ABSTRACT
Residue profile analysis techniques were developed, along with laboratory and field-based bioassays to describe the modes of insecticidal activity responsible for the control of the plum curculio, *Conotrachelus nenuphar* (Herbst), in apples (*Malus* spp.). Adult plum curculios were treated in laboratory topical bioassays to determine acute contact activity and lethal time for five insecticides. Azinphosmethyl had the highest levels of toxicity and shortest lethal time values, followed by the neonicotinoids thiacloprid, thiamethoxam, and imidacloprid, whereas indoxacarb had the highest LD$_{50}$ and LT$_{50}$ values for topical exposure. Field-based residual activity bioassays assessed adult mortality, and fruit and leaf injury from plum curculio exposed to 4 h, 7 d, and 14 d field-aged residues. All compounds caused significant levels of mortality to plum curculio when adults were exposed to fruit clusters 4 h post-application. Thiacloprid, thiamethoxam, and imidacloprid showed oviposition deterrence, antifeedant, and repellency effects in the 7- and/or 14 d residual bioassays and protected fruit in the absence of significant lethal activity. Indoxacarb maintained lethal activity throughout the study intervals, with the incidence of plum curculio feeding, suggesting that ingestion is an important mode of entry. For the neonicotinoids thiacloprid, thiamethoxam and imidacloprid plum curculio mortality was highly correlated with fruit and leaf surface residues. As surface residues declined, sublethal effects such as oviposition deterrence and antifeedant effect remained. The value of the plant–insect–chemistry triad model for describing the temporal dimensions of insecticidal modes of activity and understanding a compound’s critical performance characteristics is discussed.

KEY WORDS *Conotrachelus nenuphar*, apple, residue profile, plant–insect–chemistry triad, sublethal effects

The plum curculio, *Conotrachelus nenuphar* (Herbst), is a native beetle that occurs east of the Rocky Mountains (Chapman 1938) and is a key pest of cultivated pome and stone fruits in eastern and central North America (Racette et al. 1992, Yonce et al. 1995). In the spring, female plum curculios make crescent-shaped oviposition scars in the developing fruit, and larvac feed internally on the fruit flesh. Adult plum curculios also damage fruit by making a small puncture hole in the skin and then eating the flesh underneath the skin. Internal feeding damage can render the fruit unmarketable, as does the scar tissue resulting from oviposition and adult feeding. Developing larvac tunnel in the fruit and have been reported to cause fruit abscission (Levine and Hall 1977); however, it is difficult to determine effects on yield in apple because curculio-induced fruit abscission occurs at the same time as June drop. Left unchecked, the plum curculio can cause significant damage in apple (*Malus* spp.) orchards in a short time. In early studies of plum curculio damage potential in apple, Stedman (1904) found that a single female curculio can lay between 200 – 400 eggs and make about twice as many oviposition scars. Prokopy et al. (1993) found that plum curculio can cause economic levels of injury in a 12-h period; control measures should be well timed and broadly effective to protect the crop.

Over the past 50 yr, plum curculio control has been based almost entirely on organophosphate insecticides, primarily azinphosmethyl (Guthion, Bayer CropScience, Research Triangle Park, NC) and to a lesser extent phosmet (Imidan, Gowan Company, Yuma, AZ). In recent years, Michigan apple growers have effectively prevented plum curculio injury with two to three applications of organophosphate insecticides. The effectiveness and availability of organophosphates (OPs) have resulted in limited perceived need for research on alternative control measures. However, as a result of the Food Quality Protection Act (FQPA) (FQPA 1996), apples grown east of the Mississippi are now limited to a total of 1.8 kg (4.0 lb) active ingredient (AI) /acre of azinphosmethyl per year, leaving growers with three to four applications to cover a multitude of key pests throughout the season. Continued use of azinphosmethyl is subject to regul-
latory review, and this product may in the future become unavailable to growers.

