JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Analysis of Methoxyfenozide Residues in Fruits, Vegetables, and Mint by Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS)

GREGORY L. HALL, JO ENGEBRETSON, MATHEW J. HENGEL,* AND TAKAYUKI SHIBAMOTO

Department of Environmental Toxicology, University of California at Davis, One Shields Avenue, Davis, California 95616

Methoxyfenozide [3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide; RH-2485], in the formulation of INTREPID, was applied to various crops. Analysis of methoxyfenozide was accomplished by utilizing liquid−liquid extraction and partitioning, followed by liquid chromatography−tandem mass spectrometry (LC-MS/MS). Method validations for fruits, vegetables, and mint are reported. Methoxyfenozide mean recoveries ranged from 72 to 129% over three levels of fortification. The overall average of mean recoveries is 97 ± 10%. The limit of quantitation for fruits, artichoke, cucumber, squash, and refined sugar was 0.010 ppm, with a detection limit of 0.005 ppm. For all other crops, the limit of quantitation was 0.050 ppm, with a detection limit of 0.025 ppm. No residues were found greater than the limit of quantitation in control samples. Residues above the limit of quantitation were found in all matrices except refined sugar. Foliage (bean, beet, pea, and radish) had greater residue levels of methoxyfenozide residue than their corresponding roots or pods. Other crop matrices contained <1.0 ppm of methoxyfenozide except artichoke, which had a mean of 1.10 ppm.

KEYWORDS: Methoxyfenozide; Intrepid; insecticide; LC-MS/MS

INTRODUCTION

Methoxyfenozide [3-methoxy-2-methylbenzoicacid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethyl-ethyl) hydrazide; RH-2485] is a diacylhydrazine insecticide that was first introduced by Rohm and Haas Company in 1996 (*1*). Le presented INTREPID as an efficacious member of the diacylhydrazine class. Methoxyfenozide acts as an agonist or mimic of the insect molting hormone, 20-hydroxyecdysone (20E) (*2*−*4*). The nature of the insecticide is to mimic the hormone and bind to the sites so that precocious molting occurs (*5*−*7*).

Methoxyfenozide acts against a wide range of lepidopteron pests of cotton, corn, and other major agronomic crops (*8*−*16*). Methoxyfenozide has been shown to be more effective than tebufenozide on armyworms, *Spodoptera* (sp.) and other pests (*9*, *11*−*13*, *17*, *18*). Methoxyfenozide is an effective agent for control of codling moth *Cydia pomonella* (*19*, *20*), Southwestern corn borer, *Distraea grandiosella* (*17*, *18*), European corn borer *Ostrinia nubilalis* (*10*, *18*, *21*), rice stem borers (*5*, *22*, *23*), and cotton leaf worm (*14*, *24*). Ecdysteroid agonists can have an effect on fecundity and fertility in leaf rollers, with methoxyfenozide being the most potent (*19*, *25*). Methoxyfenozide can be very effective when interacting with other compounds such as juvenile hormone mimic (*16*).

Methoxyfenozide has been shown to be an effective pesticide for use on lepidopteron pests, yet it appears to retain a high degree of safety with respect to nontarget organisms. Lady beetles (*26*), parasitoids of leaf rollers (*27*), parasitoids of the rice borer (*23*), and big-eyed bugs (*28*) exposed to methoxyfenozide at effective levels were unharmed.

Liquid chromatography tandem−mass spectroscopy (LC-MS/MS) as an analytical tool is unsurpassed in selectivity and confidence in qualitative response (*29*−*31*). Matrix suppression has not been a factor in this study (*32*, *33*) with a quantitation limit of 0.01 or 0.05 ppm and a detection limit of 0.005 or 0.025 ppm. For particular compounds, such as methoxyfenozide, which are nonvolatile and can move readily through the LC system, LC-MS/MS provided a rapid and selective analysis in a variety of crops.

Few reports have been made on the analysis of methoxyfenozide in crops (*30*, *34*). In the present study, methoxyfenozide was analyzed in a large variety of crops. The aim of the present work is to report the general method of analysis for methoxyfenozide in various crops and matrices. Interregional Research Project 4 (IR-4) is a U.S. Department of Agriculture (USDA) program that carries out the research needed for the registration of pest control materials on minor crops. A minor crop is any individual crop grown on ≤300,000 acres. IR-4 prepares and submits petitions to the U.S. Environmental Protection Agency (EPA) requesting tolerances or exemptions for a pest control

* Author to whom correspondence should be addressed [telephone (530) 752-2402; fax (530) 754-8556; e-mail mjhengel@ucdavis.edu].

