

Technical studies in Colombia for the establishment of Maximum Residue Limits (MRLs) for Spinetoram in avocado

Edwin S. Barbosa^{1*}, Hugo A. Rodríguez², Oscar J. Soriano², Julián Ayala², René A. Castro², Adriana Castañeda², Rosana M. Brochado², Edith Lurvey³, Jason Sandahl⁴

¹Instituto Colombiano Agropecuario*; ²Instituto Colombiano Agropecuario-ICA; ³IR-4 Project Cornell University; ⁴United States Department of Agriculture-Foreign Agricultural Service.
E-mail: adriana.castaneda@ica.gov.co

*actualmente vinculado a la Corporación Colombiana de Investigación Agropecuaria-Corpoica



INTRODUCTION

Colombia is participating in a regional collaborative project for establishment of maximum residue limits (MRLs) in the minor crop, subgroup 006B for tropical fruit with inedible peel. The Global study is funded by a Standards and Trade Development Facility grant, led by the USDA with technical support from the IR-4 Project. Colombia is developing technical studies for determination of pesticide MRLs for Spinetoram, registered in the country under the trade name Exalt 60 SC, for *Thrips* control in avocado. The project is implemented under Good Laboratory Practices (GLP), and includes establishment of six field trials done in three of the main avocado production regions and the laboratory analyses to determine pesticide residue levels. The locations were selected from farms certified by ICA in Good Agricultural Practices (GAP).



EXPERIMENTAL

FIELD STUDIES

The six field trials were conducted in the second half of 2014 in the three main avocado production regions of Colombia: Antioquia, Risaralda and Cauca. The variables that differentiated the trials were: geographic location, avocado variety, application equipment; the use or not of adjuvants and spray volumes. Varieties are listed in Table 1 for each of the tests. In those trials where the same type of equipment were used, spray volumes differed by at least 25%.



Table 1. Variables for field trials

| VARIABLES | | | | | | |
|-----------------------------|-----------------|----------------------------|-----------------------------|---------------|-----------------------------|--|
| Department | Cauca | Antioquia | Cauca | Risaralda | Risaralda | Antioquia |
| Testing ID | 11400.14-CO01 | 11400.14-CO02 | 11400.14-CO03 | 11400.14-CO04 | 11400.14-CO05 | 11400.14-CO06 |
| Farm | Jireh | Los Pinares | El Porvenir | El Píramo | La Bulgaria | Comercializadora Heclmen |
| Avocado variety | Hass | Reed | Hass | Papelillo | Hass | Hass |
| Use of adjuvants | Adjuvant | No adjuvant | No adjuvant | Adjuvant | Adjuvant | No adjuvant |
| Application equipment | Stationary pump | Mist blower | Motorized back-pack sprayer | Mist blower | Motorized back-pack sprayer | Stationary pump |
| Spray volume | 1049 L/hectare | 1242 L/hectare | 1086 L/hectare | 828 L/hectare | 1425 L/hectare | 1514 L/hectare |
| SAMPLING | | | | | | |
| Number of samples at day 1 | 2 treated | 3 treated | 3 treated | 2 treated | 3 treated | Decline study: Sampling at days 0, 1, 3, 4, 7, 11, 14 and 21 |
| | 2 untreated | 3 untreated | 3 untreated | 2 untreated | 3 untreated | |
| Number of samples at day 14 | 2 treated | 3 treated | 3 treated | 2 treated | 3 treated | |
| Matriz analysis | Whole fruit | Pulp, peel and whole fruit | Pulp, peel and whole fruit | Whole fruit | Pulp, peel and whole fruit | Whole fruit |

Each trial was composed of two plots, an untreated control plot and a treated plot, contained six trees each and was separated by at least 30 meters. In the treated plot, three applications of Spinotoram were done at 7 day intervals at a rate of 60 g of a.i./hectare (1L formulated product/hectare). Before each application, the discharge volume of the equipment and operator application rate were calibrated. Coefficients of variation <5% were considered.

After application, the efficiencies were calculated, which are shown in Table 2

$$\text{Efficiency} = \frac{\text{applied dose (ml/hectare)} * 100}{\text{Dose protocol (ml/hectare)}}$$

Table 2. Efficiency application

| Testing ID | | | | | | |
|----------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Efficiency (%) | 11400.14-CO01 | 11400.14-CO02 | 11400.14-CO03 | 11400.14-CO04 | 11400.14-CO05 | 11400.14-CO06 |
| Application 1 | 103 | 104 | 101 | 102 | 102 | 103 |
| Application 2 | 101 | 103 | 104 | 100 | 100 | 101 |
| Application 3 | 102 | 106 | 100 | 101 | 101 | 100 |
| Average | 102 | 104 | 101 | 101 | 101 | 102 |

In five of six field trials, avocado samples were collected from non treated plots (control) and treated plots 1 and 14 days after the third application, in order to analyze the residues in different fractions of the fruit; whole fruit (seedless) pulp, and peel. In the trial six samples were collected at days 0, 1, 3, 4, 7, 11, 14, 21 to determine decline curve.