Since the implementation of FQPA there have been a series of new reduced-risk and OP-alternative insecticides that show promise for plum curculio control. The neonicotinoid compounds Actara (thiamethoxam), Calypso (thiacloprid), and oxadiazine Avaunt (indoxacarb) have all shown significant levels of control in field efficacy trials on apple (Wise and Gut 2000). Although such field trials are encouraging in terms of showing basic fruit protection over prescribed intervals, they do not provide much information on the basis of the insecticide’s performance. Optimizing the performance of a compound requires understanding the mode of exposure, field residual, and modes of insecticidal activity that are responsible for preventing the pest from injuring the crop. Recent research on compounds in the neonicotinoid insecticide class suggests that sublethal modes of activity (i.e., antifeedant, oviposition deterrence) can make an important contribution to their overall performance (Hu and Prokopy 1998, Kunkel et al. 2001, Liburd et al. 2003, Isaacs et al. 1999, Nauen 1995). In addition, neonicotinoid insecticides are known to have unique chemodynamic interactions with the plant, which can influence insecticide exposure to the pest (MacDonald and Meyer 1998, Wannhoff and Schneider 1999). The results of these studies suggest that the spatial and temporal dimensions of the plant–insect–chemical (PIC) interaction may be important in fully understanding the overall performance of a pest management tool.

In this study, we combined residue profile analysis with field and laboratory bioassays to describe the modes of insecticidal activity that are responsible for the control of the plum curculio. The four organophosphate-alternative insecticides included in the study were the neonicotinoids imidacloprid, thiacloprid, and thiamethoxam and the oxadiazine indoxacarb, with the organophosphate azinphosmethyl as the standard comparison. The objectives of this study were to 1) characterize the lethal activity of these compounds in terms of lethal dose and lethal time, 2) identify the nonlethal modes of insecticidal activity that contribute to performance and the temporal dimension of each, and 3) describe the relationship between surface and interior residues on the plant and the lethal activity of insecticides on the plum curculio.

**Materials and Methods**

**Insect Material.** **Northern Strain.** For trials evaluating the behavior of plum curculio, northern (diapausing) strain insects were collected in May and June 2001 with beating trays and pyramid traps (Tedders and Wood 1994) placed in orchards at the Michigan State University Trevor Nichols Research Complex. Beetles were collected 4–7 d before trial use and held together at 25°C at a photoperiod of 16:8 (L:D) h to provide time for mating. Females were sorted from males 24 h before study initiation.

**Southern Strain.** To improve consistency of results, beetle age, and history were standardized for bioassays. For these trials, southern (nondiapausing) strain weevils were used from a continuous colony at Michigan State University. This colony has been in production since 1998 with occasional additions from collaborators from the southeastern United States. Adults were reared on immature apples for food and oviposition substrate (modified from Smith 1957) and held at 25°C at a photoperiod of 16:8 (L:D) h. Larvae were placed in soil for pupation, and adults were collected in traps above these pupation containers.

**Laboratory Topical Toxicity Bioassay.** Topical bioassays were used to determine the baseline toxicity of imidacloprid (Bayer CropScience, Research Triangle Park, NC), thiacloprid (Bayer CropScience), thiamethoxam (Syngenta, Greensboro, NC), indoxacarb (DuPont, Wilmington, DE), and azinphosmethyl (Bayer CropScience) to plum curculio adults. Technical-grade material was serially diluted with acetone (98.7% purity) to eight concentrations (31,600, 10,000, 3,160, 1,000, 316, 100, 31.6, and 10 μg [AI]/ml). Adult southern strain beetles were used (age 1–2 wk post-eclosion). Beetles were treated with 1 μl of each solution on the dorsal surface of the abdomen applied with repeating dispenser (Hamilton PB600-1) equipped with a 50-μl beveled point syringe (Hamilton, Reno, NV). Beetles in the check were treated with acetone only. The actual doses of active ingredient applied to beetles were 31.6, 10, 3.16, 1, 0.316, 0.1, 0.0316, and 0.01 μg (AI) per beetle. After treatment, beetles were placed in a 100- by 15-mm polystyrene disposable sterile petri dish (VWR, West Chester, PA) lined with a Whatman no. 2 filter paper (Maidstone, England). Immature ‘Empire’ apples (∼25 mm in diameter) were sliced into 10-mm-thick sections placed inside each of the petri dishes as a food source. Apples were replaced daily. Petri plates were randomly labeled for blind readings. Mortality was recorded at 3, 6, 12, 24, 48, 72, and 120 h after application to determine lethal dose and lethal time aspects of toxicity. Each replicate contained 10 beetles, with three replications per treatment.

Mortality data were corrected for natural mortality by using Abbott’s formula (Abbott 1925). The LC50, fiducial limits, slope ± SE, and chi-square goodness-of-fit values were determined using probit analysis (SAS Institute 2002). Relative toxicity of each compound to azinphosmethyl was calculated by dividing the LD50 value of the compound by that of azinphosmethyl. Percentage of mortality data also were arcsine-transformed for analysis of variance (ANOVA) (Sokal and Rohlf 1994).

