Lethal and Sublethal Activities of Imidacloprid Contribute to Control of Adult Japanese Beetle in Blueberries

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ABSTRACT Field-based bioassays and residue profile analysis were used to determine the relative importance of lethal and sublethal effects of imidacloprid on adult Japanese beetle, *Popillia japonica* Newman, in blueberries, *Vaccinium corymbosum* L. Field-based bioassays assessed adult mortality and knockdown, and fruit and leaf injury from Japanese beetles exposed to 4-h and 7-d field-aged residues of imidacloprid, and the conventional insecticides azinphosmethyl and esfenvalerate. Azinphosmethyl and imidacloprid caused high levels of mortality when beetles were exposed to blueberry shoots with ripe fruit 4 h postapplication, and all compounds protected blueberry fruit and foliage from beetle feeding. Azinphosmethyl and esfenvalerate caused significant Japanese beetle mortality when adults were exposed to blueberry shoots 7 d postapplication, whereas imidacloprid residues caused effects that protected leaves, although not of ripe fruit. When beetles were exposed to shoots with immature green fruit, relatively more leaf feeding and mortality were observed, suggesting that earlier treatment timings may be most effective for systemic neonicotinoids. Japanese beetle mortality was highly correlated with imidacloprid fruit and leaf surface residues, whereas sublethal feeding deterrent effects were observed after the surface residues diminished. The value of the plant-insect-chemistry model for describing the spatial and temporal dimensions of insecticide modes of activity is discussed in terms of optimizing crop protection.

KEY WORDS Japanese beetle, imidacloprid, blueberry, sublethal, PIC-Triad

The Japanese beetle, *Popillia japonica* Newman, is an invasive pest of fruit and vegetable crops, turfgrass, and ornamentals in eastern and central North America (Vittum et al. 1999, Potter and Held 2002). More than 300 plant species are hosts to adults of this species (Fleming 1972, Held 2004). During the adult emergence period of June to September in Michigan beetles can be observed feeding and mating in clusters on host plants. Their phenology and behavior create a challenge for growers of crops that are attractive to beetles as well as for nonattractive crops that contain weed hosts that draw beetles into the crop planting. Much of the food industry maintains a zero tolerance standard for insect contamination at pack-out, which places added pressure on growers of fruit crops such as cherry, peach, plum (all *Prunus* spp.), and blueberry (*Vaccinium* spp.) that may be harvested when beetles are present. The majority of commercial blueberry producers use over-the-row mechanical harvesters for collecting fruit from their fields. This harvesting method does not effectively discriminate between beetles and berries, so adult Japanese beetles are a significant contamination risk in fields being harvested where Japanese beetle has not been controlled.

Blueberry growers have experienced an average $72 increase per acre in production costs (Szendrei and Isaacs 2006b) as they strive to control this pest and meet the quality standards of the food industry. Management programs are being developed for perennial fruit producers to minimize the amount of attractive oviposition sites (Szendrei et al. 2005), provide nonattractive ground covers for beetles (Szendrei and Isaacs 2006a), and use short-residual botanical insecticides to remove beetles from bushes immediately before harvest (Isaacs et al. 2004). Color sorting technology also has been adopted by many large processors to detect and remove beetles, providing >95% removal (R.I., unpublished data). Even with these management components available to help minimize the risk of fruit contamination with adult beetles, conventional insecticides remain the primary approach to in-field management of Japanese beetles in fruit crops.

The primary insecticides used to control Japanese beetle in blueberry fields are organophosphate insecticides such as azinphosmethyl, phosmet, and malathion; pyrethroid insecticides such as esfenvalerate; and the carbamates carbaryl and methomyl (Wise et al. 2006a). However, as a result of the Food Quality Protection Act passed by the U.S. Congress in 1996, and implementation of this legislation by the U.S. Environmental Protection Agency, the future avail-

