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Penetrative and Dislodgeable Residue Characteristics of ^{14}C-Insecticides in Apple Fruit

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ABSTRACT: Infinite- and finite-dose laboratory experiments were used to study the penetrative and dislodgeable residue characteristics of ^{14}C-insecticides in apple fruit. The differences in dislodgeable and penetrated residues of three radiolabeled insecticides (^{14}C-thiamethoxam, ^{14}C-thiacloprid, and ^{14}C-indoxacarb), applied in aqueous solution with commercial formulations, were determined after water and methanol wash extractions. The rate of sorption and extent of penetration into the fruit cuticles and hypanthium of two apple cultivars were measured after 1, 6, and 24 h of treatment exposure, using radioactivity quantification methods. For all three compounds, 97% or more of the treatment solutions were found on the fruit surface as some form of non-sorbed residues. For indoxacarb, sorption into the epicuticle was rapid but desorption into the fruit hypanthium was delayed, indicative of a lipophilic penetration pathway. For the neonicotinoids, initial cuticular penetration was slower but with no such delay in desorption into the hypanthium.

KEYWORDS: *dislogeable residues, cuticle penetration, neonicotinoid, oxadiazine, radioactivity*

INTRODUCTION

The Food Quality Protection Act (FQPA)[1] fundamentally changed the basis by which the Environmental Protection Agency (EPA) registers and regulates pesticides in the U.S.A. The EPA is currently implementing new standards related to dietary risk, worker exposure, and environmental impacts for the use of agricultural chemicals and, as a result, has begun to eliminate or severely restrict many conventional pesticides traditionally relied upon for agriculture production. Many of the newly registered insecticides, including the neonicotinoids, insect growth regulators, oxidiazines, spinosyns, and diamides, have been designated by the U.S. EPA as reduced-risk because they are expected to reduce pesticide risks to human health, nontarget organisms, and reduce the potential for contamination of valued, environmental resources.[2−4]

Even though extensive applied research in fruit entomology has shown that many of these reduced-risk insecticides hold promise for replacing older pesticides, their performance characteristics are different in many ways.[5,6] Research also shows that the physicochemical interactions between the plant, insect, and chemical are important for understanding the performance attributes of a given insecticide.[7,8] Residue profile analysis of the neonicotinoid insecticides, thiamethoxam, imidacloprid, and thiacloprid, shows that the extent and duration of insecticide penetration in apple fruit and leaf tissues serves to regulate lethal and sublethal modes of activity on the target pest.[6] Salgado and Saar[9] identified two nicotinic receptor subtypes (desensitizing and non-desensitizing) in cockroaches, one causing acute lethal symptoms in the presence of high concentrations of imidacloprid (nAChN type) and the other causing subacute symptoms under very low concentrations (nAChD type). Other studies[10,11] have also documented sublethal behavioral effects on insects after exposure to or detection of internal plant residues.

Research focused on understanding the systemic qualities of insecticides for controlling insect pests in apples and cherries showed that certain compounds, especially those in the neonicotinoid class, have penetrative characteristics in fruit that can kill eggs or larvae post-infestation.[12,13] Curative activity is the lethal action of an insecticide on a pest post-infestation from the transitory penetration of the compound into plant tissue.[6] This "curative" form of insecticide activity can serve to reduce the number of sprays to control internal fruit pests. The concept of moving from a purely preventative approach to an approach that incorporates curative insecticidal activity can provide growers with greater timing flexibility and the opportunity to reduce the overall number of insecticide sprays in a season. However, compounds that show curative activity on a pest on early season apples do not necessarily maintain the same effects on late season apple pests or the same pest in another fruit crop, such as cherries.[14,15]

The plant cuticle, subtending the epicuticular wax, is a nonliving and non-uniform plant structure, made of a polymeric cutin matrix and soluble waxes, that protects the plant from water loss, is a barrier against pathogens, and allows for gas exchange.[16,17] The cuticle outer surface or extracellular membrane is composed of cutin and waxes, whereas the layer

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underneath contains polar polysaccharides and small amounts of cellulose and pectins.[18] This transversal heterogeneity results in varied permeability, depending upon the property of the solute and structure of the membrane.[18] The cuticular membranes of fruits are generally thicker than those of leaves, yet fruits have higher water permeability coefficients.[19] Apple cultivars, such as *Malus domestica* Borkhausen, 'Golden Delicious', and *Malus domestica* Borkhausen, 'Red Delicious', vary in the thickness of fruit cuticles.[20] The transport of a "synthetic organic" pesticide through the fruit cuticle, following penetration of the epicuticular wax, involves sorption into the cuticular lipids, diffusion across the cuticular membrane, and desorption into the epidermal cells of the hypanthium.[21,22] Solute mobility across the cuticle is influenced by several factors, including the molecular volume of the compound, diffusion path length, wax characteristics, and temperature, with properties varying for both polar and lipophilic pathways.[18,22] The rate of penetration is influenced by the partition coefficient of the substance, the surface concentration, and the physical characteristics of the cuticle.[21]

In cherry fruit cuticles, polar penetration pathways were determined to be important for the transport of water and polar substances through the fruit cuticular membrane.[23,24] In one study, buffered forms of 2-(1-naphthyl)-acetic acid (NAA) were expected to penetrate the cuticular membrane along the lipophilic or polar pathways based on polarity and molecular dimensions of the solute.[24] There is limited fundamental knowledge, however, about the physicochemical relationships between the array of modern insecticides and apple fruit cuticles.