**Field Trials.** Plots consisted of two 9-yr-old ‘Gala’ apple, Malus domestica Borkhausen, trees. The experimental design was a randomized complete block with four replications. Tree spacing was 5.5 by 6.1 m, with one buffer tree and one buffer row separating all plots. Regular maintenance applications of fungicides were applied to all treatment blocks. Insecticide treatments were applied with an FMC 1029 airblast sprayer calibrated to deliver 935 liters/ha (100 gpa). Applications
were made on 17 May (petal fall + 3 d). A second application was made on 31 May for the efficacy trial assessment. Fruit and foliage from one tree per sprayed and untreated check plot were collected for use in residue profile analysis and the field-based bioassays. Fruit on the second tree of each plot were used for the efficacy trial.

Treatment compounds, formulations, and rates used for the applications were imidacloprid (Provado 1.6 F, Bayer CropScience) 111.83 g (AI)/ha (8 fl oz/acre), thiacloprid (Calypso 4 F, Bayer CropScience) 105.08 g (AI)/ha (3 fl oz/acre), thiamethoxam (Actara 30 WG, Syngenta) 94.57 g (AI)/ha (4.5 oz/acre), and indoxacarb (Avaunt 30 WG, DuPont) 126.09 g (AI)/ha (6.0 oz/acre). A nonionic spreader sticker, Kinetic (Helena Chemical Co., Collierville, TN) was added to the Avaunt formulation per manufacturer’s recommendation. This organophosphate azinphosmethyl (Guthion 50W, Bayer CropScience) 1,120.84 g (AI)/ha (2 lb/acre) was added to this set treatments for the field efficacy trial and bioassays related to residue profile analysis.

Field Efficacy Trial. As a measure of the overall field performance of insecticides, apples were evaluated for oviposition damage on 15 June 2001 (15 d after final treatment application). For evaluation, fruit were randomly harvested (100 fruit per plot, 400 fruit per treatment), and the number of fruit with oviposition stings was recorded. Mortality and/or moribund was defined as beetles that were dead or rendered unable to recover or function normally after being held over a 24-h period. Beetles were counted as alive if they seemed to function normally. Statistical comparisons (by date) were made using ANOVA (PROC GLM, SAS Institute 2002). Incremental behavior observations were made on beetles after 1, 6, 12, 24 and 96 h of exposure in the bioassay arenas for each of the three residual bioassays (4 h, 7 d, and 14 d postinsecticide application). Beetles were observed for 1 min at each time interval, and data were recorded on the position and/or activity of beetles, categorized as follows: beetle on leaf/stem, beetle feeding on fruit, beetle ovi-position in fruit, beetle still on fruit, beetle alive but not on plant, beetle twitching on bottom, and beetle dead/still on bottom. “Still” beetles were not manipulated to determine mortality so as to not disturb the ongoing experiment. Statistical comparisons were made for each treatment to the untreated check by using Dunnett’s test (Kerchove 2005).

Residual Activity Bioassays. Field-based bioassays were used to evaluate the modes of insecticidal activity that contribute to performance and the temporal progression of those modes as treatment residues age. Fruit clusters were collected from field treated trees 4 h after the applications and again 7 and 14 d later. Each shoot was pruned to have 10 fruit and 10 leaves and placed in water-soaked OASIS floral foam (Smithers-Oasis Co., Kent, OH) in clear plastic 946-ml containers (Fabri-Kal, Kalamazoo, MI) with lids. The foam was covered with sealing wax (Gulf Wax, distributed by Royal Oak Sales, Inc, Roswell, GA) to preserve the integrity of the fruit and foliage. Holes were punched in each container’s lid to reduce condensation of water vapor inside the container and minimize fumigation effects, if any. Each of these containers was considered an experimental unit in the bioassay.

Northern strain adults were collected, held, and sexed 24–48 h before each bioassay. Five females were placed in the bottom of each experimental arena. There were four replicates for each treatment at each of the post-application time intervals. The numbers of living and moribund beetles were recorded after 96 h of exposure. The numbers of fruit oviposition stings, fruit feeding marks, and leaf-feeding marks also were recorded. Mortality and/or moribund was defined as beetles that were dead or rendered unable to recover or function normally after being held over a 24-h period. Beetles were counted as alive if they seemed to function normally. Statistical comparisons (by
age and physiological condition in a laboratory colony. Females used in bioassays were 1–2 wk post-eclosion and were exposed to males to assure mated status. Ten female beetles were used in each experimental unit, with four replicates per treatment at each of the three postapplication time intervals (4 h, 7 d, and 14 d). Beetles were examined after 96 h of exposure to field treated fruit clusters (see bioassay container description above), and a final count was made of moribund beetles, along with the amount of leaf feeding and oviposition damage. For each treatment compound, regression (PROC GLM, SAS Institute 2002) was used to determine correlates between curculio mortality and type of residue (surface and interior leaf residues, surface and interior fruit residues).