10.1021/jf030591n CCC: $27.50
Published on Web 01/30/2004

Table 1. Formulations, PHI, and Residue Results of Methoxyfenozide Analysis in Fruits, Vegetables, and Mint

type	crop/matrix	formulation[a]	PHI[b] (days)	control (ppm)	treated samples (ppm)				
					n	low	high	mean[c]	SD
fruit	cantaloup	80WSP	3 ± 1	<0.010	14	0.050	0.255	0.138	0.05
fruit	cranberry	2F	14 ± 2	<0.010	12	0.028	0.407	0.162	0.13
fruit	strawberry	2F	14 ± 1	<0.010	16	0.125	1.154	0.405	0.29
mint	mint/fresh	2F	14 ± 2	<0.050	12	1.020	6.362	2.648	1.70
mint	mint/oil	2F	processed	<0.050	2	<0.050	0.071	N/A	N/A
vegetable	artichoke	80WSP	5 ± 1	<0.010	6	0.744	1.650	1.101	0.35
vegetable	bean (dry)	80WSP	7 ± 1	<0.050	26	<0.050	0.223	N/A	N/A
vegetable	bean (edible podded)/foliage	2F	7 ± 1	<0.050	16	3.081	31.750	8.346	8.03
vegetable	bean (edible podded)/pods	2F	7 ± 1	<0.050	16	<0.050	0.991	0.388	0.35
vegetable	beet (sugar)/dry pulp	2F	processed	<0.050	1	0.121	N/A	N/A	N/A
vegetable	beet (sugar)/molasses	2F	processed	<0.050	1	0.156	N/A	N/A	N/A
vegetable	beet (sugar)/RAC for processed samples	2F	7 ± 1	<0.050	1	0.143	N/A	N/A	N/A
vegetable	beet (sugar)/refined sugar	2F	processed	<0.010	1	<0.010	N/A	N/A	N/A
vegetable	beet (sugar)/roots	2F	7 ± 1	<0.050	20	<0.050	0.173	0.111	0.03
vegetable	beet (sugar)/tops	2F	7 ± 1	<0.050	22	0.404	10.200	4.205	2.59
vegetable	carrots	2F	14 ± 2	<0.050	10	<0.050	0.138	0.101	0.04
vegetable	cucumber	80WSP	3 ± 1	<0.010	16	<0.010	0.080	0.036	0.02
vegetable	pea/foliage	2F	7 ± 1	<0.050	12	3.031	9.612	5.945	2.19
vegetable	pea/pods	2F	7 ± 1	<0.050	12	0.103	0.454	0.215	0.15
vegetable	pea (succulent shelled)	2F	7 ± 1	<0.050	32	<0.050	0.628	0.160	0.17
vegetable	radish/roots	2F	14 ± 1	<0.050	10	<0.050	0.120	0.095	0.02
vegetable	radish/tops	2F	14 ± 1	<0.050	10	0.315	3.963	1.316	1.32
vegetable	squash (summer)	80WSP	3 ± 1	<0.010	12	<0.010	0.155	0.078	0.05

[a] Formulations: 80WSP = 141.75 g of product/acre; 2F = 473 mL of product/acre. [b] Interval in days between last application and harvest. [c] Mean represents samples that have measurable residues.

product on minor crops. Residues of methoxyfenozide found in a variety of crops collected from IR-4 testing fields have been summarized.

MATERIALS AND METHODS

Materials. Methoxyfenozide (99.7% purity) was acquired from Rohm and Haas (Philadelphia, PA). All solvents and reagents were of residue grade or better. Columns specifically used for LC analysis are cited below.

Preparation of Standard Solutions. Stock solution (1 mg/mL) was prepared by dissolving ~0.1003 g in a 100 mL volumetric flask and diluting to volume with 50% acetonitrile/water. Various dilutions were made from the stock solution in acetonitrile, or 50% acetonitrile/water, for fortification solutions and standard solutions for LC-MS/MS analysis. LC-MS/MS standards were made up as needed and typically consisted of 1000, 500, 250, 125, 100, 50, and 25 pg/μL for the 0.01 validation level. Stock solution was kept frozen, generally at <−20 °C. LC-MS/MS and fortification solutions were kept refrigerated, generally at <4 °C.