Samples were collected from different quadrants of the tree, under aseptic conditions, avoiding the edges and preventing cross-contamination. Once collected they were refrigerated, labeled, packed and shipped to the laboratory and temperature recorded. The same day the samples were coded in the lab, their seed removed and peel according to table 1 and stored at -20 °C for later analysis.

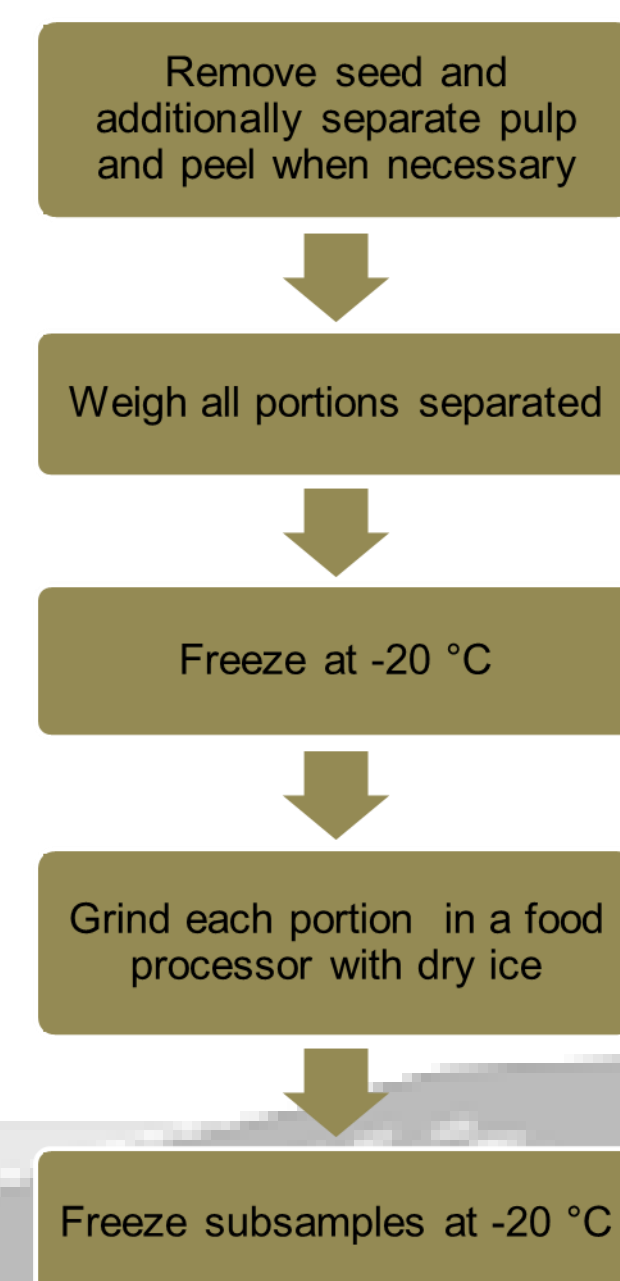
6 sub-samples of 5g of each fruit fraction were spiked with a mixture of Spinotoram J, L, Demethyl J and Formyl J, at concentration of 0,2 mg/kg in order to determine pesticide stability in stored samples.

ACKNOWLEDGMENTS

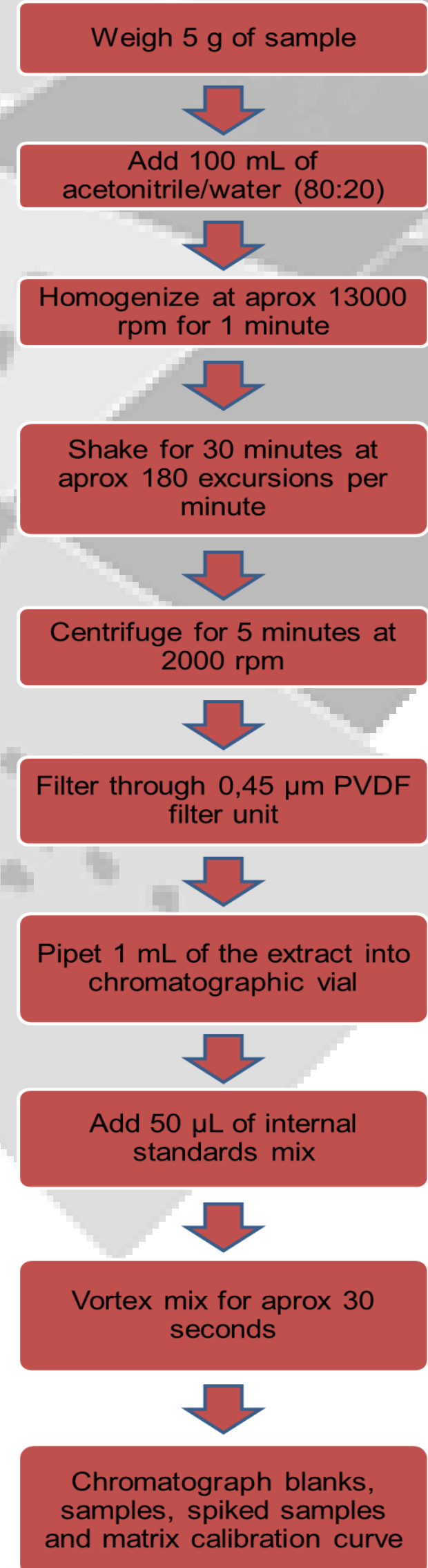
Thanks to United States Department of Agriculture- Foreign Agricultural Service
Standards and Trade Development Facility
IR-4 Project