**Results**

**Laboratory Topical Toxicity Bioassay.** Azinphosmethyl was the most toxic compound to plum curculio adults in the topical assays, with an LD$_{50}$ value of 0.16 µg per beetle (160 ppm) (Table 1). Thiacloprid and thiamethoxam were less toxic, but their 95% confidence intervals overlapped with azinphosmethyl. Imidacloprid followed thiacloprid and thiamethoxam in toxicity, and indoxacarb was the least toxic of the insecticides tested in the topical bioassays.

Time to 50% mortality (LT$_{50}$) is shown in Table 2. Guthion had the most rapid lethal activity with an LT$_{50}$ value of 0.76 h at 10 µg (AI) per beetle (highest dose). Imidacloprid had lethal activity with a calculated LT$_{50}$ value of 18.27 h at 10 µg (AI) per beetle dose. Thiamethoxam and thiacloprid had LT$_{50}$ values of 39.25 and 43.99 h, respectively, at 10 µg (AI) per beetle. Indoxacarb showed the slowest activity having an LT$_{50}$ value of 114 h at 10 µg (AI) per beetle.

**Field Trials. Field Efficacy Trial.** Untreated check trees had an average of 43.0% of fruit damaged from plum curculio oviposition. All treatments except imidacloprid provided significant levels of fruit protection compared with the untreated check ($F = 4.25$, df = 18, $P = 0.01$) (Table 3). Imidacloprid-treated trees had significantly more damage than thiacloprid and thiamethoxam but not significantly different from the other two compounds.

**Residual Activity Bioassays.** Azinphosmethyl could not be included in the treatment list because of the limited numbers of northern strain beetles collected from the field. Plum curculio adults exposed to 4-h post-application chemical residues resulted in significantly higher levels of mortality for all treatment compounds than the untreated check ($F = 3.16$, df = 15, $P = 0.045$) (Table 4). The incidence of fruit oviposition and fruit and leaf feeding was limited significantly for all compounds compared with the untreated check (oviposition: $F = 66.794$, df = 15, $P = 0.0001$; fruit feeding: $F = 31.304$, df = 15, $P = 0.0001$; and leaf feeding: $F = 25.733$, df = 15, $P = 0.0001$). In fruit feeding, thiacloprid, thiamethoxam, and indoxacarb provided better fruit protection than imidacloprid.

For plum curculio adults exposed to 7-d-old residues, only indoxacarb caused a significant level of mortality ($F = 12.84$, df = 15, $P = 0.0001$). The incidence of fruit oviposition and fruit feeding, however, was significantly reduced for all compounds compared with the untreated check (oviposition: $F = 10.06$, df = 15, $P = 0.0004$; and fruit feeding: $F = 7.929$, df = 15, $P = 0.0012$). In fruit oviposition, thiacloprid and indoxacarb maintained the lowest levels, followed by imidacloprid and thiamethoxam. For the neonicotinoid compounds, the reduced feeding and oviposition in the presence of predominantly live beetles in the canopy suggests oviposition deterrence and antifeedant modes of activity at work.

For plum curculio adults exposed to 14-d-old residues, only indoxacarb caused a significant level of beetle mortality ($F = 8.06$, df = 15, $P = 0.0011$). By day

**Table 1. Probit analysis (lethal dose) after 120 h for five chemicals applied to southern strain plum curculio**

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Slope ± SE</th>
<th>LD$_{50}$ (95% FL)</th>
<th>$\chi^2$</th>
<th>Toxicity ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azinphosmethyl</td>
<td>30</td>
<td>2.89 ± 0.83</td>
<td>0.16 (0.06–0.53)</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>30</td>
<td>0.91 ± 0.13</td>
<td>3.67 (2.05–8.31)</td>
<td>&lt;0.001</td>
<td>0.943</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>30</td>
<td>0.92 ± 0.11</td>
<td>0.255 (0.17–0.46)</td>
<td>&lt;0.001</td>
<td>0.561</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>30</td>
<td>1.30 ± 0.23</td>
<td>0.68 (0.28–1.85)</td>
<td>&lt;0.001</td>
<td>0.235</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td>30</td>
<td>1.48 ± 0.26</td>
<td>21.7 (13.8–43.7)</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
</tbody>
</table>

$^a$ Toxicity relative to azinphosmethyl.