Collection of Field Samples. INTREPID (RH-2485) 80 WSP or 2F insecticide formulation of methoxyfenozide (EPA Reg. No. pending, CAS Registry 161050-58-4) was used for application in these field studies (refer to **Table 1**). This test substance was applied in a manner that represents and/or simulates the major application techniques that are used by commercial growers. Samples were collected from IR-4 field test sites throughout the United States (for specific information contact IR-4 Project, Center for Minor Crop Pest management, Technology Centre of New Jersey, 681 U. S. Highway #1 South, North Brunswick, NJ 08902-3390).

Each test site usually consisted of one untreated (control) and one treated plot. Individual plots were of adequate size to ensure that no more than 50% of the plot would be needed to provide the necessary plant material for residue sampling. Buffer zones were employed between plots to prevent contamination.

Typically, duplicate samples were harvested from each plot. Each sample was collected in a manner to ensure a representative, impartial sample that approximates commercial practices. The sample was placed in a plastic-lined cloth bag that was labeled with complete identification. After collection, samples were usually placed in a cooler and frozen within 24 h of harvest. Samples were kept frozen during shipping and held at generally <−20 °C at the laboratory until analysis.

Sample Preparation. The crop was chopped with equal amounts of dry ice using a Hobart food chopper (Hobart Corp., Troy, OH). Each chopped sample was stored in a labeled ~1 L jar, and a lined lid was loosely closed on top to allow the dry ice to dissipate during storage at generally <−20 °C. For mint oil and molasses, aliquots were measured directly from the original containers.

Storage Stability Study. A minimum of six control samples were fortified with methoxyfenozide at 0.5 or 1.0 ppm level for each matrix. At least three samples were analyzed after a storage period equivalent to the number of days (±10%) between harvest and analysis, and the remaining samples were retained for long-term storage.

Extraction. Ten grams of crop (except mint oil, see below) were weighed into a 250 mL Erlenmeyer flask. Fortification samples were spiked at this step. One hundred milliliters of extraction solution (methanol/aqueous 0.1 N HCl, 9:1, v/v) was added to the flask. The mixture was sonicated if needed at this step, particularly for refined sugar. The sample was blended for 2−3 min at moderate speed using an Ultra-Turrax T25 (Wilmington, NC). The extract was filtered through a porcelain funnel fitted with a Whatman no. 3 filter and a small amount of Celite. The blender flask and the filtration cake were rinsed with several portions of extraction solution totaling 50 mL. The total volume of the filtrate was ~150 mL. The extract was quantitatively transferred to a 500 mL separatory funnel.

Cleanup. *Hexane Partition*. Twenty milliliters of a 10% sodium chloride solution was added to the extract in the separatory funnel. The extract was partitioned with 40 mL of hexane by shaking the separatory funnel for 1 min with venting as necessary. The phases were allowed to separate. The lower phase (methanol/water) was drained into a 250 mL beaker, and the upper phase (hexane) was discarded.

Dichloromethane Partition. The lower phase from the beaker was poured into a 500 mL separatory funnel. One hundred and seventy-five milliliters of a 10% sodium chloride solution and 100 mL of dichloromethane were added to the extract in the 500 mL separatory funnel. The extract was partitioned by shaking the separatory funnel for 1 min with venting and allowing the phases to separate. The lower dichloromethane phase was collected in a 250 mL TurboVap tube through a funnel plugged with glass wool and anhydrous sodium sulfate. The aqueous layer was partitioned again with another 100 mL of dichloromethane. Both dichloromethane fractions were pooled in the

