DOI:10.1021/jf103468d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

QuEChERS Multiresidue Method Validation and Mass Spectrometric Assessment for the Novel Anthranilic Diamide Insecticides Chlorantraniliprole and Cyantraniliprole

Timo Schwarz,[†] Timothy A. Snow,[§] Christopher J. Santee,[#] Christopher C. Mulligan,[#] Thomas Class,[†] Michael P. Wadsley,[⊥] and Sergio C. Nanita*,[⊥]

[†]PTRL Europe GmbH, Helmholtzstrasse 22, Science Park I, D-89081 Ulm, Germany, [§]DuPont Haskell Global Centers for Health and Environmental Sciences, Stine-Haskell Research Center, 1090 Elkton Road, Newark, Delaware 19714, United States, [#]Department of Chemistry, Illinois State University, Normal, Illinois 61790-4160, United States, and [⊥]DuPont Crop Protection, Stine-Haskell Research Center, 1090 Elkton Road, Newark, Delaware 19714, United States

The gas-phase dissociation reactions of chlorantraniliprole (Rynaxypyr) and cyantraniliprole (Cyazypyr) have been studied in triple-quadrupole, ion trap, and orbitrap mass spectrometers equipped with electrospray and desorption electrospray ion sources, revealing the formation of odd-electron fragment ions, the structures of which were elucidated. The odd-electron fragments were unusually abundant, and their formation is proposed to occur via a tricyclic intermediate. The applicability of the QuEChERS multiresidue method for the quantitation of chlorantraniliprole and cyantraniliprole was also assessed in this study. Four matrices representative of oily, watery, acidic, and dry crop groups were tested, with a targeted limit of quantitation (LOQ) of 0.01 mg/kg. Average recoveries ranged between 87 and 107%, with relative standard deviations (RSD) of ≤8%. Linear calibration functions with correlation coefficients $r > 0.99$ were obtained. The study provides an expansion of the QuEChERS method to include anthranilic diamides and a mass spectrometric assessment for these two novel agrochemical active ingredients.

KEYWORDS: Chlorantraniliprole; cyantraniliprole; Rynaxypyr; Cyazypyr; QuEChERS method; residue analysis; HPLC-MS/MS; odd-electron ion; electrospray ionization; desorption electrospray ionization

INTRODUCTION

Pesticide residue analysis is a tool used to ensure food safety by confirming that produce is in compliance with maximum residue limits. There are hundreds of agrochemical active ingredients used by industry to make thousands of crop protection product options for farmers around the world. The number of agrochemicals in today's market and the complex composition of produce samples make analytical determinations a challenging task. Multiresidue methods represent a solution introduced and adopted by private, government, and industry laboratories to streamline quantitation of pesticide residues in the food chain. A well-known multiresidue procedure for sample extraction and preparation is the "Quick, Easy, Cheap, Effective, Rugged, and Safe" QuEChERS method (*1*, *2*). This method has quickly become a preferred method by laboratories for residue analysis and was recently established as an official multiresidue method in Europe (European Norm EN 15662: 2009-02: Foods of plant origin − determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partition and cleanup by dispersive SPE − QuEChERS-method).

Mass spectrometry (MS) is one of the most powerful analytical technologies employed within the agrochemical industry, and an important application is quantitative residue analysis (*3*−*6*). Advances in tandem mass spectrometry have significantly improved the precision, accuracy, sensitivity, specificity, and ruggedness of instruments, particularly those equipped with triple-quadrupole and time-of-flight (TOF) mass analyzers, making these devices excellent partners for simplified sample preparation techniques, such as QuEChERS (*1*, *2*), allowing the development of methods capable of quantifying hundreds of active ingredients in a single or few analytical runs (*7*−*10*). However, technological advances in agriculture pose new analytical challenges, as the crop protection industry designs novel compounds to control pests and improve crop quality and yields. Consequently, multiresidue methods need continuous improvement to expand their capability to include emerging active ingredients. To achieve this goal, it is important to understand the solution-phase and gas-phase chemistry of new active ingredients, especially those that belong to a new class of agrochemicals. This is typically done by evaluating the applicability of known purification techniques (e.g., solid-phase extraction, QuEChERS, etc.)

*Corresponding author [phone (302) 451-5806; e-mail sergio.c.nanita@usa.dupont.com].

 Published on Web 12/31/2010

Table 1. Chromatographic Conditions Used at PTRL Europe for Evaluation of the QuEChERS Method

LC column	Phenomenex Aqua C_{18} column: length, 50 mm; i.d., 2.0 mm; particle size, 5 μm; column temperature, 30 °C			
LC gradient	solvent A: water/methanol (8:2 v/v) + 0.1% formic acid + 5 mM ammonium acetate solvent B: methanol/water (9:1 v/v) + 0.1% formic acid + 5 mM ammonium acetate injection volume: 20 μL mobile phase composition:			
	time (min)	flow rate (mL/min)	% A	% B
	0.00	0.25	95	5
	2.00	0.25	95	5
	4.00	0.25	70	30
	6.00	0.25	0	100
	11.00	0.25	0	100
	11.10	0.25	95	5
	16.00	0.25	95	5
retention time	≈9.3 min for cyantraniliprole ≈9.6 min for chlorantraniliprole			

together with ionization and precursor ion fragmentation studies to design logical sample preparation procedures and mass spectrometry experiments for instrumental analysis methods.

DuPont Crop Protection research and development efforts have recently resulted in the design of novel insecticide active ingredients. Chlorantraniliprole and cyantraniliprole are insecticides within the anthranilic diamide class recently discovered by Lahm and co-workers (*11*−*14*) and continue to be developed for a variety of applications in agriculture and home use. They control pests through a new mode of action relying on activation of insect ryanodine receptors, which play a critical role in muscle function (*11*). Cyantraniliprole and chlorantraniliprole exhibit remarkable selectivity and low toxicity to mammals because of structural differences between insect and mammalian ryanodine receptors (*12*, *13*). Chlorantraniliprole is designed for controlling lepidoptera and other selected species (*14*) and is registered for multiple agricultural uses. Cyantraniliprole is currently an experimental insecticide being developed by DuPont Crop Protection.