**Table 2. Probit analysis results for lethal time (hours) at 10 µg [AI] per beetle**

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Slope ± SE</th>
<th>LT$_{50}$ (95% FL)</th>
<th>$\chi^2$</th>
<th>Toxicity ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azinphosmethyl</td>
<td>30</td>
<td>1.98 ± 0.95</td>
<td>0.76 (9.49 × 10$^{-1}$–1.95)</td>
<td>&lt;0.001</td>
<td>0.041</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>30</td>
<td>0.55 ± 0.17</td>
<td>18.27 (10.55–29.80)</td>
<td>&lt;0.001</td>
<td>0.017</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>30</td>
<td>2.37 ± 0.46</td>
<td>43.99 (27.18–78.25)</td>
<td>&lt;0.001</td>
<td>0.019</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>30</td>
<td>1.41 ± 0.39</td>
<td>30.25 (16.43–208)</td>
<td>&lt;0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Indoxacarb$^b$</td>
<td>30</td>
<td>9.07 ± 2.18</td>
<td>114 (103–132)</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td>30</td>
<td>7.63 ± 1.57</td>
<td>107 (96–124)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

FL, fiducial limit.

$^a$ Toxicity relative to azinphosmethyl.

$^b$ 3.16 µg (AI) per beetle.
14, under the no-choice bioassay conditions, there were no significant reductions in oviposition injury for any treatments ($F = 2.256, df = 15, P = 0.1082$). Thiacloprid and indoxacarb significantly reduced leaf feeding, whereas thiamethoxam reduced fruit feeding compared with the untreated check (leaf feeding: $F = 3.92, df = 15, P = 0.0225$; and fruit feeding: $F = 4.058, df = 15, P = 0.02$).

Behavioral observations of northern strain plum curculio adults in the 4-h field-aged bioassays (evaluations made after 1, 6, 12, 24, and 96 h of beetle exposure) resulted in documented patterns of behavior and/or canopy positions that were abnormal in contrast to the beetles in the untreated check (Fig. 1). The most pronounced abnormal behavior occurrence throughout the five observation periods of the 4-h bioassay setup was that of beetles being dead/still on bottom of the bioassay chamber. This condition was significantly different from the check for thiacloprid in all five observations periods, for imidacloprid and thiamethoxam in four of the five periods, and for indoxacarb for two of the periods (1-h exposure: $F = 2.55, df = 15, P = 0.0824$; 6-h exposure: $F = 7.69, df = 15, P = 0.0014$; 12-h exposure: $F = 11.00, df = 15, P = 0.0002$; 24-h exposure: $F = 12.27, df = 15, P = 0.0001$; and 96-h exposure: $F = 4.49, df = 15, P = 0.013$). The beetles in the three neonicotinoid bioassays reached their mortality midpoint after 6–12 h of exposure, whereas beetle mortality in the indoxacarb treatment progressed very slowly until the 96-h observation (particularly when including the prelethal “twitching” condition included in the moribund measures). The mortality–exposure patterns observed in these field-based bioassays are relatively consistent with the LT50 values produced from the topical bioassay study (Table 2), reflecting the speed of activity differences between the compounds. There were only two occasions of significantly higher levels of beetles twitching on bottom, one occasion being in the thiacloprid treatment at the 1-h observation and the other occasion being in the indoxacarb treatment at the 96-h observation (1-h exposure: $F = 4.25, df = 15, P = 0.017$; and 96-h exposure: $F = 3.03, df = 15, P = 0.0001$). Generally, the beetles “twitching on bottom” eventually died, although for imidacloprid and thiacloprid in the

### Table 3. Percentage of fruit with oviposition injury from plum curculio in a field efficacy trial

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Product rate (g [AI]/ha)</th>
<th>% fruit injury (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated check</td>
<td>43.0 (14.7)a</td>
<td>43.0 (14.7)a</td>
</tr>
<tr>
<td>Azinphosmethyl</td>
<td>1,120.84 7.0 (3.1)bc</td>
<td>1,120.84 7.0 (3.1)bc</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>111.83 26.0 (7.6)ab</td>
<td>111.83 26.0 (7.6)ab</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>105.08 5.5 (1.7)c</td>
<td>105.08 5.5 (1.7)c</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>94.57 4.5 (2.6)c</td>
<td>94.57 4.5 (2.6)c</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td>126.09 8.5 (5.4)bc</td>
<td>126.09 8.5 (5.4)bc</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different ($P < 0.05$; least significant difference [LSD]).