Table 2. Average Recoveries of Methoxyfenozide in Fruits, Vegetables, and Mint

type	crop/matrix	level 1 (ppm)	% ± SD[a]	level 2 (ppm)	% ± SD	level 3 (ppm)	% ± SD
fruit	cantaloup	0.01	106 ± 11 (n = 4)	0.1	102 ± 10 (n = 5)	1.0	92 ± 15 (n = 6)
fruit	cranberry	0.01	97 ± 13 (n = 5)	0.1	82 ± 4 (n = 3)	1.0	81 ± 15 (n = 9)
fruit	strawberry	0.01	129 ± 9 (n = 3)	0.05	109 ± 3 (n = 3)	1.0	96 ± 6 (n = 6)
mint	mint/fresh	0.05	99 ± 4 (n = 6)	1.0	101 ± 19 (n = 8)	15	97 ± 3 (n = 4)
mint	mint/oil	0.05	100 ± 2 (n = 3)	1.0	95 ± 2 (n = 7)	15	102 ± 3 (n = 4)
vegetable	artichoke	0.01	94 ± 8 (n = 3)	0.1	96 ± 11 (n = 4)	1.0	101 ± 10 (n = 7)
vegetable	beans (dry)	0.05	96 ± 11 (n = 9)	0.1	84 ± 2 (n = 3)	0.5	96 ± 7 (n = 12)
vegetable	bean (edible podded)/foliage	0.05	100 ± 8 (n = 6)	1.0	109 ± 15 (n = 6)	15	95 ± 6 (n = 3)
vegetable	bean (edible podded)/pods	0.05	102 ± 14 (n = 6)	1.0	98 ± 5 (n = 9)	15	81 ± 1 (n = 3)
vegetable	carrots	0.05	103 ± 5 (n = 6)	0.5	100 ± 2 (n = 3)	1.0	96 ± 4 (n = 6)
vegetable	beet (sugar)/dry pulp	0.05	124 ± 12 (n = 3)	1.0	91 ± 6 (n = 3)	5.0	81 ± 4 (n = 6)
vegetable	beet (sugar)/molasses	0.05	93 ± 7 (n = 9)	5.0	90 ± 2 (n = 3)	20	96 ± 1 (n = 3)
vegetable	beet (sugar)/refined sugar	0.01	98 ± 21 (n = 9)	5.0	104 ± 4 (n = 3)	20	99 ± 10 (n = 3)
vegetable	beet (sugar)/roots	0.05	97 ± 2 (n = 12)	1.0	88 ± 4 (n = 15)	10	85 ± 6 (n = 3)
vegetable	beet (sugar)/tops	0.05	100 ± 11 (n = 12)	5.0	86 ± 10 (n = 12)	20	79 ± 6 (n = 3)
vegetable	cucumber	0.01	85 ± 6 (n = 3)	0.1	95 ± 8 (n = 4)	1.0	120 ± 18 (n = 7)
vegetable	pea/foliage	0.05	102 ± 9 (n = 6)	1.0	94 ± 11 (n = 9)	15	83 ± 2 (n = 3)
vegetable	pea/pods	0.05	105 ± 10 (n = 6)	1.0	91 ± 12 (n = 9)	15	95 ± 4 (n = 3)
vegetable	pea (succulent shelled)	0.05	94 ± 5 (n = 9)	0.5	91 ± 1 (n = 3)	1.0	92 ± 10 (n = 12)
vegetable	radish/roots	0.05	99 ± 10 (n = 6)	0.5	86 ± 8 (n = 9)	20	96 ± 2 (n = 3)
vegetable	radish/tops	0.05	107 ± 6 (n = 6)	0.5	97 ± 8 (n = 9)	20	94 ± 3 (n = 3)
vegetable	squash (summer)	0.01	72 ± 8 (n = 4)	0.1	97 ± 5 (n = 3)	1.0	105 ± 7 (n = 4)

[a] Values are mean percent recovered ± standard deviation; n is the number of replications.

TurboVap tube. The sodium sulfate in the funnel was rinsed with 10 mL of dichloromethane. The extract was evaporated to dryness on a Turbovap Nitrogen evaporator (Zymark Corp., Hopkinton, MA) at ~55 °C. The sample was redissolved in an appropriate amount of 50% acetonitrile/water (v/v) for LC-MS/MS analysis. If needed, the sample was filtered through a Millipore 0.45 μm nylon filter into an autosampler vial.

Mint Oil Analysis. For oil analysis, 1 g of oil was measured into a 250 mL Erlenmeyer flask. Then 150 mL of extraction solution (methanol/0.1 N HCl, 9:1, v/v) was added and mixed well. The sample was transferred to a 500 mL separatory funnel. Steps for hexane partition and dichloromethane partition were followed as above. The residue sample was redissolved in an appropriate amount of acetonitrile for analysis.