Dislodgeable foliar residue (DFR) is defined as the amount of pesticide residue that can be dislodged from the two-sided foliar surface of a plant during a well-defined procedure.[25] We are defining dislodgeable residues more broadly to represent the proportion of a compound that has not penetrated the plant cuticle sufficiently to resist physical removal from the plant surface. The stability of a material on a particular plant surface is dependent upon its affinity to the cuticle and epicuticular wax, environmental factors, such as temperature, moisture, and drying time, and the form of physical contact causing removal.[26−29] Studies of pyrethroid and organophosphate insecticides on leaf surfaces have shown that bioavailability of residues varies among plant species, suggesting that, in some cases, there is sufficient sorption into the plant cuticle to modify contact toxicity from surface exposure to the pest.[7,30] Buchholz and Nauen[31] compared the translocation and translaminar movement of two neonicotinoid insecticides, imidacloprid and acetamiprid, on cotton and cabbage leaves. Even though the intrinsic toxicity of these two compounds to green peach aphid, *Myzus persicae* (Sulzer), and cotton aphid, *Aphis gossypii* (Glover), were equal, differences were found in the ultimate pest efficacy based on the translaminar and acropetal patterns of insecticide movement in each of the two plant types. When penetration and persistence of chlorpyrifos-methyl in apples, grapefruit, and strawberries were compared, epicuticular waxes were found to inhibit the extent of penetration into the fruits.[32] How an insecticide is formulated or combined with commercial adjuvants can additionally impact the duration and extent of penetration in plant tissue. These studies have resulted in key insights into the contribution of penetrated and dislodgeable residues on the overall activity of an insecticide on the target pest.

A range of solvents and procedures, including cheese cloth, water, methanol, and sodium dioctyl sulfosuccinate have been used to estimate degrees of dislodgeability, with the relevance of each method depending upon the research question being addressed. Those conducting dislogeable residue studies for human exposure assessments, however, are generally restricted from using organic solvents for residue extraction (according to EPA/OPP dislodgeable residue guidelines).

Infinite- and finite-dose systems are the two most commonly used laboratory methods for studying the penetrative characteristics of pesticides in plants.[21,24] In an infinite-dose system, the solute is in constant aqueous contact with the plant surface for a defined exposure period and is generally viewed as more robust for studying cuticular penetration of pesticides. Finite-dose systems deliver a definitive dose of pesticide to the plant surface, which is allowed to air-dry and diffuse over the exposure period. This method is generally viewed to be more reflective of field-sprayed conditions, but the resulting data are more difficult to generalize.

The purpose of this study was to determine the rate and extent of penetration of three radiolabeled insecticides (^{14}C-thiamethoxam, ^{14}C-thiacloprid, and ^{14}C-indoxacarb) of different water−lipid partitioning properties, into the fruit cuticle and hypanthium of two apple cultivars, and measure the extraction characteristics of the remaining dislodgeable residues. We selected thiamethoxam, thiacloprid, and indoxacarb because they represent modern insecticide chemistries used in commercial apple production from early season through preharvest timings, and their partitioning values span the range of solubilities needed for advancing fundamental knowledge in this area of research. The specific objectives were to determine (1) the rate of insecticide sorption and desorption into two fruit cultivars under finite- and infinite-dose systems, (2) the differences in dislodgeable and penetrated residues between three insecticides, and (3) the depth of penetration into apple fruit between three insecticides.

■ MATERIALS AND METHODS

Insecticides. Three ^{14}C-insecticides were used for the study, two neonicotinoids, ^{14}C-thiamethoxam (thiazolyl-2-^{14}C-CGA-293343, purity of 99.6% and specific activity of 58.9 μCi/mg) and ^{14}C-thiacloprid (cyanamide,*N*-[3-[(6-chloro-3-pyridinyl) methyl]-2-thiazolidinylidene]-thiazoli dene-4,5-^{14}C, purity of 100% and specific activity of 26.7 μCi/mmol), and an oxidiazine, ^{14}C-indoxacarb (indanone-1-^{14}C) (Figure 1). Each radiolabeled compound was mixed with its nonlabeled counterpart to form a 100 ppm solution. The nonlabeled compounds including thiamethoxam (Actara 25 WG, Syngenta Crop Protection, Greensboro, NC), thiacloprid (Calypso 4 F, Bayer CropScience, Kansas City, MO), and indoxacarb (Avaunt 30 WG, DuPont, Wilmington, DE). The dilutions were performed in a phosphate buffer solution with a pH of 6.4, and a spreader sticker was added (Latron B-1956, 0.15%).

Core Preparation. Cuticular permeability and the resultant depth of penetration were measured using a modified disk method similar to that described by Flore and Bukovac. Two varieties of apples with different wax content were used: a low-wax cuticle apple variety, *M. domestica* Borkhausen, 'Golden Delicious', and a high-wax cuticle apple variety, *M. domestica* Borkhausen, 'Red Delicious'. On September 1, 2007 (approximately 110 days after full bloom), fruit samples were randomly picked from apple trees that had not previously received insecticides that season from the Michigan State University Trevor Nichols Research Center (42.5951° N, −86.1561° W), Fennville, MI. The fruit were approximately 5 cm in diameter, and maturity was about 30 days from the normal commercial harvest time. Apple cores were prepared by the methodology of Wise et al.,[14] by slicing apples in