In this study, the QuEChERS multiresidue method was evaluated for quantitation of chlorantraniliprole and cyantraniliprole residues in a variety of crop matrices, together with a systematic mass spectrometric assessment of these active ingredients to elucidate their gas-phase dissociation mechanisms, providing fundamental knowledge about this new pesticide family. Desorption electrospray ionization (DESI) (*15*) analysis is also reported, demonstrating the ability to directly analyze the target insecticides as surface-bound residues.

MATERIALS AND METHODS

Mass Spectrometric Assessment. Analytical Standard grade reagents (purity > 97%) synthesized by DuPont Crop Protection were used. Individual analyte 100 μg/mL stock standard solutions were prepared in acetonitrile. Dilutions with 100 mM NH_4Ac in methanol were made to get 1−10 μg/mL solutions, which were used to record mass spectra, unless indicated otherwise.

Electrospray (ESI) triple-quadrupole experiments were performed with an Applied Biosystems API-5000 mass spectrometer. An 1100 series HPLC (Agilent Technologies, Wilmington, DE), which was coupled to the mass spectrometer, was used in flow injection mode to make 5 μL injections into the instrument with a carrier solvent (methanol) flow of 400 μL/min. Additional parameters were set as follows: ESI source voltage = 4.5 kV; CUR = 10 psi; GS1 = 70 psi; GS2 = 70 psi; ion source temperature = 450 °C; CAD pressure = 5.0 psi. Various collision energies were tested for all compounds. The exact collision energy values used to record the mass spectra displayed in figures appear in the captions as appropriate.

Experiments involving three stages of mass analysis (MS/MS/MS) were conducted using a Finnigan LCQ Classic equipped with an ESI source. Conditions were as follows: spray voltage, 3.55 kV; sheath gas, 65 arbitrary units (arb); auxiliary gas, 50 arb; capillary voltage, 12 V; capillary temperature, 275 °C; tube lens voltage, 5 V. Solutions were infused at 10 μL/min using a syringe pump.

DESI experiments were conducted with a Thermo LCQ Fleet equipped with an Omnispray ionization source (Prosolia, Inc.). Conditions were as follows: spray voltage, 5.0 kV; capillary voltage, 15 V; capillary temperature, 275 °C; tube lens voltage, 110 V. DESI parameters were similar to those previously reported (*16*). Aliquots (1 μL) of a 500 μg/mL solution were individually spotted (500 ng per spot) onto porous Teflon (Small Parts, Inc.). Upon drying, spots were analyzed using 100 mM NH_4Ac in 1:1 $MeOH/H_2O$ as the spray solution.

High-resolution mass spectra were recorded on a LTQ-Orbitrap (Thermo Fisher Scientific) by infusing analyte solutions at 13 − 23 μL/min. Source conditions were as follows: spray voltage, 5.0 kV; vaporizer temperature, 65 °C; sheath gas, 45 arb; auxiliary gas, 15 arb; sweep gas, 15 arb; capillary voltage, 30 V; tube lens voltage, 50 V; capillary temperature, 330 °C.

QuEChERS Method Testing. The analytical method validated within this study was based on a QuEChERS multiresidue method as established at PTRL Europe for pesticide residue analysis. All plant materials were homogenized using a moulinette/laboratory mixer prior to use. Lettuce and whole orange specimens were stored frozen until analysis; wheat grain and maize grain were stored at room temperature in the dark. Analyte fortification was done by spiking the solid/homogeneous matrices with fortification solutions of the analytes prior to extraction. For lettuce and whole orange, 10 g samples were extracted by adding 10 mL of acetonitrile and shaking vigorously for 1 min. For wheat grain and maize grain, 5.0 g samples were soaked for 5 min with 5.0 mL (wheat grain) or 10 mL (maize grain) of water prior to acetonitrile extraction. After the addition of $MgSO_4$, NaCl, and buffering citrate salts (pH 5−5.5), each mixture was shaken intensively and centrifuged for phase separation. An aliquot of the organic phase (6.0 mL) was cleaned up by dispersive SPE with PSA, $MgSO_4$, and additionally C_{18} material (for whole orange only). The purified extracts were acidified with formic acid and diluted 1:1 with water (0.50 mL purified/acidified extract aliquot mixed with 0.50 mL of water) in final preparation for chlorantraniliprole and cyantraniliprole residue determination.

Instrumental analysis was performed by HPLC-MS/MS. The liquid chromatography equipment was an Agilent 1100 HPLC system equipped with a vacuum solvent degasser, a binary HPLC pump, a column oven, and a CTC Analytics HTC-Pal autosampler (see **Table 1** for HPLC instrument conditions). It was coupled to an Applied Biosystems MDS Sciex API 3000 triple-quadrupole system with Turbo IonSpray (ESI) source. Positive ion detection was used for both compounds. Ion source conditions were set as follows: source temperature = 430 °C; nebulizer gas = 14 arb; curtain gas =12 arb; collision-activated dissociation (CAD) = 5 arb; entrance potential = 10 V; ion spray voltage = 5.5 kV; focusing potential = 150 V; resolution Q1 = unit; resolution Q3 = unit. Additional MS parameters appear in **Table 2**.

Two MS/MS ion fragmentation reactions were monitored for each analyte and used for residue quantitation and confirmation. The protonated ions $[M + H]^+$ were the isolated targets in MS/MS data acquisition

(see **Table 2**). Note that the nominal *m*/*z* values were not used. Instead, and for optimum sensitivity, the *m*/*z* of isolated precursors (Q1) and detected fragments (Q3) corresponded to the most abundant isotopomers of chlorine- and bromine-containing ions. The LC-MS/MS acquisition method allowed detection of both analytes at concentrations as low as 0.50 or 1.0 ng/mL with 20 μL injections, therefore providing sufficient sensitivity to determine and to confirm residues of the analytes in extracts obtained from lettuce, wheat grain, whole orange, and maize grain.