Instrumentation. The LC-MS/MS system consisted of a PE Sciex (PE Biosystems) API 2000 tandem mass spectrometer with a Perkin-Elmer series 200 autosampler and micropump. Detector conditions included a heated nebulizer (at 425 °C), nitrogen curtain gas pressure at 45 psi, nitrogen collision gas pressure at 3 psi, nebulizer current at −4, nitrogen ion source gas 1 at 60 psi and gas 2 at 15 psi, Q_1 mass, 367.2 amu, Q_3 mass, 149.2 amu, run in negative ion mode with the m/z 367 ⇒ 149 transition monitored. The mobile phase ranged from 50:50 to 60:40 acetonitrile/water (type I, v/v, 10 mM ammonium acetate) depending on column conditions, with a flow of 800 μL/min. The HPLC column was a Restek Allure C18 (Restek Corp., Bellefonte, PA), 50 × 3.2 mm i.d., 5 μm particle size.

RESULTS AND DISCUSSION

The USDA IR-4 Program (USDA Interregional Research Project 4, Minor Use Pesticide Registration Program) initiated these projects in 1999 to obtain residue data for submission of registration petitions to the EPA. All field and laboratory work was conducted as close as possible to the Good Laboratory Practice Standards mandated by the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), *Federal Register* 40 CFR Part 160. The *Federal Register* has established tolerances for methoxyfenozide on the minor crops apple pomace, wet, of 7.0 ppm and pome fruits, crop group, of 1.5 ppm (*35*).

Average recoveries and levels of fortification for methoxyfenozide are shown in **Table 2** for all crops studied. Fruits were fortified at 0.01 ppm as the lowest level. For fruits, methoxyfenozide mean recoveries ranged from 81 to 129%. Vegetable crops were fortified for the lowest level at 0.05 or 0.01 ppm. Average methoxyfenozide mean recoveries for vegetables

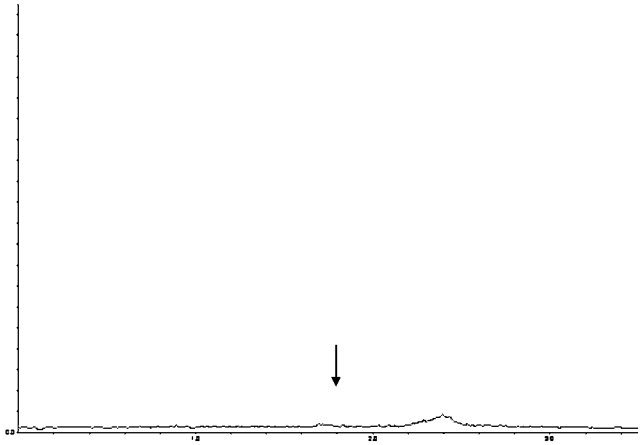

Figure 1. Sample chromatogram of control sugar beet tops, 10.0 mg injected, which shows no quantifiable level of methoxyfenozide.

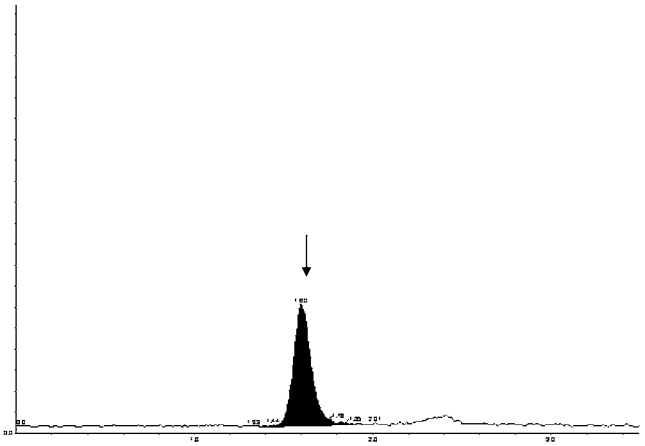

Figure 2. Sample chromatogram of methoxyfenozide standard, 100 pg/μL × 5 μL, retention time = 1.8 min.

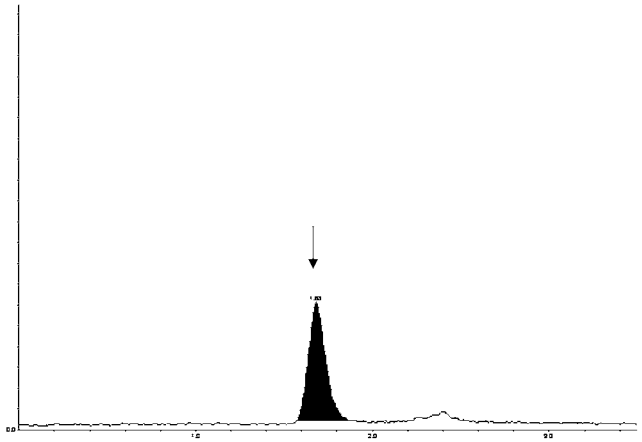

Figure 3. Sample chromatogram of fortified sugar beet top (10.0 mg injected, 93% recovery).