* Insecticide applications made on 17 and 31 May, delivered with 935 liters/ha water.

** Raw data square root transformed [SQR(X + 0.5)] before ANOVA. Untransformed means shown for comparison.
6-h exposure observation there seemed to be beetle recovery before the 12-h period. There were fewer beetles observed ovipositing in fruit at the 6- and 12-h observations for all compounds, except not imidacloprid at the 12-h period (6-h exposure: $F_{15} = 112.39$, $P = 0.0001$; and 12-h exposure: $F_{15} = 4.47$, $P = 0.014$). Similarly there was a lower incidence of beetles observed feeding on fruit at the 1- and 24-h periods for all compounds (1-h exposure: $F_{15} = 7.56$, $P = 0.0015$; 24-h exposure: $F_{15} = 6.75$, $P = 0.0026$).

Behavioral observations for the 7-d bioassay setup documented some noteworthy changes in behavior and/or canopy position patterns (Fig. 1). The most dramatic pattern change from the 4-h bioassay was in the incidence of beetles with a dead/still condition. There was little or no lethal response to exposure to imidacloprid, thiacloprid, and thiamethoxam in the 7-d bioassay setup. Even so, on two occasions (1- and 6-h observation periods), there were significantly fewer beetles observed ovipositing in fruit (1-h exposure: $F = 7.56$, $P = 0.0015$; 24-h exposure: $F = 6.75$, $P = 0.0026$).

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Behavioral observations for the 14-d field-aged bioassays documented far fewer instances of abnormal behaviors and/or canopy position patterns. At the 1- and 6-h periods for all insecticide treatments there were still fewer beetles observed ovipositing in fruit than in the untreated check (1-h exposure: $F = 14.49$, $df = 15$, $P = 0.0001$; and 6-h exposure: $F = 9.0$, $df = 15$, $P = 0.0006$). At the 6-h observation thiacloprid and thiamethoxam treatments also had significantly lower incidences of beetles feeding on fruit, indicating some antifeedant activity (6-h exposure: $F = 6.0$, $df = 15$, $P = 0.0003$). Imidacloprid again showed a higher number of beetles alive but not on plant, suggesting repellency activity (96-h exposure: $F = 5.20$, $df = 15$, $P = 0.0078$).

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Residue Profile Analysis. The residue profile analysis shows azinphosmethyl to have predominantly surface residues on fruit and leaves, with total active ingredient declining over time (Fig. 2). The more dramatic decline in fruit residues between the 4-h and 7-d samples compared with leaf residues is probably related to the growth dilution effect of the rapidly expanding fruit tissue at this time of the growing season (Borchert et al. 2004). The residue profiles of imidacloprid and thiamethoxam are similar in that
large proportions of their active ingredient move into fruit and leaf tissue within 4h of the field application, and surface residues diminished within the first 7d of field exposure. Thiacloprid was seen to have more persistent surface residues on fruit and leaves, with some penetration observed into fruit. Indoxacarb was seen to have relatively equal proportions of active ingredient on the leaf surface as in or below the leaf cuticle, and the residue pattern remained stable over time. On fruit, there were higher proportions of indoxacarb on the fruit surface than within the fruit.

Residue Correlation Bioassays. The regression \( r^2 \) correlations of fruit (surface, internal) and leaf (surface, internal) residues and plum curculio adult mortality (southern strain beetles) showed several significant relationships. For azinphosmethyl the strongest significant relationship was between surface leaf residue and mortality (\( R^2 = 0.8379, \) model significant at \( \alpha = 0.001 \) \( \)) (Table 5). This suggests that the contact exposure of plum curculio adults to azinphosmethyl leaf surface residues in the tree canopy is the most important contributor to mortality as the primary mode of activity for this compound. For thiamethoxam and thiacloprid, there were strong significant relationships between mortality and leaf and fruit surface residues (\( r^2 \) values >0.70, model significant at \( \alpha = 0.001 \)) as well as internal leaf residue for thiamethoxam. Imidacloprid showed a similar pattern of correlation for surface and internal leaf residue and surface fruit, except that the relationships between residue and mortality were generally weaker (\( r^2 \) values >0.60, model significant at \( \alpha = 0.05 \)). This suggests that plum curculio contact exposure to the neonicotinoid surface residues on leaf and fruit are responsible for the important lethal mode of activity seen in the first week after application. In the original indoxacarb data set, no correlations were found to be significant for any of the plant residue profiles. There was one mortality data rep in the bioassay that is strongly suspected to be an outlier, but no clear explanation for its occurrence can be determined. When that data rep is eliminated and the data set reanalyzed, several noteworthy correlation relationships emerge. With the corrected indoxacarb data there were significant relationships with mortality for surface and internal leaf and fruit residues (\( r^2 \) values >0.50, model significant at \( \alpha = 0.05 \)), the internal residues being generally stronger than the surface residues. The significant relationship between internal residues and mortality suggests that exposure through ingestion may be an equally important contributor to its lethal activity as contact with surface residues. This is supported by the feeding patterns documented in the behavior observations in the field-based bioassays.