Table 2. Applied Biosystems MDS/Sciex API 3000 Triple-Quadrupole MS/MS Conditions Used for Evaluation of the QuEChERS Method

MS/MS conditions	chlorantraniliprole	cyantraniliprole
first MS/MS transition	*m*/*z* 484 → 286	*m*/*z* 475 → 286
collision energy (CE)	19 V	19 V
cell exit potential (CXP)	20 V	20 V
declustering potential	66 V	76 V
second MS/MS transition	*m*/*z* 484 → 453	*m*/*z* 475 → 444
collision energy (CE)	21 V	21 V
cell exit potential (CXP)	32 V	33 V
declustering potential	66 V	76 V
dwell time per transition	50 ms	100 ms

RESULTS AND DISCUSSION

Mass Spectrometric Assessment. Initial investigations on anthranilic diamide insecticides were conducted using a triple-quadrupole instrument. ESI-MS spectra were recorded, which, under optimized instrumental conditions, mainly showed the protonated adducts. Panels **a** and **d** of **Figure 1** display expanded regions of the chlorantraniliprole and cyantraniliprole spectra, respectively, to show the isotopic distributions, which are consistent with the corresponding bromine and chlorine atom content of the active ingredients (i.e., 1Br and 2Cl in chlorantraniliprole and 1Br and 1Cl in cyantraniliprole). Tandem mass spectra of the protonated adducts were subsequently recorded to evaluate gas-phase fragmentation reactions. At relatively low collision-induced dissociation (CID) energy, for example, 10 V, shown in **Figure 1b**,**e**, $(M + H)^+$ ions fragment almost exclusively via cleavage of the amide bonds almost exclusively via cleavage of the amide bonds. The ions at *m*/*z* 451 and 442 in **Figure 1**, panels **b** and **e**, respectively, are the product of the loss of neutral methylamine, whereas *m*/*z* 284 results from the inner amide bond cleavage. Extensive fragmentation is observed when the collision energy is increased to 70 V (**Figure 1c**,**f**), where a fragment at *m*/*z* 205 is

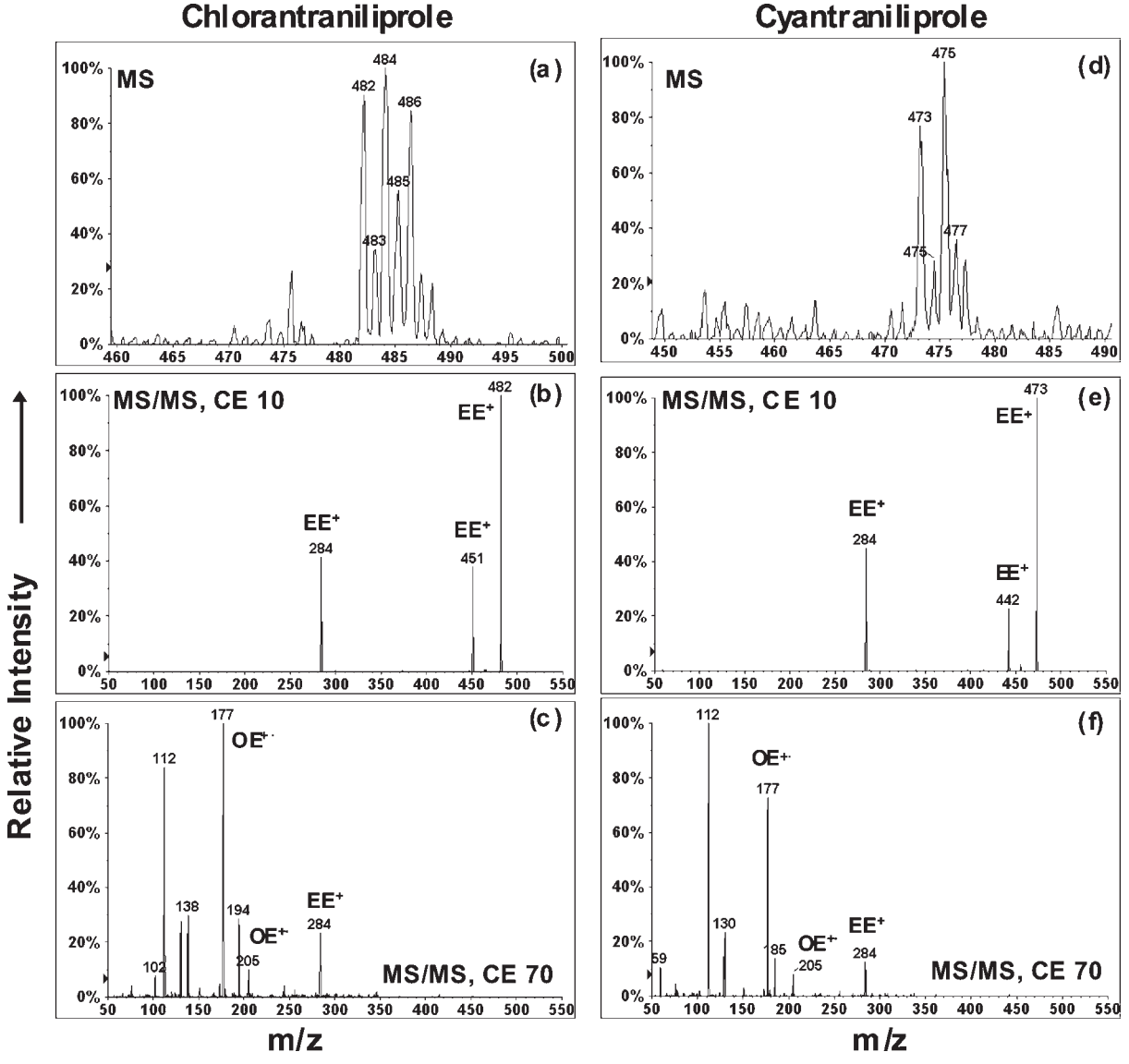

Figure 1. ESI mass spectra of anthranilic diamide insecticides recorded in a triple-quadrupole instrument. Chlorantraniliprole: (**a**) MS and tandem mass spectra at collision energies of (**b**) 10 V and (**c**) 70 V. Cyantraniliprole: (**d**) MS and tandem mass spectra at collision energies of (**e**) 10 V and (**f**) 70 V. EE^+ = even-electron cation; $OE^{\bullet+}$ = odd-electron cation.