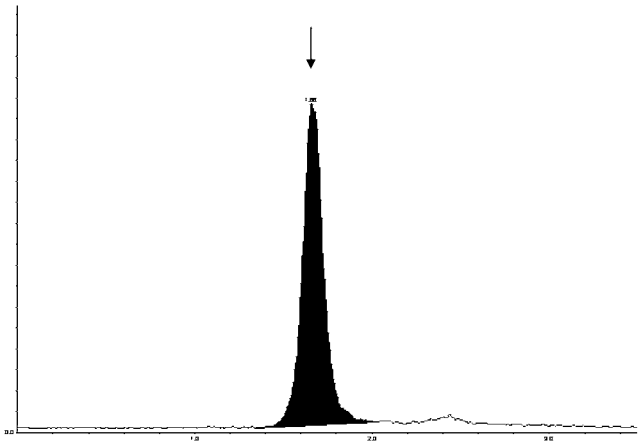

Figure 4. Sample chromatogram of treated sugar beet top (0.20 mg injected, 8.9 ppm found).

ranged from 72 to 124%. Mint foliage mean recoveries ranged from 97 to 101%, and mint oil mean recoveries ranged from 95 to 102%, both starting at the 0.05 ppm fortification level.

Residue results of methoxyfenozide analysis in fruits, vegetables, and mint are shown in **Table 1**. Residues were found greater than the limit of quantitation for some samples in all matrices except refined sugar. Foliage (bean, beet, pea, and radish) samples had greater levels of methoxyfenozide residue than their corresponding roots or pods. Other crops parts showed <1.0 ppm of methoxyfenozide except artichoke, which had a mean of 1.10 ppm.

The most interesting results were obtained with analysis of beets (sugar). Raw agricultural commodity (RAC) had residues of 0.143 ppm, and other collected roots had mean residues of 0.111 ppm. Sugar beet tops had a mean residue level of 4.205 ppm, consistent with other results where foliage matrix had significantly more residue than roots or pods. Processed samples showed 0.121 ppm for a dry pulp sample, 0.156 ppm for the molasses sample, and <0.010 ppm for refined sugar. The process of refining the sugar beet roots to sugar appears to reduce or degrade methoxyfenozide, so there is essentially no residue remaining. Representative chromatograms of sugar beet control, methoxyfenozide standard, recovery sample, and sugar beet treated top sample are shown in **Figures 1−4**.

Stability study samples were analyzed for all crops but are not reported here. The storage interval was comparable to the harvest (or processing) to analysis date. No significant degradation of methoxyfenozide was found during freezer storage (generally <−20 °C) on any crop.

We feel that the above method was rugged and versatile, and gave good recoveries for a variety of crops. Residues were found in the analyzed minor crops that were below the *Federal Register* established tolerances for apple pomace and pome fruit. In the analyzed crops, foliage contained higher residues of methoxyfenozide than the corresponding roots or pods. Methoxyfenozide could make a powerful addition to pest management strategies for control of lepidopteron pests on minor crops.

ACKNOWLEDGMENT

We thank the Rohm and Haas Co. and Jay Holmdal for providing analytical standards and assistance for this research. We also thank Keith Dorschner as Study Director and the IR-4 Minor Crop Use Pesticide Registration Program. We are grateful to Riza Reyes for laboratory assistance.

LITERATURE CITED

(1) Le, D. P.; Thirugnanam, M.; Lidert, Z.; Carlson, G. R.; Ryan, J. B. RH-2485: A new selective insecticide for caterpillar control. *Brighton Crop Prot. Conf.—Pests Dis.* **1996**, 481−486.

(2) Smagghe, G.; Carton, B.; Wesemael, W.; Ishaaya, I.; Tirry, L. Ecdysone agonists—Mechanism of action and application on *Spodoptera* species. *Pestic. Sci.* **1999**, *55*, 386−389.

(3) Dhadialla, T. S.; Carlson, G. R.; Le, D. P. New insecticides with ecdysteroidal and juvenile hormone activity. *Annu. Rev. Entomol.* **1998**, *43*, 545−569.