Discussion

The objective of this research was to identify the modes of insecticidal activity critical to each compound’s performance and to describe the spatial and temporal dimension of these control mechanisms. To do this we used a spectrum of evaluations, including
laboratory topical bioassays to determine acute contact activity and lethal time; field-based residual bioassays to identify lethal and sublethal modes of activity contributing to adult control and fruit protection; behavioral observations to identify temporal occurrences of mortality, repellency, oviposition deterrence, and antifeedant effects; and residue profile analysis to understand the contribution of fruit and leaf surface and internal residues to these lethal and sublethal modes of insecticidal activity. No one form of evaluation alone can provide sufficient information to fully describe the natural interactions between the plant, insect and chemical. Rather, each research component reveals a critical and complementary piece of the puzzle. We are convinced that this PIC model of investigation is noteworthy and can be applied to other pests and crop systems. For simplicity, we can refer to this model as the PIC triad, representing the importance of measuring the three-way interaction between the plant, insect and chemistry.

The use of residue profile analysis in this initial research experience was limited in part by our dependence on composite residue samples. Future studies can be strengthened with fully replicated residue sampling and further measurement of plant tissue attributes.

In the pursuit of crop protection, control of a pest can be achieved through more than just lethal means. The basis for pest management with OPs such as azinphosmethyl is reliance on their fast-acting contact toxicity. In this study, we see that for many new insecticides, other modes of activity are important contributors to the overall crop protection seen in the field. In addition, these sublethal modes of activity may work in concert with lethal modes based on a temporal sequence of activity. Residue profile analysis of the neonicotinoid insecticides thiamethoxam, imidacloprid, and thiacloprid reveals a dynamic interaction with fruit and leaf tissues that serves to regulate the mode of activity at hand. Strong lethal activity occurs as long as sufficient residues are present on fruit and foliage surface. As these surface residues degrade, a range of sublethal behavioral effects, such as oviposition deterrence, repellency, and antifeedant activity are generated in the presence of the remaining residues. The temporal sequence of lethal and sublethal modes in this case is sufficient to provide the crop protection demonstrated in field efficacy trials. Other studies (Nauen et al. 1998, Isaacs et al. 1999) also have documented sublethal behavioral effects after exposure to or detection of internal plant residues. Two nicotinic receptor subtypes (desensitizing and non-desensitizing) have been identified in cockroaches (Salgado and Saar 2004), one subtype causing acute lethal symptoms in the presence of high concentrations of imidacloprid (nAChN-type), and the other subtype causing subacute symptoms under very low concentrations (nAChD-type). In addition to being responsible for antifeedant activity reported by Nauen and Salgado, nAChD receptor inhibition may also be responsible for the oviposition deterrence observed in this study.

Indoxacarb represents an example of the importance of further characterizing the nature of lethal activity to understand a compound’s performance. Although field-based bioassays showed indoxacarb, of all the tested OP-alternative compounds, to be the most similar to azinphosmethyl in terms of lethal activity, behavior observations and topical bioassays were needed to properly describe the nature of its activity. The very high LD<sub>90</sub> and LT<sub>90</sub> values seen in the topical bioassays indicate that the lethal activity of indoxacarb is very slow compared with azinphosmethyl and that dermal contact alone is not an optimal means of delivering the poison. The plum curculio feeding documented in the field-based bioassays and behavioral studies supports what others have published about ingestion being an important mode of entry for the bioactivation of formulated DPX-JW062 to its decarbomethoxylated metabolite (DCJW) (Wing et al. 1997, Tillman et al. 2001). The correlation relationship between internal residues and mortality is an additional indicator that ingestion is important for enhanced lethal activity of indoxacarb. We cannot assume, however, that surface residues do not contribute to the toxicity of ingestion-activated compounds such as indoxacarb. Plum curculios have to chew through surface tissue residues to reach internal plant tissues, and dislogeable surface residues also can be ingested through grooming behavior. This is the