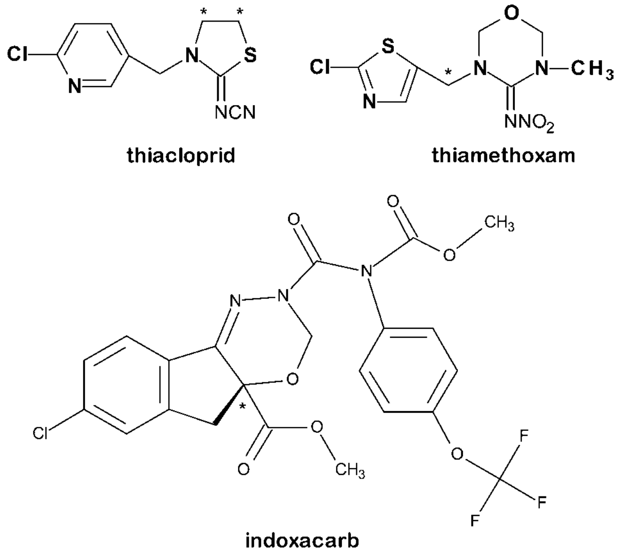

Figure 1. Chemical structures of thiamethoxam, thiacloprid, and indoxacarb and the location of ^{14}C in the structures.

half laterally from the calyx to stem end and taking the cores from each half from the ovary to the cuticle with a cork borer (Science, Art and More, Seattle, WA). Finished apple cores were approximately 20 mm in diameter × 16 ± 2 mm in length.

Exposure of the Cores to Insecticide Solutions. Glass vials (1 mm thickness, 10 mm high, 12 mm external diameter, and 10 mm internal diameter, with an internal area of 78.5 mm^2) opened on both ends were attached to the outer cuticle surface of fruit cores with silicone rubber. Cores were placed upright in culture cell boxes lined with moistened filter paper at the bottom. Boxes of the culture cell containing the cores were placed in a Plexiglas box that was placed over water in a model YB-521 water bath (American Scientific Products, Columbus, OH) to maintain the moisture integrity of the fruit cores. Two methods to expose the cores to insecticides were performed: finite- and infinite-dose methods.

Infinite-Dose Method. The infinite-dose method consisted of applying a volume of 150 μL of insecticide solution to each core (the glass vial held the insecticide solution, and the surface of the fruit exposed to the compound was equal to the internal area of the glass vial as defined above). The amount of radioactivity applied per insecticide core was 0.349 μCi for thiamethoxam, 0.308 μCi for indoxacarb, and 0.085 μCi for thiacloprid. The total amount used in the experiment was 14.658 μCi for thiamethoxam, 12.936 μCi for indoxacarb, and 3.37 μCi for thiacloprid (1 core × 7 replications × 2 apple varieties × 3 times exposure). Fruit sample cores were exposed to insecticides for 1, 6, and 24 h. Seven replicate cores were used per insecticide at each time exposure period. Samples were held at a temperature of 25 °C, with illumination at 250 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR) (PAR is in the spectral range of solar radiation from 400 to 700 nm). After the insecticide exposure period, the remaining insecticide solution was removed with a pipet and placed in a 20 mL scintillation vial. To measure the amount of non-sorbed residues, the surface cuticle of the apple cores was washed with distilled water (200 μL) and a second wash was performed for each with 150 μL of methanol. Each wash consisted of dispensing water or methanol over the core of the fruit and then removing the water or methanol with a pipet. This process was repeated 2 additional times using the same liquid fraction. Each fraction of water and methanol was separately transferred to a scintillation vial for radioactivity quantification. After the washed cores were dried at room temperature, razor blades were used to separate apple core samples into segments: the apple cuticle, outside 2 mm of hypanthium, next 2 mm of hypanthium, center 4 mm of hypanthium, and the remaining 8 ± 2 mm to the seed cavity (modified from ref 14). Blades were cleaned with acetone after each cut. Each apple segment was placed in its own envelope (Brown Kraft 2 $^1/_4$ × 3 $^1/_2$, Bloomington, IL) and immediately dried in an oven (model 1326, Sheldon Manufacturing, Cornelius, OR) for 24 h at 70 °C.

Finite-Dose Method. In the finite-dose method, the amount of radioactivity applied per insecticide on the fruit surface of each core was 0.038 μCi for thiamethoxam, 0.037 μCi for indoxacarb, and 0.013 μCi for thiacloprid. The total amount used in the experiment was 1.596 μCi for thiamethoxam, 1.554 μCi for indoxacarb, and 0.556 μCi for thiacloprid (1 core × 7 replications × 2 apple varieties × 3 times exposure). To the fruit surface of each core a 1.5 μL volume of insecticide solution was applied (three drops of 0.5 μL placed in the center 78.5 mm^2 area of the fruit surface) and then allowed to dry. There was no removal of insecticide solution, but there were two cuticle washes: first with water (200 μL) and then with methanol (150 μL), as described with the infinite-dose treatment. The number of replications, environment conditions, counting of radioactivity in water and methanol, slicing of the cores, and drying of the apple segments were conducted similarly as described in the infinite-dose treatment above.

Sample Combustion and Autoradiograms. The dried apple segments were combusted in a biological tissue oxidizer (OX-300, R.J. Harvey Instrument Corp., Tappan, NJ). The resulting $^{14}CO_2$ was trapped in a scintillation vial with 15 mL of cocktail fluid (Safety Solve, Research Product International Corp., Mount Prospect, IL). Radioactivity was measured in a liquid scintillation counter (LKB Wallac, Turku, Finland), and values were corrected for the efficiency of the oxidizer and liquid scintillation counter. Autoradiograms were taken by placing the cuticle samples face-down (inner and outer surfaces) in a phosphor screen (Amershan Biosciences, Sunnyvale, CA) after 1, 6, and 24 h treatment exposure times. The screens were scanned in a phosphorimager analyzer (Quantity One, BioRad) for 24 h of exposure, and digital images of the distribution of the ^{14}C-insecticide in the inner and outer cuticle surfaces were obtained.