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ability of these tools for insect control in blueberries is in doubt. The lethal activity of many conventional insecticides, including organophosphates, carbamates, and pyrethroids, has been well established in field trial and bioassay studies over the years, but in only a few cases have the speed of activity and knockdown effects been measured (Hamilton et al. 1971, Sullivan et al. 1972). Biorational insecticides, such as neem extracts are not generally lethal to Japanese beetle adults, but they have demonstrated feeding deterrent effects with field residual activity up to 7 d (Ladd et al. 1978, Witt et al. 1999). Recent research on neonicotinoid insecticides suggests that sublethal effects, such as feeding deterrence, can make important contributions to their overall performance (Nauen 1995, Isaacs et al. 1999, Kunkel et al. 2001, Liburd et al. 2003). In addition, neonicotinoid insecticides are known to have unique physicochemical properties that can result in systemic movement into the treated plant, which can influence the pest's exposure to the insecticide (Nauen et al. 1999, Sur and Stork 2003, Tomizawa and Casida 2005, Wise et al. 2007). Recent studies involving imidacloprid have shown positive correlative relationships between the concentration and location of the parent compound in the plant and the resulting lethal or sublethal effects on the target pest (Wang et al. 2005, Wise et al. 2006c).

Optimizing the performance of new insecticides requires understanding their lethal and sublethal modes of activity, length of field residual, and the speed of activity for each compound. The neonicotinoid imidacloprid has shown good control of adult Japanese beetles in blueberries in small plot efficacy trials and field-scale implementation projects (Wise et al. 2006b; R.I., unpublished data). Even though such trials demonstrate basic crop protection, they do not provide information on the key attributes of this insecticide's performance. In this study, we combined field-based bioassays with residue profile analysis to determine the key attributes that contribute to the performance of imidacloprid on Japanese beetles. The objectives of this study were to 1) characterize the lethal and sublethal effects of imidacloprid over time, compared with azinphosmethyl and esfenvalerate; 2) describe the speed of activity of each insecticide on Japanese beetle in terms of mortality and knockdown; 3) compare the performance of imidacloprid when Japanese beetles were exposed to ripe fruit versus immature fruit; and 4) determine the relationship between surface and subsurface plant residues and the lethal activity of imidacloprid on Japanese beetles.

Materials and Methods

Insects. Japanese beetle adults were collected from grass fields at the Michigan State University Trevor Nichols Research Complex in Fennville, MI, during July 2001. Beetles were captured using yellow and green canister traps with a floral lure (Great Lakes IPM Inc, Vestaburg, MI) during the 24-h period before each study. After collection, beetles were held in cages with sassafras, *Sassafras albidum* (Nutt.), foliage at ≈25°C and a photoperiod of 16:8 (L:D) h. Healthy beetles exhibiting mobility on the foliage were kept for use in experiments.

Field Plots and Treatment Applications. Each plot consisted of 11 mature highbush blueberry, *Vaccinium corymbosum* L., 'Rubel' bushes at the Trevor Nichols Research Complex, and these were plots replicated four times in a completely randomized design. Bush spacing was 3.05 by 1.83 m with two buffer rows separating all plots. Insecticide treatments were applied at labeled rates by using an FMC 1029 airblast sprayer calibrated to deliver 467.5 liters of water/ha (50 gal/acre). Applications were made to bushes that had ripe and unripe fruit clusters on 19 July 2001. These plots served as the source of fruit and foliage for use in bioassays and residue analysis. Daily high and low temperatures and precipitation volumes were recorded with an automated weather station (Michigan Agriculture Weather Network) located within 1 km of the field plots.

Treatment compounds, formulations and rates (labeled for Japanese beetle control) used for the applications were as follows: imidacloprid (Provado 1.6 F, Bayer CropScience, Research Triangle Park, NC) at 83.8 g of active ingredient (AI)/ha (6 oz/acre), azinphosmethyl (Guthion 50W, Bayer CropScience) at 672.5 g (AI)/ha (1.2 lb/acre), and esfenvalerate (Asana XL 0.66 EC, DuPont, Wilmington, DE) at 20.6 g (AI)/ha (9.6 oz/acre). Control plots were not sprayed.