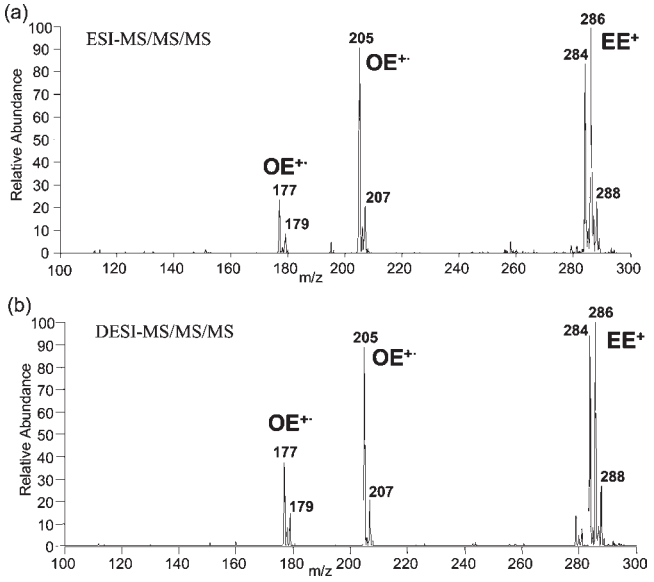

Figure 2. MS^3 of protonated chlorantraniliprole recorded using LCQ ion trap mass spectrometers equipped with (**a**) ESI and (**b**) DESI ion sources. The collision energies were set at 25 and 40% for the first (*m*/*z* 482) and second (*m*/*z* 284) ion isolation events, respectively. Large ion isolation windows of *m*/*z* 20 units were used to fragment the entire parent isotope distribution. The most abundant fragment ions, *m*/*z* 177 and 205, are radical cations (odd electron, $OE^{\bullet+}$) formed sequentially through the fragmentation of the (even electron, EE^{+}) precursor at *m*/*z* 284.

observed. This ion can be qualitatively explained by the loss of a neutral bromine radical (79 Da) from *m*/*z* 284, which would make the ion at *m*/*z* 205 an odd-electron species. The ion at *m*/*z* 177 (**Figure 1c**,**f**) can be explained by the loss of neutral carbon monoxide from *m*/*z* 205; thus, it could also be a radical cation.

Thurman et al. (*17*) evaluated the fragmentation of 100 pesticide active ingredients and reported that approximately 7% of fragment ions measurable in ESI mass spectra of protonated (even-electron) pesticides were radical cations. However, most radical cations were observed in low abundance (as suggested by the even-electron rule (*18*, *19*)); only methiocarb sulfone and nitenpyram yielded radical cation fragments with > 50% relative intensity (*17*). The odd-electron ions, that is, *m*/*z* 205 and 177, are of interest because they highlight important gas-phase dissociation reactions for this new class of agrochemicals. Ion trap mass spectrometers equipped with ESI and DESI sources were employed to evaluate the dissociation of chlorantraniliprole in MS^3 experiments, which could confirm or rule out the hypothesis of homolytic C−Br bond cleavage (radical formation) while elucidating the mechanism by which the ion at *m*/*z* 205 is formed. Panels **a** and **b** of **Figure 2** show representative ESI-MS^3 and DESI-MS^3 spectra obtained for protonated chlorantraniliprole. In this experiment, the ions at *m*/*z* 482 $(M + H)^+$ and *m*/*z* 284 (fragment) were sequentially isolated (with large ion selection windows, 20 *m*/*z* units, to include the entire parent isotope distribution), collisionally activated, and fragmented, yielding the ions at *m*/*z* 205 and 177. The isotopic distribution of these fragment ions confirms that a bromine radical was lost. These ions (**Figure 2**), including their ^{37}Cl isotopologues, account for ~90% of the fragment ion signal intensity resulting from the dissociation of *m*/*z* 284. Analysis of agrochemicals by DESI-MS continues to be an area of high interest, and the ability to detect active ingredients directly from vegetation (*20*), fruit surfaces (*21*), and aqueous solutions (*22*) has been recently reported. Although outside the scope of the study, the results reported here (**Figure 2**) demonstrate that DESI-MS can be applied to anthranilic diamides. Most importantly, the preferred ionization mechanism observed with DESI is identical to electrospray ionization (*15*, *16*); the analytes form the protonated adducts, which dissociate inside the mass spectrometer regardless of the ionization method employed (DESI or ESI), and thus the same fragment ions and MS/MS experiments could be used for quantitative determinations.

The structures of ions shown in **Figure 3** were further evaluated in MS^n experiments performed with a LTQ-Orbitrap (mass spectrometric resolution (*R*) of approximately 100,000). Accurate mass measurements were within 0.04−0.96 mDa of the theoretical exact masses of proposed structures, supporting the fragmentation pathways in **Figure 3**. In particular, the radical cation-forming reactions were confirmed. The mass difference measured for the neutral Br radical loss to form *m*/*z* 205 was 78.9185 Da (absolute error = 0.12 mDa) and for the CO loss to form *m*/*z* 177 was 27.9950 (absolute error = 0.04 mDa). The data indisputably support the homolytic cleavage of the C−Br bond. However, this reaction seems unlikely to occur from the *m*/*z* 284 fragment formed initially (**Figure 3**, center structure), because the loss of CO (28 Da) would be expected. The unusual experimental observations can be explained by a cyclization reaction, which would stabilize *m*/*z* 284, leaving the halogens as the only tricyclic intermediate substituent groups that could be lost as neutrals (see **Figure 3**). Because of its lower bond energy, homolytic cleavage of C−Br is preferred.