(4) Nakagawa, Y.; Minakuchi, C.; Ueno, T. Inhibition of [^{3}H]-ponasterone A binding by ecdysone agonists in the intact Sf-9 cell line. *Steriods* **2000**, *65*, 537−542.

(5) Dhadialla, T. S.; Jansson, R. K. Non-steroidal ecdysone agonists: New tools for IPM and insect resistance management. *Pestic. Sci.* **1999**, *55*, 357−359.

(6) Carlson, G. R.; Dhadialla, T. S.; Hunter, R.; Jansson, R. R.; Jany, C. S.; Lidert, Z.; Slawecki, R. A. The chemical and biological properties of methoxyfenozide, a new insecticidal ecdysteroid agonist. *Pest Manag. Sci.* **2001**, *57*, 115−119.

(7) Oberlander, H.; Silhacek, D. L.; Porcheron, P. Non-steroidal ecdysteroid agonists: Tools for the study of hormonal action. *Arch. Insect Biochem. Physiol.* **1995**, *28*, 209−223.

(8) Smagghe, G.; Carton, B.; Heirman, A.; Tirry, L. Toxicity of four dibenzoylhydrazine correlates with evagination-induction in the cotton leafworm. *Pestic. Biochem. Physiol.* **2000**, *68*, 49−58.

(9) Smagghe, G.; Wesemael, W.; Carton, B.; Tirry, L. Tebufenozide and methoxyfenozide against the beet armyworm, *Spodoptera exigua*. *Brighton Conf.—Pests Dis.* **1998**, 311−312.

(10) Trisyono, A.; Goodman, C. L.; Grasela, J. J.; Mcintosh, A. H.; Chippendale, G. M. Establishment and characterization of an *Ostrinia nubilalis* cell line, and its response to ecdysone agonists. *In Vitro Cell. Dev. Biol.—Anim.* **2000**, *36*, 400−404.

(11) Smagghe, G.; Nakagawa, Y.; Carton, B.; Mourad, A. K.; Fujita, T.; Tirry, L. Comparative ecdysteroid action of ring-substituted dibenzoylhydrazines in *Spodoptera exigua*. *Arch. Insect Biochem. Physiol.* **1999**, *41*, 42−53.

(12) Adamczyk, Jr., J. J.; Leonard, B. R.; Graves, J. B. Toxicity of selected insecticides to fall armyworms (Lepidoptera: Noctuidae) in laboratory bioassay studies. *Fla. Entomol.* **1999**, *82* (2), 230−236.

(13) Adel, M. M.; Sehnal, F. Azadirachtin potentiates the action of ecdysteroid agonist RH-2485 in *Spodoptera littoralis*. *J. Insect Physiol.* **2000**, *46*, 267−274.

(14) Smagghe, G.; Carton, B.; Decombel, L.; Tirry, L. Significance of absorption, oxidation, and binding to toxicity of four ecdysone agonists in multi-resistant cotton leafworm. *Arch. Insect Biochem. Physiol.* **2001**, *46*, 127−139.