Residual Activity Bioassays. Bioassays were used to compare the lethal and sublethal effects of the three insecticides and to determine the temporal progression of these effects as the residues aged in the field. Blueberry shoots (≈15 cm in length) were collected from field plots described above 4 h after the applications and then again 7 and 14 d later (14 d samples used for residue profile analysis only). Each shoot was pruned to have 10 ripe fruit and 10 leaves, and then they were placed in water-soaked OASIS floral foam (Smithers-Oasis Co., Kent, OH) in clear plastic 950-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 the lid to reduce condensation of water vapor inside the container and minimize potential fumigation effects. Each of these containers was considered an experimental unit in the bioassays.

As soon as bioassay arenas were prepared, 10 Japanese beetle adults were placed in the bottom of each arena, and the containers were held in the laboratory at ≈21°C and a photoperiod of 16:8 (L:D) h. There were four replicates for each treatment at each of the postapplication time intervals. The number of beetles that were live, dead, or in a knockdown condition were recorded after 96 h of exposure. The knockdown condition was defined as beetles that were twitching in a nonupright position at the bottom of the container. Beetles were counted as live if they seemed to behave normally. The percentage of fruit and leaf-

feeding damage also was recorded by estimating the relative surface area damaged per berry and leaf by using 10% increments, with a common set of reference samples throughout the evaluation to ensure consistency. Statistical comparisons among treatments were made for each date by using analysis of variance (ANOVA) (Kerchove 2005) of arcsine-transformed and square root $(x + 0.5)$-transformed data.

Behavioral Response. Behavior observations were made on beetles after 24 h of exposure in the bioassay arenas to the blueberry shoots aged for 4 h and 7 d under field conditions. Beetles were observed for 1 min, and data were recorded on the condition, position, and activity of beetles, categorized as beetle still on plant, beetle feeding on fruit, beetle feeding on leaf, beetle alive but not on plant, beetle twitching on bottom of arena, or beetle immobile on bottom of arena. Beetles observed as being immobile were motionless, but were not manipulated to confirm death so as not to disturb the experiment. The proportion of beetles exhibiting each behavior in each treatment was compared with the untreated check, by using Dunnett's test (Kerchove 2005).

Speed of Activity. Incremental measurements of the number of beetles in immobile and knockdown conditions were made after 1, 24, and 96 h of exposure to 4-h aged residues. Immobile beetles were considered dead, but they were not manipulated to confirm mortality. The percentage of beetles exhibiting knockdown symptoms and immobile symptoms at the three time increments were compared among treatments by using ANOVA on arcsine-transformed values (Kerchove 2005).

Immature Fruit Bioassay. Bioassays were used to evaluate the performance of imidacloprid when Japanese beetles were exposed to blueberry shoots with green fruit. The methods were the same as described above in the residual activity bioassays, except that fruit clusters with green fruit were used in the study.

Imidacloprid Residue Profile Analysis. A parallel series of fruit and foliage samples were taken from field plots at each of three postapplication timings (4 h and 7 and 14 d). Composite samples of 40 leaves and 40 fruit each were frozen, and samples were transported to the Michigan State University Pesticide Analytical Laboratory in East Lansing, MI. Samples were placed in a −20°C freezer until extraction. A pesticide extraction procedure was used to separate dislodgeable residues on the surface of the fruit and leaf samples from the subsurface residues (in the plant cuticle and internal tissues) to provide separate quantification of surface and subsurface residues over time.

To determine the amount of residue on the fruit and leaf surfaces, 10-g composite samples of blueberry fruit and leaves were placed in 150 ml of high-performance liquid chromatography (HPLC)-grade acetonitrile (EMD Chemicals, Inc., Gibbstown, NJ) and sonicated for 10–15 s. The acetonitrile was decanted through 5 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water. The sample was dried via rotary evaporation and brought up in acetonitrile for HPLC analysis.

To determine the subsurface residues from the fruit and leaves the remaining solid fruit and leaf samples were ground in 200 ml of HPLC grade dichloromethane (Burdick & Jackson, Muskegon, MI). The extracts were then vacuum filtered, and the filtrate was passed through 5 g of anhydrous sodium sulfate. The samples were dried via rotary evaporation and brought up in acetonitrile. Any remaining particulates were removed by passing the sample through a 0.45-μm Acrodisc 13-mm syringe filter (Pall, East Hills, NY).