Additional ESI-MS/MS experiments were conducted to study the homolytic bond cleavage and radical cation-forming reactions. Chlorantraniliprole and cyantraniliprole chlorine analogues, 3-chloro-*N*-[4-chloro-2-methyl-6-[(methylamino)carbonyl]-phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide and 3-chloro-1-(3-chloro-2-pyridinyl)-*N*-[4-cyano-2-methyl-6-[(methylamino)carbonyl]phenyl]-1*H*-pyrazole-5-carboxamide, respectively, were available at the time of the experiment. These chemicals were ideal candidates to evaluate the effect of the halogen atom in the reactions that yield odd-electron ions. Ion breakdown plots were recorded for protonated adducts of chlorantraniliprole, cyantraniliprole, and their corresponding chlorine analogues. Panels **a** and **b** of **Figure 4** show the ion formation/breakdown plots obtained for *m*/*z* 205 and 177 when formed by homolytic cleavage of carbon−bromine (the active ingredients) or carbon−chlorine (the Cl-analogues) bonds. The much lower abundance obtained for the products of homolytic cleavage of C−Cl bonds is again in agreement with the known energies of carbon−halogen bonds (C−Cl > C−Br). The careful mass spectrometric assessment of protonated anthranilic diamides reported here provides gas-phase ion fragmentation reactions not previously reported for this class of chemicals. The development of a MS/MS-based acquisition method was sought using the transition from the protonated analytes to the tricyclic ion (nominal *m*/*z* 284) for quantitative calibration and analysis as part of the QuEChERS/HPLC/MS/MS method testing, which is discussed below.

QuEChERS Method Testing. The objective of this portion of the study was to assess the applicability of the QuEChERS (*1*, *2*) multiresidue method for the determination of residues of chlorantraniliprole and cyantraniliprole in crops using LC-MS/MS, with a target limit of quantitation (LOQ) of 0.01 mg/kg for each analyte (see Experimental Procedures for method description). The target LOQ was defined as the concentration that yielded a signal-to-noise ratio of approximately 10 to 1 for the least responsive analyte (cyantraniliprole). The analytical method was tested in matrices representative of four crop groups: lettuce (watery), maize grain (oily), wheat grain (dry), and whole orange (acidic). The validation trials were conducted according to regulatory guidelines (*23*, *24*),

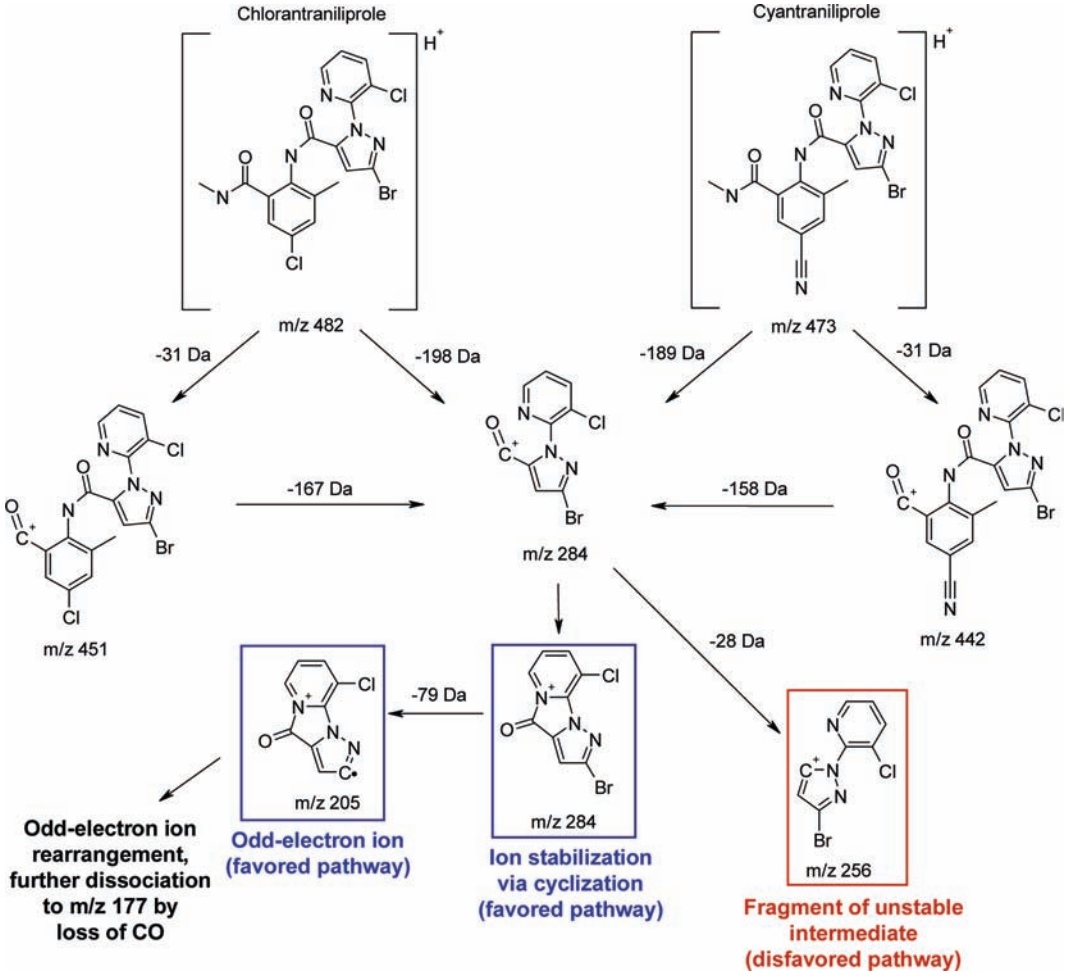

Figure 3. Proposed anthranilic diamide fragmentation reactions (based on mass spectra). The preferred fragmentation route leads to formation of odd-electron ions. Note that the fragment structures shown are not necessarily the most stable and several resonance structures are possible.