(15) Olsza, R. W.; Pluciennik, Z. Preliminary investigations on effectiveness of two modern insecticides in controlling codling moth, plum fruit moth, and leaf rollers. *Brighton Crop Prot. Conf.—Pests Dis.* **1998**, 57−62.
(16) Oberlander, H.; Silhacek, D. L.; Leach, C. E. Interactions of ecdysteroid and juvenoid agonists in *Plodia interpunctella* (Hübner). *Arch. Insect Biochem. Physiol.* **1998**, *38*, 91−99.
(17) Trisyono, A.; Chippendale, G. M. Effect of the ecdysone agonists, RH-2485 and tebufenozide, on the Southwestern corn borer, *Diatraea grandiosella. Pestic. Sci.* **1998**, *53*, 177−185.
(18) Trisyono, Y. Effect of the ecdysone atgonists, methoxyfenozide and tebufenozide, on European and Southwestern corn borers *in vivo* and *in vitro*. Ph.D. Dissertaion, University of Missouri, 1999; 149 pp.
(19) Sun, X.; Barrett, B. A. Fecundity and fertility changes in adult codling moth (Lepidoptera: Tortricidae) exposed to surfaces treated with tebufenozide and methoxyfenozide. *J. Econ. Entomol*. **1999**, *92*, 1039−1044.
(20) Charmillot, P. J.; Gourmelon, A.; Fabre, A. L.; Pasquier, D. Ovicidal and larvicidal effectiveness of several insect growth inhibitors and regulators on the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae). *J. Appl. Entomol*. **2001**, *125*, 147−153.
(21) Trisyono, A.; Chippendale, G. M. Effect of the nonsteroidal ecdysone agonists, methoxyfenozide and tebufenozide, on the European corn borer (Lepidoptera: Pyralidae). *J. Econ. Entomol*. **1997**, *90*, 1486−1492.
(22) Nakagawa, Y.; Hattori, K.; Minakuchi, C.; Kugimiya, S.; Ueno, T. Relationships between structure and molting hormonal activity of tebufenozide, methoxyfenozide, and their analogs in cultured integument system of *Chilo suppressalis* Walker. *Steroids* **2000**, *65*, 117−123.
(23) Legaspi, J. C.; Legaspi, Jr., B. C.; Saldaña, R. R. Laboratory and field evaluations of biorational insecticides against the Mexican rice borer (Lepidoptera: Pyralidae) and a parasitoid (Hymenoptera: Braconidae). *J. Econ. Entomol*. **1999**, *92*, 804−810.
(24) Ishaaya, I.; Yablonski, S.; Horowitz, A. R. Comparative toxicity of two ecdysteroid agonists, RH-2485 and RH-5992, on susceptible and pyrethroid-resistant strains of the Egyptian cotton leafworm, *Spodoptera littoralis*. *Pytoparasitica* **1995**, *23* (2), 139−145.
(25) Sun, X.; Barrett, B. A.; Biddinger, D. J. Fecundity and fertility reductions in adult leafrollers exposed to surfaces treated with the ecdysteroid agonists tebufenozide and methoxyfenozide. *Entomol. Exp. Appl.* **2000**, *94*, 75−83.
(26) Trisyono, A.; Puttler, B.; Chippendale, G. M. Effect of the ecdysone agonists, methoxyfenozide and tebufenozide, on the lady beetle, *Coleomegilla maculata*. *Entomol. Exp. Appl*. **2000**, *94*, 103−105.
(27) Brunner, J. F.; Dunley, J. E.; Doerr, M. D.; Beers, E. H. Effect of pesticides on *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), parasitoids of leafrollers in Washington. *J. Econ. Entomol*. **2001**, *94*, 1075−1084.
(28) Elzen, G. W.; Elzen, P. J. Lethal and sublethal effects of selected insecticides on *Geocoris punctipes*. *Southwest. Entomol*. **1999**, *24* (3), 199−205.
(29) Niessen, W. M. H.; Tinke, A. P. Liquid chromatography−mass spectrometry general principles and instrumentation. *J. Chromatogr., A* **1995**, *703*, 37−57.
(30) Choi, B. K.; Gusev, A. I.; Hercules, D. M. Quantitative LC-ESI-MS analysis for pesticides in a complex environmental matrix using external and internal standards. *Int. J. Environ. Anal. Chem*. **2000**, *77* (4), 305−322.
(31) Slobodnik, J.; van Baar, B. L. M.; Brinkman, U. A. Th. Column liquid chromatography−mass spectrometry: selected techniques in environmental applications for polar pesticides and related compounds. *J. Chromatogr., A* **1995**, *703*, 81−121.
(32) Choi, B. K.; Hercules, D. M.; Gusev, A. I. LC-MS/MS signal suppression effects in the analysis of pesticides in complex environmental matrices. *Fresenius' J. Anal. Chem*. **2001**, *369*, 370−377.
(33) Choi, B. K.; Hercules, D. M.; Gusev, A. I. Effect of liquid chromatography separation of complex matrices on liquid chromatography−tandem mass spectrometry signal suppression. *J. Chromatogr., A* **2001**, *907*, 337−342.
(34) Stein, R.; Wu, S. *Tolerance Enforcement Method for Parent RH-2485 in Pome Fruit*; Rohm and Haas: Spring House, PA, 1998.
(35) U.S. Environmental Protection Agency. Methoxyfenozide; Benzoic Acid, 3-methoxy-2-methyl-2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide; Pesticide Tolerance, Final Rule. *Fed. Regist.* **2000**, *65* (139), 41355−41365.

Received for review August 11, 2003. Revised manuscript received December 8, 2003. Accepted December 10, 2003.

JF030591N