Statistical Analysis. Percentage of the ^{14}C compound in apple cultivars, time of exposure, method of insecticide application, fractions, and extent of penetration were analyzed by a factorial analysis (PROC GLM, SAS, Inc., Cary, NC). Levels for each factor were apples (yellow and red), time of exposure (1, 6, and 24 h), and fractions (insecticide solution, water wash, methanol wash, and recovery from apple core tissues in the infinite-dose method and water wash, methanol wash, and apple core tissues in the finite-dose method). Normality of data was determined by inspection of normal probability plots. When necessary, data were normalized by either square root or logarithmic transformation. For both the infinite- and finite-dose experiments, data were analyzed to determine (1) the extent of penetration into fruit for each compound and cultivar over the three exposures times (1, 6, and 24 h), (2) differences in dislodgeable non-sorbed and penetrated residues between the three insecticides at 24 h, and (3) depth of insecticide penetration into the apple cuticle and hypanthium across compounds at 24 h (apple cuticle, outside 2 mm of hypanthium, next 2 mm of hypanthium, center 4 mm of hypanthium, and the remaining 8 ± 2 mm to the seed cavity). Because of limited access to radioactive material, doses applied per core were not uniform across insecticides. However, the samples were analyzed as percentage or proportional data to compare radioactivity across cultivars, over exposure times, and among non-sorbed and fruit fractions.

■ RESULTS

Infinite-Dose Experiment. No differences were seen in the penetration or distribution of any of the insecticides between the two cultivars (Red Delicious and Golden Delicious) (indoxacarb, $F = 0.17$, df = 1, and $p = 0.6791$; thiacloprid, $F = 0.11$, df = 1, and $p = 0.7380$; and thiamethoxam, $F = 0.0000$, df = 1, and $p = 0.9751$). Because there were no statistical differences in the variety effects, all sample data from Red Delicious and Golden Delicious were combined for the remaining analysis.

For infinite-dose treatment, the proportion of thiamethoxam, thiacloprid, and indoxacarb recovered varied by fraction (insecticide solution, water wash, methanol wash, and apple core: indoxacarb, $F = 1309$, df = 2, and $p = 0.0001$; thiacloprid, $F = 5141$, df = 2, and $p = 0.0001$; and thiamethoxam, $F = 7454$, df = 2, and $p = 0.0001$), time after treatment (indoxacarb, $F = 72$, df = 2, and $p = 0.0001$; thiacloprid, $F = 86$, df = 2, and $p = 0.0001$; and thiamethoxam, $F = 33$, df = 2, and $p = 0.0001$), and fractions × time (indoxacarb, $F = 107$, df = 2, and $p = 0.0001$; thiacloprid, $F = 642$, df = 4, and $p = 0.0001$; and thiamethoxam, $F = 226$, df = 4, and $p = 0.0001$). After 24 h of exposure, the significantly highest radioactivity values for the remaining insecticide solution were from the thiamethoxam treatments, followed by thiacloprid, and then indoxacarb (Figure 2A). This pattern directly follows the solubility sequence of the three compounds, with thiamethoxam being the most hydrophilic (log $K_{ow} = -0.13$), followed by thiacloprid (log $K_{ow} = 1.26$), and then indoxacarb being the most lipophilic (log $K_{ow} = 4.65$). The water wash removed more thiacloprid than indoxacarb, with the least amount being thiamethoxam. Conversely, the methanol wash removed more residues from the indoxacarb-treated fruit than either of the two neonicotinoid treatments. There were no differences in the total amount of ^{14}C-insecticide found in the apple core (combined five segments) among the three insecticides.

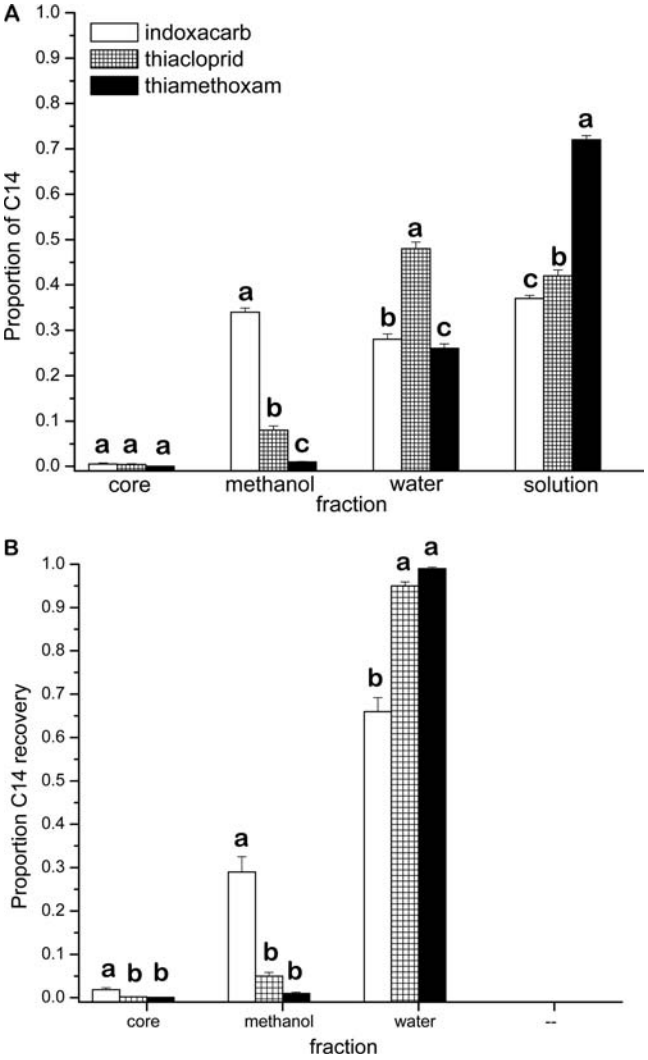

Figure 2. Proportion (mean ± SE) of ^{14}C-insecticide recovery in different fractions (A, core, methanol, water, and insecticide solution in the infinite-dose treatment; B, core, methanol, water, and insecticide solution in the finite-dose treatment) of three compounds, 24 h after treatment. Mean proportions of compounds within a given fraction sharing the same letter are not significantly different (Tukey $\alpha = 0.05$).