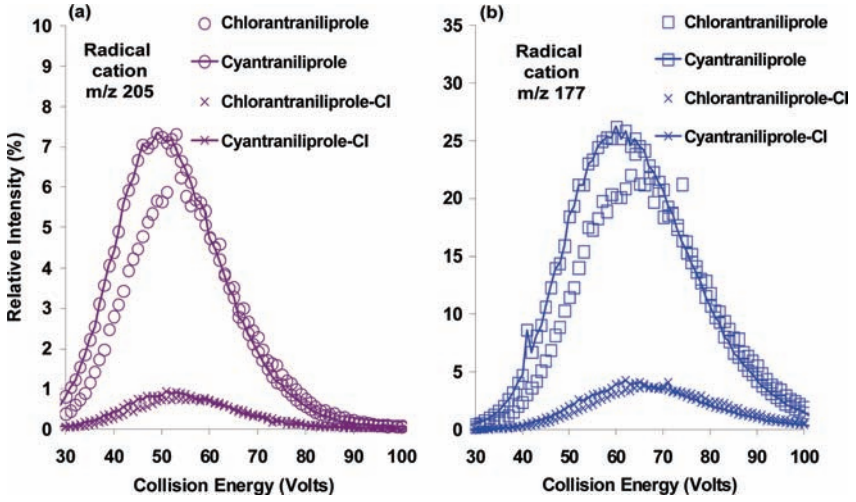

Figure 4. Ion formation/breakdown plots for odd-electron species (**a**) *m/z* 205 and (**b**) *m/z* 177 recorded in a triple-quadrupole instrument by isolation/fragmentation of $(M + H)^+$ ions of chlorantraniliprole, cyantraniliprole, and their chlorine analogues.

and the data acceptance criteria described in the OECD and SANCO documents were followed (*23*, *24*). Briefly, acceptable analytical methods are expected to provide average percent recoveries, (analyte found/analyte added) × 100, between 70 and 110% for the individual analytes in each matrix tested, with relative standard deviation (RSD) no greater than 20%. External calibration standards must yield an acceptable correlation coefficient $R > 0.99$ over the targeted calibration range. For each matrix tested, validation trials consisted of 10 homogenized control samples fortified with the analytes of interest (5 at the method LOQ and 5 at a higher level) prior to extraction (see Experimental Procedures for details). Two unfortified control samples were also analyzed.

The validation data generated for the quantitation of chlorantraniliprole and cyantraniliprole in lettuce, maize grain,

Table 3. Performance of the QuEChERS/HPLC/MS/MS Method during Validation Trials

			chlorantraniliprole		cyantraniliprole	
matrix type	fortification level	recovery results	*m/z* 286, %	*m/z* 453, %	*m/z* 286, %	*m/z* 444, %
lettuce	0.01 mg/kg LOQ	av	105	104	102	96
		RSD	3	4	7	8
	0.10 mg/kg 10 × LOQ	av	101	101	102	102
		RSD	4	5	3	5
	overall	av	103	102	102	99
		RSD	4	5	5	7
wheat grain	0.01 mg/kg LOQ	av	91	93	89	89
		RSD	2	3	3	4
	0.10 mg/kg 10 × LOQ	av	91	93	88	87
		RSD	2	3	4	4
	overall	av	91	93	88	88
		RSD	2	3	3	4
whole orange	0.01 mg/kg LOQ	av	100	104	103	104
		RSD	5	4	4	3
	0.10 mg/kg 10 × LOQ	av	101	98	103	102
		RSD	1	2	5	8
	overall	av	101	101	103	103
		RSD	4	4	4	6
maize grain	0.01 mg/kg LOQ	av	107	105	107	100
		RSD	3	5	3	6
	0.10 mg/kg 10 × LOQ	av	104	105	103	105
		RSD	5	6	6	5
	overall	av	106	105	105	103
		RSD	4	5	5	6

RSD = relative standard deviation; av = average percent recovery.

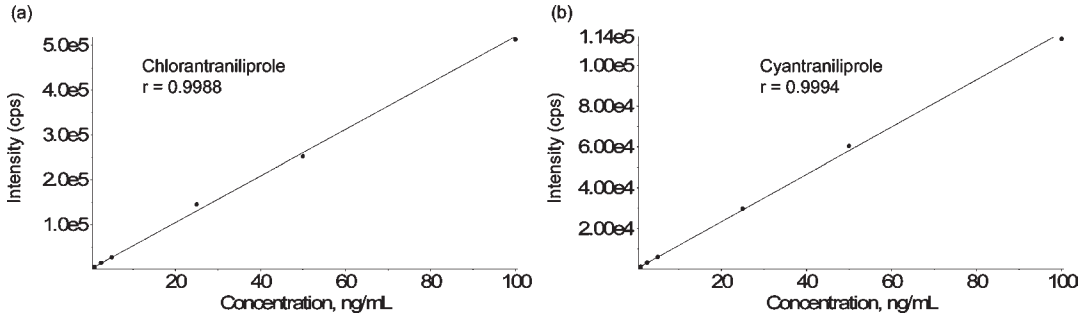

Figure 5. Representative lettuce matrix-matched standard calibration curves obtained for (**a**) chlorantraniliprole and (**b**) cyantraniliprole.

wheat grain, and whole orange appear in **Table 3**. The average recoveries obtained for both compounds in all matrices tested were within the acceptable range. Chlorantraniliprole and cyantraniliprole are relatively nonpolar and partitioned well into the acetonitrile layer during the QuEChERS "salt out" step. They also remained in solution during dispersive SPE purification, yielding very good recoveries for the overall sample preparation method. Acceptable linearity ($r > 0.998$) was observed for calibration standards from 0.50 or 1.0 to 100 ng/mL for both compounds with $1/x$ weighting (see example calibration curves in **Figure 5**). The RSDs for matrix-matched external standard responses were consistently $< 20\%$ for chlorantraniliprole and cyantraniliprole. Example chromatograms recorded for the lettuce matrix are provided in **Figure 6**. Chromatograms recorded for the other matrices tested (i.e., maize grain, wheat grain, and whole orange) were similar to those presented in **Figure 6**. No interfering signals in blank control specimens were detected at the retention time of the analytes. The absence of interferences and similarity observed in chromatograms recorded for all matrices is expected due to the high specificity of tandem mass spectrometry detection, especially when fragmentation reactions are carefully selected to enhance selectivity.