LABORATORY

Preliminary processing



Analytical processing



In Laboratorio Nacional de Insumos Agrícolas (LANIA) from Instituto Colombiano Agropecuario (ICA), the analisis method was standardized for residue analysis of Spinotoram (XDE-175)-J, Spinotoram (XDE-175)-L and their metabolites Spinotoram (XDE-175)-N-Demethyl-J and Spinotoram (XDE-175)-N-Formyl-J, analyzed by HPLC-MS/MS with ESI positive, using deuterated internal standards for the calibration curve in matrix. All standard substances were supplied by Dow Agrosiences. Testing accuracy of the method are being evaluated using four concentrations of each analyte at 0,01; 0,02; 0,2, and 2 mg/kg, in three avocado matrices (whole fruit except seed, pulp and peel separately). In the samples from three field tests Spinotoram residues and their metabolites will be evaluated only in the whole fruit while in the samples from the remaining three trials the residuality in the whole fruit, the pulp and peel will be evaluated separately.

Table 3. Chromatographic conditions

| Condition | Description | | |
|------------------------|----------------------------|--|----|
| Instrument | HPLC Agilent 1200 QQQ 6400 | | |
| Chromatographic column | Agilent zorbax eclipse XBD | | |
| | Stationary phase | C18 in reverse phase | |
| | Length | 150 mm | |
| | Internal diameter | 4,6 mm | |
| | Particle size | 5 µm | |
| Injection volume | 50 µL | | |
| Analysis time | 20 min | Flow (mL/min) | 1 |
| Mobil phase | Phase A | ACN/MeOH (1:1) with amonium acetate 2 mM | |
| | Phase B | Amonium acetate 2 mM | |
| Gradient | Time (min) | %A | %B |
| | 0 | 67 | 33 |
| | 10 | 100 | 0 |
| | 15 | 100 | 0 |
| | 17 | 67 | 33 |
| | 20 | 67 | 33 |

Table 4. Spectrometric conditions

| Analyte | Retention time (min) | Precursor ion (m/z) | Product ion (m/z) | |
|-----------------------------------|----------------------|---------------------|-------------------|-----------|
| | | | Quantifier | Qualifier |
| Spinotoram (XDE-175)-J | 13,5 | 748,6 | 142,2 | 98,0 |
| Spinotoram (XDE-175)-L | 14,0 | 760,5 | 142,2 | 98,2 |
| Spinotoram (XDE-175)-N-Demethyl-J | 12,2 | 734,5 | 128,2 | 84,2 |
| Spinotoram (XDE-175)-N-Formyl-J | 11,0 | 784,5 | 629,4 | 517,4 |
| I.S. Spinotoram J | 13,4 | 757,9 | 146,2 | 102,4 |
| I.S. Spinotoram L | 13,9 | 769,9 | 146,2 | 102,6 |
| I.S. N-demetyl-spinetoram J | 12,2 | 739,9 | 128,2 | 84,2 |

QUALITY ASSURANCE UNIT

The quality assurance unit, to date, has made a total of 19 inspections and audits (random and programmed)

Field test: It were inspected and audited with tracking SOPs, the six assays (plots untreated and treated) the stages of installation, test substance application (review of calculations) sampling package of samples, and transporting them to the laboratory facilities. (Total inspections: 16)

Testing laboratory: inspections have been conducted to: laboratory facilities, storage of samples, maintenance of equipment, reference substances, the analytical sample preparation stage, the study of decline (revised estimates) and advances in some validation attributes such as recovery rates and precision of the analytical method approved in the plan. (Total inspections: 3)

RESULTS

Results obtained for accuracy in whole fruit for all analytes, met criteria established with recoveries between 70 and 120%, see table 5. Typical chromatograms are showed in graph 1. It is possible to see a good resolution in the chromatogram TIC. For each analyte chromatographic signals representative of their product ions were obtained, allowing identification and quantification of analytes.