Samples were analyzed for imidacloprid residue (parent compound) with a Waters 2690 Separator Module HPLC equipped with a Waters 2487 Dual Wavelength Absorbance Detector (Waters, Milford, MA) set at 270 nm, and a C18 reversed phase column (150 by 4.6 mm bore, 5 μm particle size, Restek, Bellefonte, PA) (Bayer 1998). The mobile phase was water/acetonitrile (80:20) at 55°C. The HPLC level of quantification was 0.457 μg/g (ppm) of active ingredient, and level of detection was 0.138 ppm.

To determine the relationship between lethal effects on beetles and imidacloprid residues, correlation analysis was performed on 96-h beetle mortality data from the three postapplication residual activity bioassays and the imidacloprid residue data for each sample date (PROC GLM, SAS Institute 2002). Regression analysis (PROC GLM, SAS Institute 2002) was used to determine the relationship between the amount of surface and subsurface residues of leaves and fruit and Japanese beetle mortality.

Results and Discussion

Residual Activity Bioassays. Japanese beetle adults exposed to blueberry shoots with 4-h old residues exhibited significantly higher levels of mortality on the imidacloprid and azinphosmethyl treatments than the untreated shoots ($F = 52.33$; df = 3, 12; $P = 0.0001$) (Table 1). Azinphosmethyl showed the greatest lethal activity, imidacloprid was intermediate, whereas esfenvalerate was not significantly different from the control. There was a significant effect of treatment on the level of knockdown symptoms (i.e., twitching on bottom) when beetles were exposed for 96 h to 4-h-old residues ($F = 3.58$; df = 3, 12; $P = 0.047$), with no significant difference among the three insecticides (Table 1). The levels of fruit feeding damage from beetles exposed to 4-h-old residues was significantly lower for all insecticides than the untreated control, with azinphosmethyl and imidacloprid providing similar levels of fruit protection ($F = 13.68$; df = 3, 12; $P = 0.0004$). Esfenvalerate provided intermediate fruit protection, not significantly different from the other insecticides. All three insecticides completely prevented beetles from feeding on leaves ($F = 44.48$; df = 3, 12; $P = 0.0001$) (Table 1).

For Japanese beetle adults exposed to 7-d old residues, only azinphosmethyl and esfenvalerate caused significant levels of mortality, with azinphosmethyl showing the greatest lethal effects ($F = 50.47$; df = 3,

Table 1. Mean ± SE percentage of plant tissue damage[a] and number of dead or knocked down Japanese beetle adults after exposing 10 beetles to insecticide residues aged in the field for 4 h or 7 d. Beetles were exposed to treated fruit clusters each with 10 blue (ripe) fruit and 10 leaves for 96 h

Treatment	4-h Residue				7-d Residue			
	No. dead beetles[b]	Knocked down beetles[b]	% fruit damage[c]	% leaf damage[c]	No. dead beetles[b]	Knocked down beetles[b]	% fruit damage[c]	% leaf damage[c]
Control	0.5 (0.3)c	0.0b	41.8 (6.2)a	45.0 (10.4)a	0.0c	0.0a	31.5 (4.0)a	88.7 (4.3)a
Imidacloprid	3.5 (0.5)b	2.0 (0.7)a	6.5 (2.9)b	0.0b	0.0c	1.5 (0.3)a	22.6 (6.8)ab	8.8 (1.3)b
Esfenvalerate	1.8 (0.5)bc	0.8 (0.5)ab	15.7 (2.4)b	0.0b	1.5 (0.3)b	1.0 (0.7)a	11.9 (2.4)b	10.0 (7.1)b
Azinphosmethyl	9.5 (0.5)a	0.5 (0.5)ab	6.9 (3.2)b	0.0b	7.7 (1.9)a	0.3 (0.3)a	11.8 (2.2)b	3.3 (1.7)b

Data in columns followed by the same letter are not significantly different ($P < 0.05$).

[a] Percentage of fruit- and leaf-feeding damage are surface area estimates in 10% increments.

[b] Data were square root ($x + 0.5$) transformed before ANOVA. Mean separations calculated using LSD. Data shown are nontransformed means.

[c] Data were arcsine square-root percent-transformed before ANOVA. Mean separations calculated using the least significant difference (LSD) test. Data shown are nontransformed means.