When the rate of penetration into fruit after three time exposures was compared, the insecticide concentration increased with time for all three compounds, with maximum percent concentrations reaching after 24 h of exposure (Figure 3A). For thiacloprid and thiamethoxam, the concentrations were significantly higher at 24 h, over those residues found after the 1 or 6 h exposure periods (thiacloprid, $F = 4.52$, df = 2, and $p = 0.0129$; and thiamethoxam, $F = 3.63$, df = 2, and $p = 0.03$). For indoxacarb, however, concentrations found in the fruit at 6 h were statistically similar to those at 24 h, while residues after 1 h of exposure were lower ($F = 3.90$, df = 2, and $p = 0.023$). The autoradiograms, although not quantitative, show substantial indoxacarb sorption into the outer cuticle surface within 1 h, with desorption at the inner surface of the cuticle delayed until 24 h (Figure 4). This pattern shows that, for indoxacarb, the initial epicuticle sorption is rapid, but diffusion across the

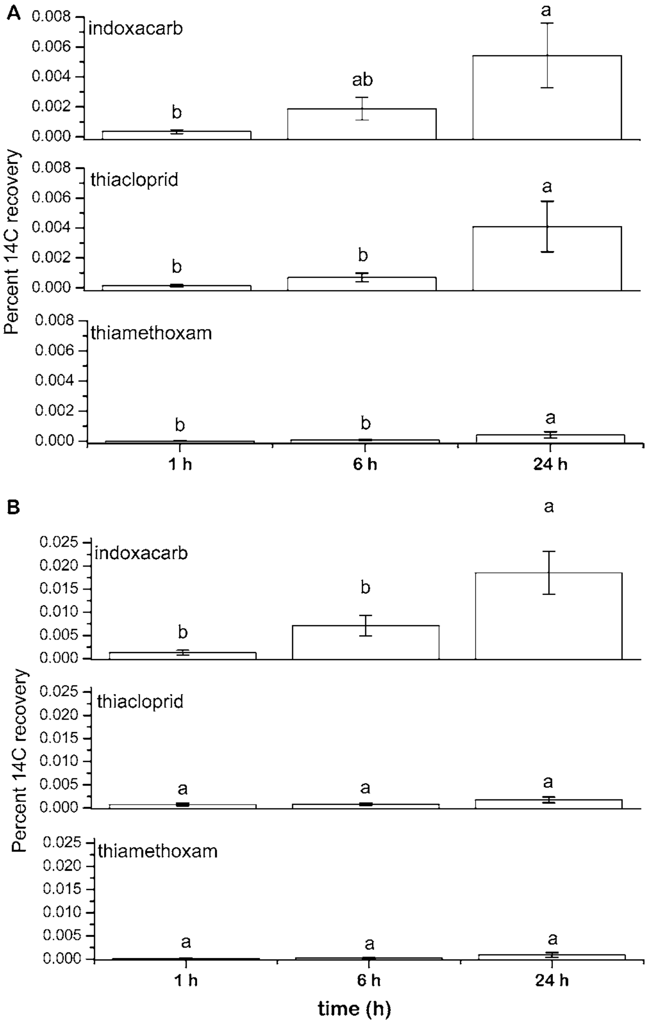

Figure 3. Percentage of ^{14}C-insecticide recovery from the apple core after different times of exposure (A, infinite-dose treatment; B, finite-dose treatment) of three compounds. Letters that are the same are not significantly different (Tukey $\alpha = 0.05$).

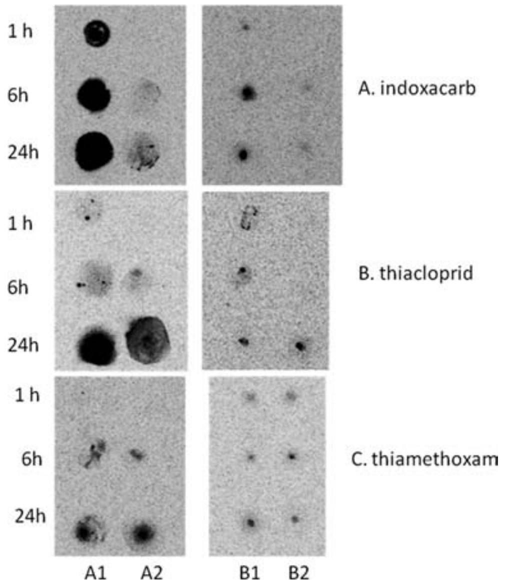

Figure 4. Sorption and desorption of ^{14}C-insecticides in the cuticle of the fruit at different times of exposure (1, 6, and 24 h) under two treatment methods: infinite-dose (A1 and A2) and finite-dose (B1 and B2). A1 and B1 are the external surface of the cuticle, and A2 and B2 are the internal surface of the cuticle.

cuticular membrane is slower, which delays desorption into apple hypanthium. For thiamethoxam and thiacloprid, the cuticular sorption is more gradual, with maximum concentrations seen at 24 h. For the neonicotinoids, however, desorption at the inner cuticle surface was first evident in the autoradiograms at 6 h and then 24 h at higher concentrations. Even though they did not enter the outer cuticle surface as readily as indoxacarb, they were transported through the membrane without the lag time seen with indoxacarb.