It is estimated that the ongoing research developed in Colombia will be completed by December 2015 and results of the collaborative work for Spinotoram on the minor crop group of tropical fruit with inedible peel (avocado, banana and mango) will be submitted to the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) by December 2016.

Graph 1. Chromatograms

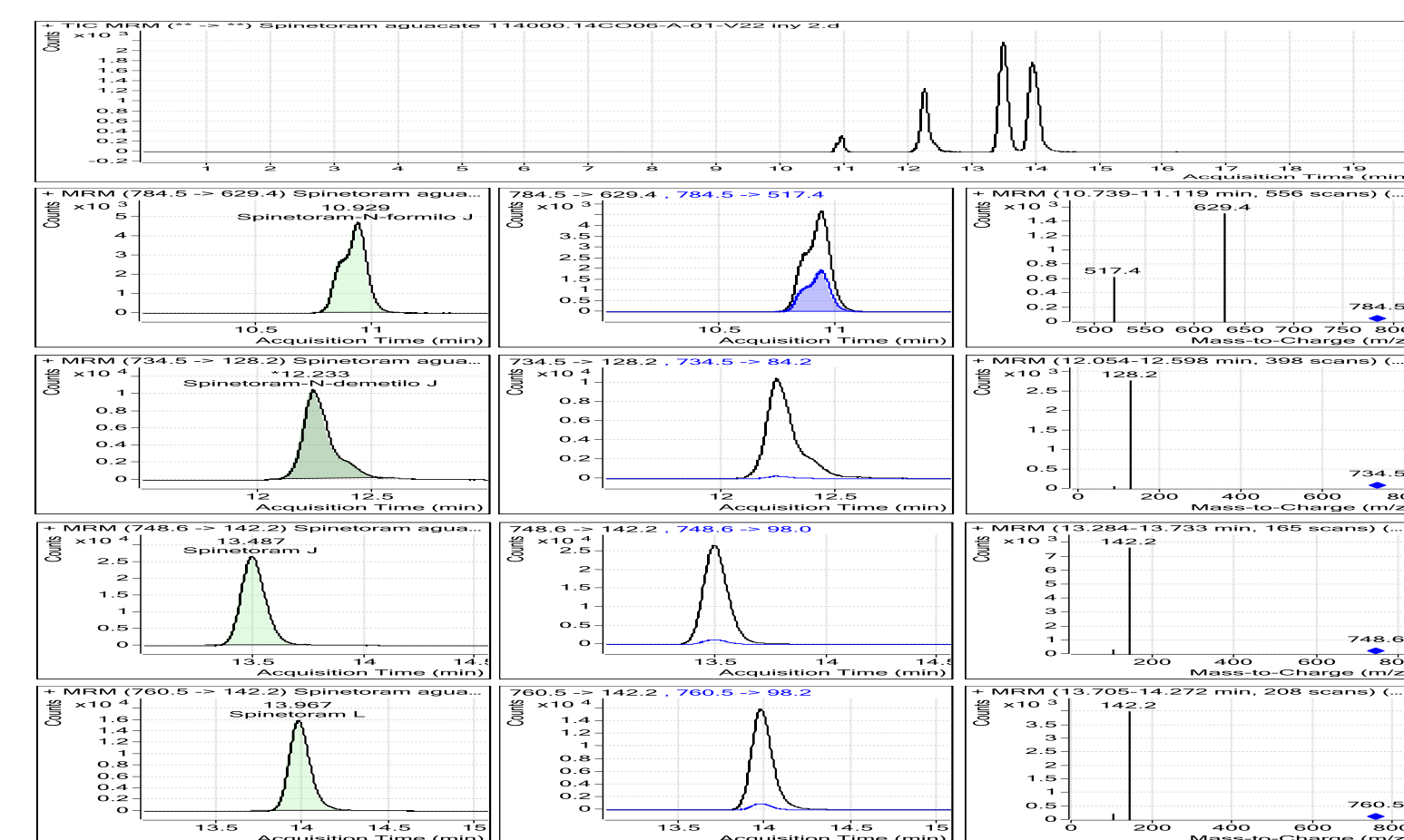


Table 5. Recoveries

| Analyte | Recovery (%) |
|-----------------------------------|--------------|
| Spinotoram (XDE-175)-N-Formyl-J | 72 – 98 |
| Spinotoram (XDE-175)-N-Demethyl-J | 90 – 111 |
| Spinotoram (XDE-175)-J | 85 – 100 |
| Spinotoram (XDE-175)-L | 87 – 103 |