12; $P = 0.0001$) (Table 1). All compounds significantly reduced Japanese beetle leaf feeding ($F = 30.45$; df = 3, 12; $P = 0.0001$), but only azinphosmethyl and esfenvalerate caused a significant reduction in the levels of fruit feeding compared with the untreated check ($F = 5.27$; df = 3, 12; $P = 0.015$). Given the lack of mortality from imidacloprid residues, the leaf protection provided by this treatment may be a result of antifeedant activity.

Behavioral Response. Behavioral observations of Japanese beetle adults in arenas with 4-h field-aged insecticide residues on blueberry shoots revealed patterns of condition, position, and activity that were significantly different to those seen on untreated shoots (Fig. 1A). The most pronounced difference was in the proportion of beetles immobile on the bottom of the bioassay chamber. This condition was significantly more common than the untreated control for all insecticides ($F = 85.91$; df = 3, 12; $P = 0.0001$). A significant increase in the proportion of beetles in the esfenvalerate treatment also was observed twitching on the bottom of the arena after 24 h of exposure ($F = 3.59$; df = 3, 12; $P = 0.046$). For all insecticides, there were lower numbers of beetles observed feeding on fruit ($F = 31.00$; df = 3, 12; $P = 0.0001$) or leaves ($F = 13.13$; df = 3, 12; $P = 0.0004$) after 24 h of exposure (Fig. 1A), which coincides with the results of the residual activity bioassay with 4-d old residues.

Observations of beetles on treated blueberry shoots aged for 7 d under field conditions revealed changes in beetle condition, position, and activity patterns compared with the untreated control (Fig. 1B). The greatest change from the 4-h residual bioassay was in the incidence of immobile beetles on the bottom of the bioassay chamber. Whereas many beetles continued to be observed immobile in the azinphosmethyl and esfenvalerate treatments, no beetles were observed in this condition when exposed to imidacloprid-treated blueberry shoots ($F = 7.58$; df = 3, 12; $P = 0.0042$). Even so, there were significantly fewer beetles observed feeding on leaves in the imidacloprid treatment, as well as the esfenvalerate treatment ($F = 6.39$; df = 3, 12; $P = 0.0078$), compared with the untreated control. For the esfenvalerate-treated shoots, there were also reduced numbers of beetles feeding on fruit compared with the untreated control ($F = 5.36$; df = 3, 12; $P = 0.014$). The fact that normal levels of beetles were observed feeding on azinphosmethyl treated fruit and leaves suggests that as residues age, and in the absence of feeding deterrence, ingestion is an important delivery mechanism for this compound's lethal activity. For imidacloprid and esfenvalerate, a significantly greater proportion of beetles were observed motionless on the blueberry shoots than on the untreated shoots ($F = 8.21$; df = 3, 12; $P = 0.0031$).

Speed of Activity. Incremental measurements of beetle mortality showed that azinphosmethyl was the overall most toxic compound to Japanese beetle adults and also had the shortest lethal time (Fig. 2A). Azinphosmethyl was the only treatment to cause significant beetle mortality after 1 h exposure to 4-h old residues ($F = 9.31$; df = 3, 12; $P = 0.0019$). This treatment also caused the highest level of beetle mortality after 96 h of exposure ($F = 52.33$, df = 3, 12, $P = 0.0001$). Esfenvalerate and imidacloprid both caused significant beetle mortality after 24 h of exposure ($F = 85.91$; df = 3, 12; $P = 0.0001$) (Fig. 2A), but in the esfenvalerate treatment the percentage of beetles in the immobile condition declined between 24 and 96 h of exposure to esfenvalerate. This is consistent with the knockdown and recovery that is often seen with pyrethroid poisoning (Sullivan et al. 1972).

