actions include :
-Monitoring by traps and fruit sampling.
-Use of SIT - Chemical Control (Malathion + Protein, Spinosad, Clorpiriphos) and Cultural Practices -Quarantine Barriers -Diffusion, Training, Communication, Raising Consciousness.
The aim of this work is to show the important growth of the National Program, its development in the different areas and its results.

PROCEM MENDOZA:
4.690 Number of Traps Installed
3.641 Jackson
1.049 Mc Phail
202 Total personnel

PROCEM SAN JUAN:
1.530 Number of Traps Installed
1.104 Jackson
426 Mc Phail
155 Total personnel

PROCEM LA RIOJA:
367 Number of Traps Installed
166 Jackson
201 Mc Phail
56 Total personnel

PROCEM PATAGONIA:
2.346 Number of Traps Installed
2.034 Jackson
312 Mc Phail
80 Total personnel

PROCEM MISIONES:
105 Number of Traps Installed
45 Jackson
60 Mc Phail
15 Total personnel

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