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### Table 5. Regression R-Squared correlations between mortality (arcsine-transformed) and fruit and leaf residue for southern strain plum curculio

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surface leaf</th>
<th>Surface fruit</th>
<th>Surface total</th>
<th>Interior leaf</th>
<th>Interior fruit</th>
<th>Interior total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azinphosmethyl</td>
<td>0.8379**</td>
<td>0.4732*</td>
<td>0.6884**</td>
<td>0.6613*</td>
<td>0.4969*</td>
<td>0.7160**</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>0.6437*</td>
<td>0.6440*</td>
<td>0.6353*</td>
<td>0.0574</td>
<td>0.0160</td>
<td>0.0483</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>0.7550**</td>
<td>0.7431**</td>
<td>0.8953**</td>
<td>—*</td>
<td>0.4372*</td>
<td>0.4372*</td>
</tr>
<tr>
<td>Thiamefoxam</td>
<td>0.7353**</td>
<td>0.7264**</td>
<td>0.7240**</td>
<td>0.7348**</td>
<td>0.5263*</td>
<td>0.7208**</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td>0.1083</td>
<td>0.1894</td>
<td>0.1499</td>
<td>0.1915</td>
<td>0.2347</td>
<td>0.1993</td>
</tr>
<tr>
<td></td>
<td>0.3479</td>
<td>0.5328*</td>
<td>0.4473*</td>
<td>0.5372*</td>
<td>0.6159*</td>
<td>0.5626*</td>
</tr>
</tbody>
</table>

Data are derived from field-based bioassays and three parallel postapplication composite residue samples. All regression models have 1 df (model). Model used is mortality = residue. P values shown are for the overall model ANOVAs. *, model significant at α = 0.05; **, model significant at α = 0.001.

* Analysis not possible owing to lack of variation in residue profile.

* Correlations for indoxacarb with suspect replication (4-h residue, no mortality) removed.
The best explanation of the differences in mortality seen in the field-based bioassays compared with laboratory-based topical bioassays. The general term “contact activity” is typically associated with the mortality resulting from insects being in contact with surface residues. This specific mode of exposure may be further refined as “transcuticular activity” to more accurately describe movement of the insecticide through the insect cuticle to internal target sites. This is distinct, however, from inadvertent ingestion through insect grooming behavior, which also may result in lethal activity.

Identifying the modes of activity that contribute to insecticide performance allows us to optimize the timing of field sprays for each compound based on its critical performance characteristics. Azinphosmethyl is the least sensitive to application timing because of its contact toxicity and long-lasting surface residues. That is why petal fall sprays of this compound have been largely effective for control of the plum curculio in apples. Our data suggest that the optimal timing for neonicotinoids will be different from that of organophosphates. To capture both the short-lived lethal activity and the longer lived oviposition deterrence of these compounds, it will important to cover as much fruit surface area as possible. This can be best achieved by waiting several days after petal fall for fruit to begin sizing. Careful field scouting of plum curculio for the initiation of oviposition is also important so that fruit injury does not occur before the neonicotinoids are applied. To optimize the performance of indoxacarb, the application timing should be sequenced with plum curculio feeding behavior. Because plum curculio adult feeding on plant tissue starts before the bloom period and diminishes as oviposition behavior ensues (unpublished data), traditional petal fall timing is probably the best timing to maximize beetle presence and feeding behavior.

The critical performance characteristics of a compound should include knowledge of its modes of activity, life stage activity for the target pest, mode of exposure, and its residue profile on the respective crop. Compiling these characteristics for each insecticide, pest and crop will serve as a basis for confident pest management decisions that optimize control, conserve beneficial species, and minimize costs. It also can serve to predict chemistry combinations with incompatible modes of activity, such as neonicotinoids (anti-feedant activity) and indoxacarb (ingestion activity) or suggest as-yet unidentified synergistic combinations.

The introduction of several new classes of insecticides in the last decade has the potential for advancing integrated pest management within fruit production systems. But there is increased risk for growers if these new tools are promoted as “OP replacements” that can be reflexively switched into their spray programs without consideration of the diversity of performance characteristics among them. The burden is on the research community to thoroughly compile and extend the knowledge in a meaningful manner for the long-term sustainability of U.S. fruit production.

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