Measurements of Japanese beetles in a knocked down condition after 1 h of exposure showed that all insecticides were significantly different from the untreated control (Fig. 2B) but that esfenvalerate had the most pronounced knockdown effects at that earliest time interval ($F = 22.32$; df = 3, 12; $P = 0.0001$). Similar to the levels of immobile beetles reported above, beetles exposed to esfenvalerate showed recovery by 24 h after treatment and even greater recovery by 96 h after treatment ($F = 3.59$; df = 3, 12; $P = 0.046$). Imidacloprid was the only treatment that produced a cumulative increase in beetle knockdown over the three time intervals of this study, resulting in significantly higher levels than the untreated control at 96 h of exposure ($F = 3.58$; df = 3, 12; $P = 0.047$). Unlike esfenvalerate, the progressive increase of beetles in the knocked down condition after imidacloprid

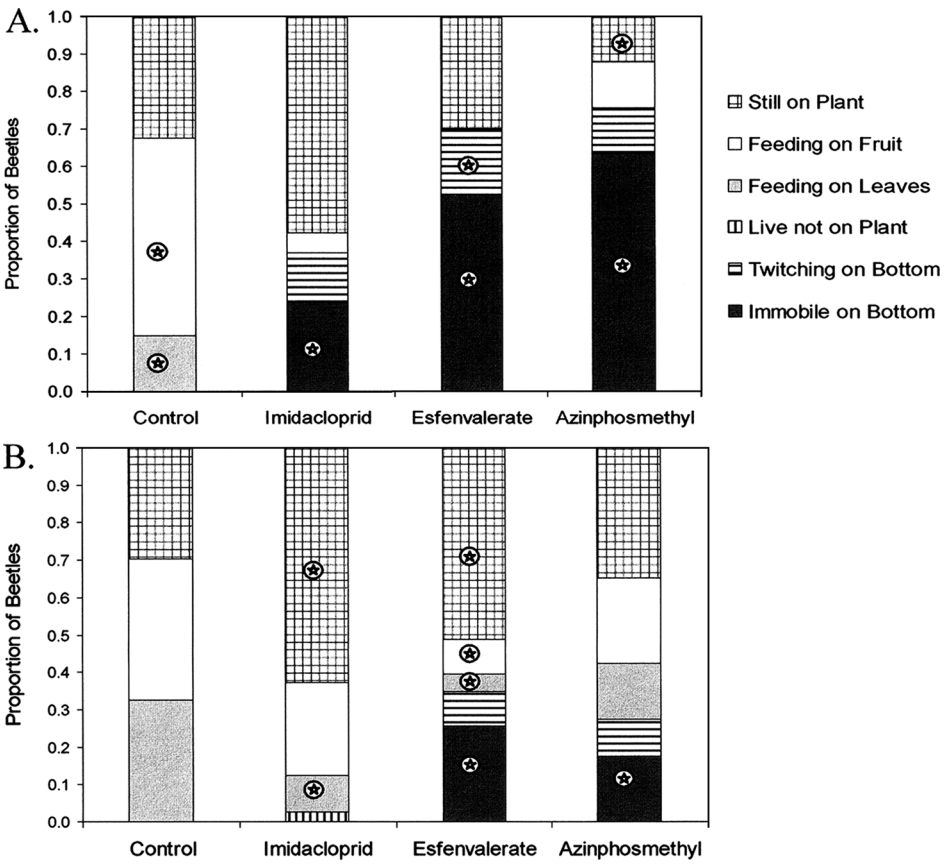

Fig. 1. Proportion of Japanese beetle adults observed in different behavior, condition, or canopy positions at 24 h postexposure observations for 4 h (A) and 7 d (B) posttreatment residual activity bioassays. The ✪ symbol within individual behavior/position bars for each treatment compound indicate statistically different incidence compared with the untreated control (Dunnett's versus control), whereas the ✪ placed within an untreated behavior/position bar indicates significantly lower incidence across all treatments.

exposure suggests that these symptoms are a prelude to mortality.