For individual compounds in the infinite-dose treatments, the proportion of insecticide recovered varied with the depth of penetration in the apple core (indoxacarb, $F = 18$, df = 1, and $p = 0.0001$; thiacloprid, $F = 19$, df = 1, and $p = 0.0001$; and thiamethoxam, $F = 36$, df = 1, and $p = 0.0001$), but concentrations did not vary with the length of exposure (i.e., 1, 6, and 24 h) (indoxacarb, $F = 0.01$, df = 1, and p = NS; thiacloprid, $F = 0.06$, df = 1, and p = NS; and thiamethoxam, $F = 0.06$, df = 1, and p = NS). The interaction or depth and time was not significant (indoxacarb, $F = 0.000$, df = 1, and p = NS; thiacloprid, $F = 19$, df = 1, and p = NS; and thiamethoxam, $F = 0.04$, df =1, and p = NS). When the three compounds in the depth of penetration into fruit after 24 h of treatment exposure were compared, there were no significant differences (Figure 5A).

Finite-Dose Experiment. As in the infinite-dose treatment experiment, no statistical differences were seen between the two cultivars in spatial or temporal patterns of distribution of the insecticides; therefore, data were combined for further analysis (indoxacarb, $F = 0.09$, df = 1, and $p = 0.7716$; thiacloprid, $F = 0.52$, df = 1, and $p = 0.0473$; and thiamethoxam, $F = 0.01$, df = 1, and $p = 0.9225$). For finite-dose treatments, all three compounds individually showed a significant effect by fraction (water wash, methanol wash, and apple core: indoxacarb, $F = 593$, df = 2, and $p = 0.0001$; thiacloprid, $F = 107$, df = 2, and $p = 0.0001$; and thiamethoxam, $F = 2371$, df = 2, and $p = 0.0001$) and fractions × time (indoxacarb, $F = 6.76$, df =2, and $p = 0.0001$; thiacloprid, $F = 5$, df = 2, and $p = 0.0001$; and thiamethoxam, $F = 6.67$, df = 2, and $p = 0.0001$), but only thiamethoxam and thiacloprid were significant by the interaction time after treatment (thiamethoxam, $F = 9.67$, df = 2, and $p = 0.0001$; and thiacloprid, $F = 107$, df = 2, and $p = 0.0001$). After 24 h of treatment exposure, the significantly highest amounts of non-sorbed residues were removed with the water wash from the thiamethoxam- and thiacloprid-treated fruits, followed by that from the indoxacarb treatment (Figure 2B). Conversely, there were more residues removed with the methanol wash from the indoxacarb-treated fruit than from the fruits of the two neonicotinoid treatments. There were no differences in the total amount of ^{14}C-insecticide found in the apple core between thiacloprid- and thiamethoxam-treated fruit, but the indoxacarb treatment showed significantly higher levels of ^{14}C than the two neonicotinoid compounds.

When the rate of fruit penetration after three time exposures was compared, concentrations in the fruit were highest for all three compounds after 24 h of exposure. For indoxacarb, the concentrations were significantly higher at 24 h, over those found after the 1 or 6 h time periods (Figure 3B) ($F = 8.66$, df = 2, and $p = 0.0003$). For thiamethoxam and thiacloprid, however, concentrations found in the fruits at 1 and 6 h were statistically similar to those at 24 h. The autoradiograms showed evidence of sorption into the epicuticle within 1 h for all three compounds, but desorption at the inner cuticle surface was evident only for thiamethoxam at the 1 h exposure time period (Figure 4). Indoxacarb desorption at the inner cuticle surface was visible at the 6 and 24 h exposure time periods. Thiacloprid desorption at the inner cuticle surface was seen only after 24 h of exposure but with indication of higher concentrations.

For finite-dose treatments, all three compounds individually showed a highly significant effect by depth of penetration (proportion) in the apple core but no differences were seen by time or depth × time (indoxacarb, $F = 14.08$, df = 1, and $p = 0.0001$; thiacloprid, $F = 15.6$, df = 1, and $p = 0.0001$; and thiamethoxam, $F = 23.36$, df = 1, and $p = 0.0001$).

When the depth of penetration at 24 h treatment exposure was compared, there were significantly higher proportions of thiamethoxam in the cuticle than thiacloprid and more thiacloprid than indoxacarb ($F = 19.21$, df = 2, and $p = 0.0001$) (Figure 5B). There were significantly higher proportions of indoxacarb in the first 2 mm of hypanthium than for the other two compounds ($F = 26.13$, df = 2, and $p = 0.0001$). There were no differences between the three compounds in the proportion of radioactivity in the next two segments of hypanthium. There were, however, significantly higher proportions of thiacloprid in the innermost segment of apple hypanthium than what was measured for indoxacarb ($F = 4.88$, df = 2, and $p = 0.0194$).

Recovery of radioactivity was high, and there were no significant losses (Table 1). Total radioactivity values in nanograms of ^{14}C for all three compounds showed higher recovery from infinite-dose treatment samples than from finite-dose treatment samples (Table 1). Relatively very low levels of active ingredients were detected in the core after washes, meaning that the vast majority of insecticide remained on the cuticle surface of the fruit. The percentage of recovered compound in cores for both methods ranged from 0.04 to 2.13. While a relatively similar percentage of radioactivity was recovered from the thiamethoxam cores among the two treatment methods, the percentage of indoxacarb radioactivity