In summary, the work described in this study expands the analytical chemistry knowledge about anthranilic diamide insecticide beyond existing analytical methods (*25*, *26*). The gas-phase dissociation reactions of protonated chlorantraniliprole and protonated cyantraniliprole have been characterized, revealing the formation of abundant radical cation fragments. Chlorantraniliprole and cyantraniliprole are unique in the way their protonated adducts dissociate to yield odd-electron ions, that is, via the tricyclic intermediate of nominal *m/z* 284. Computational chemistry studies on these systems are of interest and could provide the thermochemical details that favor the radical cation products over possible even-electron fragment alternatives. The careful characterization of protonated anthranilic diamide fragmentation reported here provides unusual dissociation mechanisms, useful for quantitative method development and structure elucidation for future compounds belonging to this novel class of insecticides. The QuEChERS multiresidue method with HPLC-MS/MS detection

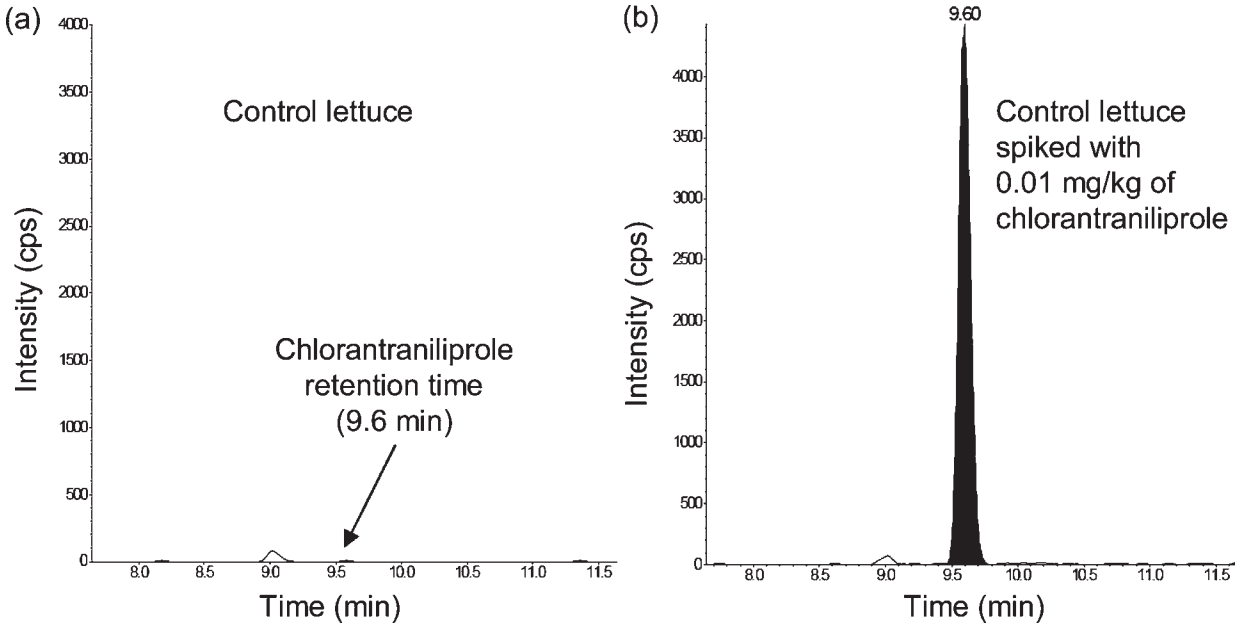

Figure 6. Example chromatograms for the determination of chlorantraniliprole (ion transition *m/z* 484 → 286): (**a**) lettuce control; (**b**) lettuce sample fortified at 0.01 mg/kg.

was successfully validated for the determination of chlorantraniliprole and cyantraniliprole in lettuce, wheat grain, whole orange, and maize grain with a LOQ of 0.01 mg/kg for each analyte. Reliable pesticide residue determinations can be difficult to achieve (*27*), and adequate selectivity is one key factor. Highly specific ion fragmentation reactions are particularly important in mass spectrometry methods that do not use chromatography, such as DESI (*21*) and DART (*28*). In addition, high specificity is becoming even more important as universal methods are designed without cleanup to obtain satisfactory recoveries for a wide range of chemicals, such as the generic extraction method introduced by Mol and co-workers (*29*) and the recently developed fast extraction and dilution flow injection mass spectrometry (FED-FI-MS) method (*30*, *31*).

ACKNOWLEDGMENT

We thank Thomas Selby, Mary Ellen McNally, Stephen Platz, James Stry, William Zimmerman, and John May (DuPont Co.) for their valuable suggestions.

LITERATURE CITED

(1) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412−431.

(2) Lehotay, S. J.; Mastovska, K.; Lightfield, A. R. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC Int.* **2005**, *88*, 615−629.

(3) Klein, J.; Alder, L. Applicability of gradient liquid chromatography with tandem mass spectrometry to the simultaneous screening for about 100 pesticides in crops. *J. AOAC Int.* **2003**, *86*, 1015−1037.

(4) Rodriguez-Mozaz, S.; Lopez De Alda, M. J.; Barceló, D. Picogram per liter level determination of estrogens in natural waters and waterworks by a fully automated on-line solid-phase extraction−liquid chromatography−electrospray tandem mass spectrometry method. *Anal. Chem.* **2005**, *76*, 6998−7006.

(5) Fernández-Alba, A. R.; García-Reyes, J. F. Large-scale multiresidue methods for pesticides and their degradation products in food by advanced LC-MS. *Trends Anal. Chem.* **2008**, *27*, 973−990.

(6) Nanita, S. C.; Pentz, A. M.; Grant, J.; Vogl, E.; Devine, T. J.; Henze, R. M. Mass spectrometric assessment and analytical methods for quantitation of the new herbicide aminocyclopyrachlor and its methyl analogue in soil and water. *Anal. Chem.* **2009**, *81*, 797−808.

(7) Greulich, K.; Alder, L. Fast multiresidue screening of 300 pesticides in water for human consumption by LC-MS/MS. *Anal. Bioanal. Chem.* **2008**, *391*, 183−197.

(8) Lehotay, S. J.; De Kok, A.; Hiemstra, M.; Van Bodegraven, P. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *J. AOAC Int.* **2005**, *88*, 595−614.

(9) Alder, L.; Greulich, K.; Kempe, G.; Vieth, B. Residue analysis of 500 high priority pesticides: better by GC−MS or LC−MS/MS? *Mass Spectrom. Rev.* **2006**, *25*, 838−865.