Immature Fruit Bioassay. Japanese beetle adults exposed to green fruit with 4-h-old imidacloprid residues resulted in significantly higher levels of mortality ($F = 13.71$; df = 1, 6; $P = 0.01$) and knockdown ($F = 37.17$; df = 1, 6; $P = 0.0009$) than the untreated control (Table 2). Imidacloprid also provided significant levels of protection from leaf feeding ($F = 187.43$, df = 1, 6, $P = 0.0001$) compared with the untreated control. In contrast to the results when ripe fruit were used in the assays, when Japanese beetles were exposed to green fruit and leaves with 7-d-old imidacloprid residues, there was a higher level of mortality than in the

Table 2. Mean ± SE percentage of plant tissue damage[a] and numbers of dead and knocked down Japanese beetle adults after exposing ten beetles to field-aged insecticide residues (beetles were exposed to treated fruit clusters each with 10 green [immature] fruit and 10 leaves for 96 h)

Treatment	4-h Residue				7-d Residue			
	No. dead beetles[b]	Knocked down beetles[b]	% fruit damage[c]	% leaf damage[c]	No. dead beetles[d]	Knocked down beetles[b]	% fruit damage[c]	% leaf damage[c]
Control	0.8 (0.3)b	0.5 (0.3)b	14.6 (5.6)a	80.0 (4.1)a	0.0	1.8 (0.5)a	18.7 (7.8)a	97.5 (2.5)a
Imidacloprid	2.8 (0.5)a	4.8 (0.8)a	4.7 (1.0)a	1.3 (1.3)b	1.0 (0.0)	2.8 (0.9)a	12.3 (6.2)a	62.5 (2.5)b

Data in columns followed by the same letter are not significantly different ($P < 0.05$).

[a] Percentage of fruit- and leaf-feeding damage are surface area estimates in 10% increments.

[b] Data were square root ($x + 0.5$) transformed before ANOVA. Mean separations calculated using LSD. Data shown are nontransformed means.

[c] Data were arcsine square-root percent-transformed before ANOVA. Mean separations calculated using LSD. Data shown are nontransformed means.

[d] ANOVA analysis could not be run because of lack of between-replicate variation.

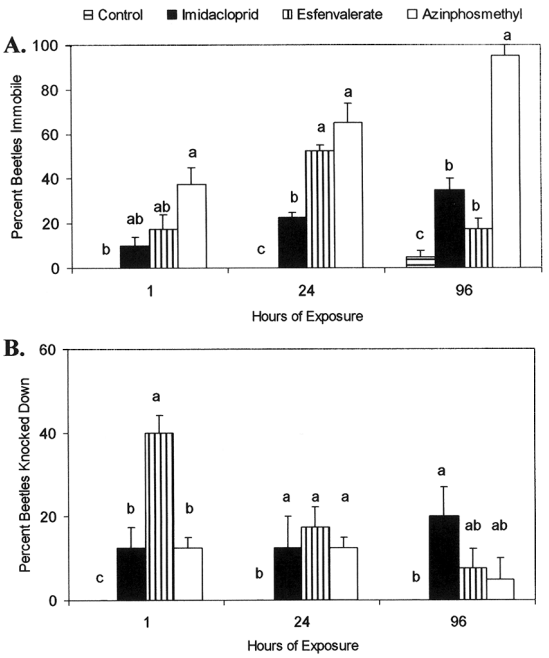

Fig. 2. Lethal time and knockdown time for insecticides on Japanese beetle adults. Mean ± SE percentage of beetles in immobile condition (A) and knocked down condition (B) measured after 1, 24, and 96 h of exposure to treated blueberry leaves and fruit 4 h after field application. Comparative means for a given exposure interval followed by the same letter are not significantly different ($P < 0.05$; Tukey's honestly significant difference test). Percent data were arcsine square-root percent-transformed before ANOVA, and the untransformed means are shown.

untreated check (ANOVA analysis could not be run for lack of between replicate variation) (Table 2). Even though proportionally more feeding occurred on 7-d imidacloprid leaves in the immature fruit bioassay than in the ripe fruit bioassay, imidacloprid still provided significant levels of protection compared with the untreated control ($F = 46.660$; df = 1, 6; $P = 0.0005$). Although this study was not designed as a choice test, these data suggest that the relative feeding preference for leaves versus green fruit may have resulted in sufficient ingestion of imidacloprid to cause the observed mortality.