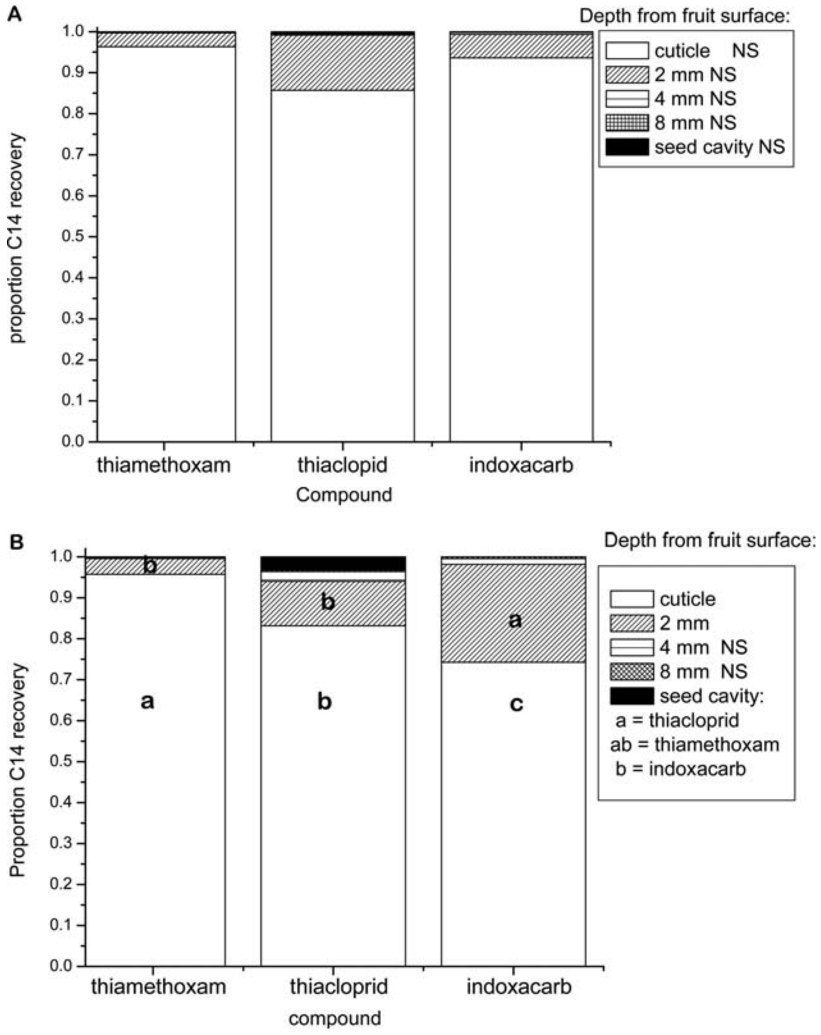

Figure 5. Percentage of ^{14}C-insecticide recovery from different segments of apple core after 24 h of exposure (A, infinite-dose treatment; B, finite-dose treatment) of three compounds. Mean proportions of compounds within a given core segment sharing the same letter are not significantly different (Tukey α = 0.05). NS = not significant.

Table 1. Nanograms of ^{14}C-Insecticide in the Different Fractions for Finite- and Infinite-Dose Treatments of the Three Different Compounds, 24 h after Treatment[a]

fraction	indoxacarb	percent of recovery (%)	thiacloprid	percent of recovery (%)	thiamethoxam	percent of recovery (%)
			Finite-Dose Treatment (ng of ^{14}C/Fraction)			
water	41.99	68.00	118.56	95.00	632.33	99.00
methanol	18.62	30.00	6.34	5.00	7.72	1.21
core	1.32	2.13	0.19	0.15	0.49	0.08
			Infinite-Dose Treatment (ng of ^{14}C/Fraction)			
solution	1381.40	37.00	347.00	43.00	4335.60	72.00
water	1045.30	28.00	397.66	49.00	1587.40	27.00
methanol	1279.10	34.00	66.94	8.00	57.14	1.00
core	21.58	0.58	3.20	0.39	2.38	0.04

[a] Solution fraction, solution of ^{14}C-insecticide removed from the infinite-dose treatment; water fraction, first rinse of the cuticle of the apple with 150 μL of water; methanol fraction, second rinse of the apple cuticle with 150 μL of methanol; and core, combined total amount of ^{14}C-insecticide in all sliced segments of the apple core.

recovered from finite-dose treatment was 4-fold higher than the corresponding infinite-dose treatment. Thiacloprid was the only compound where higher percentages were found in fruits under infinite-dose conditions, although only 2-fold higher than what was recovered in the finite-dose treatment. These data indicate that the patterns of wet and dry field conditions over a given season can result in differential levels of insecticide penetration after a spray.

DISCUSSION

The results of this study provide important insights into the fruit penetrative characteristics of insecticides in relation to apple cultivar, treatment condition, and active ingredient plus formulated material. Insecticide absorption into fruit was similar between the two apple cultivars included in the study. This was somewhat unexpected given differences in cuticle characteristics but is also reassuring as it relates to regulatory policy. One key similarity across all three compounds and both treatment conditions is that over 97% of treatment solution was found on the fruit surface as some form of non-sorbed residue, as opposed to remaining in the fruit tissue. There were some important differences seen in the nature of the non-sorbed residues between the three compounds. For the two neonicotinoid insecticides, thiacloprid and thiamethoxam, non-sorbed residues were primarily in water-extracted forms. For indoxacarb, the non-sorbed residues included water-extracted forms but comparatively more residues were removed with methanol than for the neonicotinoids. This follows the higher water solubility of the neonicotinoid insecticides compared to indoxacarb. It also suggests, however, that neonicotinoid surface residues on apple fruit may be more susceptible to wash-off from precipitation. The methanol-extracted indoxacarb may also represent the portion of residues that are embedded in epicuticular wax and remain bioavailable for transcuticular transfer to target insects on the fruit surface. This transcuticular bioavailability is viewed as a key delivery mechanism for indoxacarb activity on insects, such as the plum curculio *Conotrachelus nenuphar* Herbst.[12] Chowdhury et al.[7] similarly found increased insecticidal activity with lipophilic compounds when insects were exposed to leaves with higher levels of cuticular waxes.