(10) Klein, J.; Alder, L. Applicability of gradient liquid chromatography with tandem mass spectrometry to the simultaneous screening for about 100 pesticides in crops. *J. AOAC Int.* **2003**, *86*, 1015−1037.

(11) Lahm, G. P.; Stevenson, T. M.; Selby, T. P.; Freudenberger, J. H.; Cordova, D.; Flexner, L.; Bellin, C. A.; Dubas, C. M.; Smith, B. K.; Hughes, K. A.; Hollingshaus, J. G.; Clark, C. E.; Benner, E. A. Rynaxypyr: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6274−6279.

(12) Lahm, G. P.; Cordova, D.; Barry, J. D. New and selective ryanodine receptor activators for insect control. *Bioorg. Med. Chem.* **2009**, *17*, 4127−4133.

(13) Lahm, G. P.; Selby, T. P.; Freudenberger, J. H.; Stevenson, T. M.; Myers, B. J.; Seburyamo, G.; Smith, B. K.; Flexner, L.; Clark, C. E.; Cordova, D. Insecticidal anthranilic siamides: a new class of potent ryanodine receptor activators. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4898−4906.

(14) Cordova, D.; Benner, E. A.; Sacher, M. D.; Rauh, J. J.; Sopa, J. S.; Lahm, G. P.; Selby, T. P.; Stevenson, T. M.; Flexner, L.; Gutteridge, S.; Rhoads, D. F.; Wu, L.; Smith, B. K.; Tao, Y. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pestic. Biochem. Physiol.* **2006**, *84*, 196−214.

(15) Cooks, R. G.; Ouyang, Z.; Takáts, Z.; Wiseman, J. M. Ambient mass spectrometry. *Science* **2006**, *311*, 1566−1577.

(16) Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. Mass spectrometry under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306*, 471−473.

(17) Thurman, E. M.; Ferrer, I.; Pozo, O. J.; Sancho, J. V.; Hernandez, F. The even-electron rule in electrospray mass spectra of pesticides. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3855−3868.
(18) Levsen, K.; Schiebel, H.-M.; Terlouw, J. K.; Jobst, K. J.; Elend, M.; Preiss, A.; Thiele, H.; Ingendoh, A. Even electron ions: a systematic study of the neutral species lost in the dissociation of quasi-molecular ions. *J. Mass Spectrom.* **2007**, *42*, 1024−1044.
(19) Schäfer, M.; Drayss, M.; Springer, A.; Zacharias, P.; Meerholz, K. Radical cations in electrospray mass spectrometry: formation of open-shell species, examination of the fragmentation behaviour in ESI-MS^n and reaction mechanism studies via detection of transient radical cations. *Eur. J. Org. Chem.* **2007**, *31*, 5162−5174.
(20) Mulligan, C. C.; Talaty, N.; Cooks, R. G. Desorption electrospray ionization with a portable mass spectrometer: in-situ analysis of ambient surfaces. *Chem. Commun.* **2006**, 1709−1711.
(21) Garcia-Reyes, J. F.; Jackson, A. U.; Molina-Diaz, A.; Cooks, R. G. Desorption electrospray ionization mass spectrometry for trace analyses of agrochemicals in food. *Anal. Chem.* **2009**, *81*, 820−829.
(22) Mulligan, C. C.; MacMillan, D. K.; Noll, R. G.; Cooks, R. G. Fast analysis of ordnance-related compounds and agricultural chemicals in water with desorption electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3729−3736.
(23) EEC Directive 91/414/EEC, Annex IIA 4.2.2 as amended by EC Directive 96/46/EC; SANCO/825/00 rev.7 (17/03/2004) Guidance Document on Residue Analytical Methods.
(24) OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 13/08/07).
(25) Grant, J.; Rodgers, C. A.; Chickering, C. D.; Hill, S. J.; Stry, J. J. The determination of chlorantraniliprole residues in crops using liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry/mass spectrometry. *J. AOAC Int.* **2010**, *94*, 1293−1301.
(26) Caboni, P.; Sarais, G.; Angioni, A.; Vargiu, S.; Pagnozzi, D.; Cabras, P.; Casida, J. E. Liquid chromatography−tandem mass spectrometric ion-switching determination of chlorantraniliprole and flubendiamide in fruits and vegetables. *J. Agric. Food Chem.* **2008**, *56*, 7696−7699.
(27) Nanita, S. C.; Ziegler, M.; Giammarrusti, L.; Huber, A.; Astor, E.; Ruhl, J. C. Error propagation in pesticide residue measurements estimated by computational simulations and inter-laboratory sample analysis. *J. Environ. Sci. Health B* **2009**, *44*, 640−648.
(28) Schurek, J.; Vaclavik, L.; Hooijerink, H.; Lacina, O.; Poustka, J.; Sharman, M.; Caldow, M.; Nielen, M. W. F.; Hajslova, J. Control of strobilurin fungicides in wheat using direct analysis in real time accurate time-of-flight and desorption electrospray ionization linear ion trap mass spectrometry. *Anal. Chem.* **2008**, *80*, 9567.
(29) Mol, H. G. J.; Plaza-Bolaños, P.; Zomer, P.; de Rijk, T. C.; Stolker, A. A. M.; Mulder, P. P. J. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. *Anal. Chem.* **2008**, *80*, 9450−9459.
(30) Nanita, S. C.; Pentz, A.; Swaim, L.; Plastridge, B. Flow injection mass spectrometry for high-throughput pesticide residues measurements; 47th Florida Pesticide Residue Workshop (FPRW), St. Pete's Beach, FL, July 18−21, **2010**; (O-27).
(31) Nanita, S. C. High throughput chemical residue analysis by fast extraction and dilution flow injection mass spectrometry. *Analyst* **2011**, *136*, 285−287.

Received for review September 7, 2010. Revised manuscript received December 1, 2010. Accepted December 10, 2010. C.C.M. acknowledges research support through the Research Corporation for Scientific Advancement Cottrell College Science Award (RCSA-CCSA) Program.