Residue Profile Analysis. The residue analysis of blueberry shoots treated with imidacloprid showed this insecticide to have predominantly surface residues on fruit and leaves 4 h after the application (Table 3). A large portion of the imidacloprid residue moved to the subsurface of fruit and leaf tissue within 7 d, whereas surface residues diminished from environmental exposure and partitioning into the plant. Imidacloprid residues persisted through the 14-d sample period in fruit and leaf tissues, with greater proportions found inside the leaf and fruit tissues than on the surface. Ambient field temperature conditions ranged from 9 to 32°C over the study period. Three precipitation events totaling 62 mm between the 4 h and 7 d sampling periods likely contributed to the surface residue decline.

Table 3. Temporal profile of imidacloprid residues on blueberry leaves and fruit. Residues[a] measured in micrograms per gram (ppm) of active ingredient per leaf and fruit tissue, taken at different times after field application

Time postapplication	Leaf		Fruit	
	Surface	Subsurface	Surface	Subsurface
4 h	5.787	0.973	0.883	0.008
7 d	0.008	0.438	0.005	0.775
14 d	0.001	0.375	0.001	0.908

[a] HPLC detection levels; limit of quantitation = 0.457, limit of detection = 0.138.

Residue Correlation Bioassays. The regression analyses of fruit (surface, subsurface) and leaf (surface, subsurface) residues and Japanese beetle adult mortality showed several significant relationships. There were strong positive correlations between imidacloprid leaf and fruit surface residues and beetle mortality (leaf: $r^2 = 0.69$, $F = 21.47$, df = 1, 10, $P = 0.001$; fruit: $r^2 = 0.68$, $F = 21.28$, df = 1, 10, $P = 0.001$; and total surface: $r^2 = 0.69$, $F = 21.44$, df = 1, 10, $P = 0.001$). To a lesser extent, this was also seen for subsurface leaf residues ($r^2 = 0.61$; $F = 15.95$; df = 1, 10; $P = 0.003$). This suggests that Japanese beetle exposure to the surface residues on leaves and fruit is largely responsible for the lethal activity of imidacloprid seen in the first days after application. The significant relationship between subsurface leaf residues and beetle mortality suggests that ingestion also contributes to imidacloprid's lethal activity, especially after surface residues diminish. This is supported by the feeding patterns documented from behavior observations in the bioassays of field-aged residues. The correlation between imidacloprid fruit subsurface residues and beetle mortality was significant, but R values were negative (leaf: $r^2 = 0.58$; $F = 13.98$; df = 1, 10; $P = 0.004$). This represents an inverse relationship and indicates that beetle feeding on subsurface fruit residues does not contribute to mortality.

This study has shown that the activity of conventional broad-spectrum insecticides such as azinphosmethyl and esfenvalerate on Japanese beetle is based primarily on their fast-acting contact toxicity. The organophosphate azinphosmethyl was highly lethal to beetles via contact and ingestion, whereas the pyrethroid esfenvalerate caused high levels of sublethal knockdown followed by beetle recovery. For imidacloprid, we documented that several modes of activity were important contributors to the overall performance of the compound. There was significant lethal activity on beetles in the first days after application, as long as sufficient residues were present on fruit and leaf surfaces. As the surface residues diminished and mortality declined, feeding deterrence was sufficient to protect the blueberry plants. The temporal sequence of imidacloprid's lethal and sublethal effects worked in concert to provide crop protection from Japanese beetle.

The bioassay with immature fruit demonstrated the important influence of plant phenology on Japanese

beetle behavior, which can serve as a basis for optimizing the performance of imidacloprid against this pest. Even though there is a tendency for blueberry growers to use imidacloprid for Japanese beetle control close to harvest to reduce the risk of contamination, its lethal activity can be enhanced by timing applications when there are a greater proportion of immature berries. This timing also coincides with an earlier phase of Japanese beetle emergence, when the feeding deterrence effects of imidacloprid may also disrupt the early aggregation of beetles in blueberry bushes.

Capturing the spatial and temporal dimensions of the plant-insect-chemical (PIC Triad) (Wise et al. 2007) interaction provided important insight into the problem and ways to optimize the performance of imidacloprid as a tool for blueberry pest management. The use of residue profile analysis in this initial research experience was limited in part by our dependence on composite residue samples, and the analysis of only the imidacloprid parent compound. Future studies can be strengthened with fully replicated residue sampling and measurements of the bioactivity of imidacloprid metabolites on the target pest.

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