For the small proportion of residues found to penetrate the fruit, over 74% were found in the fruit cuticle for all three compounds and both dosing systems. The majority of remaining residues were found in the first 2 mm of hypanthium below the cuticle. For all three compounds under the infinite-dose treatments, approximately 84% of fruit residues were found in the fruit cuticle and no residues were detected beyond 4 mm depth. In the context of the limited fruit-penetrated residues, finite-dose treatments resulted in somewhat different penetration profiles than the infinite-dose treatment. For indoxacarb, whereas less than 6% of core-penetrated (cuticle + hypanthium) residues were found in the hypanthium under infinite-dose conditions, under finite-dose treatments, the percentage of residues found in the hypanthium was nearly 25%. This suggests that finite-dose conditions are a more favorable environment for indoxacarb transport. For thiacloprid, whereas infinite-dose treatment resulted in 15% of core residues in the fruit hypanthium, with most being in the first 2 mm of fruit flesh, under finite-dose treatment conditions, nearly 10% of the residues penetrated the first 2 mm of apple hypanthium and 3% reached the seed cavity segment. This penetration profile is similar to that documented in field-based studies and is credited for the curative activity of thiacloprid on insects post-infestation.[12,14,15] The thiamethoxam penetration profiles for finite- and infinite-dose treatments were very similar, suggesting that overall fruit penetration is not affected by the form of treatment. Under finite-dose conditions, however, the autoradiogram indications of rapid desorption may be a result of differences in the concentration gradient of the two exposure methods.

Results from the rate of penetration experiments show for indoxacarb that sorption into the epicuticle is rapid but desorption into the fruit hypanthium is delayed. This pattern of fruit cuticle mobility is consistent with that described for a lipophilic pathway.[22] The observed lag time for indoxacarb may also be a function of the greater sorption capacity in the cuticle membrane, thus delaying desorption into the hypanthium. The neonicotinoids behave differently, where initial cuticular sorption is slower, but there is no such delay as they desorp into the hypanthium. This pattern of cuticle penetration resembles that for materials that preferentially use a polar pathway, but it is not clear whether these neonicotinoids with molecular weights ranging from 252 to 291 g mol^{-1} would be smaller than the exclusion limit of a polar pathway in the apple exocarp.[18,24] In addition, polar pathways have not yet been described for apple fruit. Conversely, the more limited cuticular sorption of thiamethoxam seen under the infinite-dose supports the partitioning conditions of a lipophyllic pathway route, thus a relative tendency of this compound to stay in aqueous solution. Isolated cuticle studies, using test systems that ensure maximum driving force, are needed for confirmation of the physical processes involved. For both of the neonicotinoids in our study, the log *p* is expected to have been a dominating influence in the desorption process.

We must address the question of what possible influence the addition of the formulated product to the radiolabeled compounds may have had on the results of the study. We included the formulated material because we wanted the study to reflect "real world" conditions as much as possible. Indoxacarb and thiamethoxam are formulated as water-dispersible granules (WGs), also known as dry flowables. The WG formulation contains a toxicant, dispersant, binder, and diluents, and once in the spray tank, the granules disintegrate and disperse in water for sprayer application. Thiacloprid is formulated as a flowable (F), also known as a suspendable concentrate. The F formulaton suspends a very fine wettable powder toxicant in water, along with various surfactants and additives. Because indoxacarb and thiamethoxam are the same formulation, we do not expect that any significant treatment effects were influenced by the formulation. It is impossible to determine if the flowable formulation of thiacloprid caused comparatively greater or lesser penetrative mobility than the WG formulation of the other two insecticides. Insecticide formulations are developed primarily with product manufacturing and packaging, safety for pesticide handlers, and reliability in field application for the farmer in mind. A farmer will typically add an adjuvant to the spray solution if further penetrative attributes are desired. Thus, we believe that the results of this study predominantly reflect the active ingredients tested.

The results of this study confirm the general patterns of insecticide penetration in apple fruit documented in descriptive field studies.[14,32] In one field study on late season apples, thiacloprid showed higher proportions of penetrated residues in the inside-to-core section of hypanthium, resulting in greater curative activity on apple maggot, *Rhagoletis pomonella* (Walsh), larvae post-infestation.[14] The incidence of indoxacarb residues in apples were shown to be more concentrated in the cuticle and outside 2 mm of hypanthium adjacent to the cuticle. Several field studies have shown thiamethoxam fruit penetration to be more limited, especially when high-performance liquid chromatography (HPLC) detection excluded data associated with clothianidan as a metabolic byproduct. Our laboratory results are consistent with the penetrative patterns seen in these descriptive field studies. Field studies, however, are always subjective to period-specific weather conditions, which cannot be perfectly replicated in the laboratory, and can influence the observed outcomes.

It is worth noting that, under operational field conditions, the transport of insecticide residues into fruit would not likely be limited to a 24 h period. Even though environmental degradation would gradually reduce the surface residues available for cuticular sorption, the persistence of most insecticides would ensure an active source for at least several days.[8] The majority of cuticle penetration, on a temporal basis, will occur under conditions resembling finite-dose conditions compared to an infinite-dose model. The infinite-dose treatment conditions demonstrated in this study would be extremely limited under typical orchard environmental conditions because most insecticide sprays dry within minutes and only rarely would wet conditions from precipitation persist for 24 h. Canopy wetting and redrying resulting from fog and dew are more common but with durations only for 1−3 h in most cases. Nonetheless, these repeated episodes of wetting and drying may be important in reactivating the sorption process. Given regional variability in weather conditions, particularly the incidence of precipitation, dew, and fog, insecticide penetration patterns can be expected to vary depending upon where apples are grown.

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