Soil-Applied Imidacloprid Is Translocated to Nectar and Kills Nectar-Feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae)

VERA A. KRISCHIK,[1] ALYSON L. LANDMARK, AND GEORGE E. HEIMPEL

Department of Entomology, University of Minnesota, St. Paul, MN 55108

Environ. Entomol. 36(5): 1238–1245 (2007)

ABSTRACT Behavior was altered and survivorship was reduced when parasitoids, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), were fed flowers from buckwheat, *Fagopyrum esculentum* L. (Polygonaceae), treated with soil applications of imidacloprid (Marathon 1% G). Parasitoids at 1 d had significantly reduced survivorship of 38 ± 6.7% on label rate and 17 ± 4.2% on twice label rate compared with 98 ± 1.2% on untreated flowers. Parasitoids trembled 88% on label rate and 94% on twice label rate compared with 0% on untreated flowers. Residue analysis on a composite sample of 425 flowers showed that imidacloprid concentration was 6.6 ± 1.0 ppm (16 ppb/flower) in label rate, 12.3 ± 2.7 ppm (29 ppb/flower) in twice label rate, and 0 ppb in untreated flowers. The hydroxy metabolite concentration was 1.1 ppm (2.4 ppb/flower) in label rate, 1.9 ppm (4.4 ppb/flower) in twice label rate, and 0 ppm in untreated flowers. The olefin metabolite concentration was 0.2 ppm (0.5 ppb/flower) in label rate, 0.5 ppm (1.1 ppb/flower) in twice label rate, and 0 ppm in untreated flowers. Soil-applied imidacloprid used at flowering may be translocated to nectar in higher concentration compared with the imidacloprid seed treatment Gaucho. Considerable research has studied effects of Gaucho-treated canola, sunflower, and maize on behavior and mortality of *Apis mellifera* L. In our laboratory, we showed that translocation of imidacloprid to flowers reduced survivorship and altered behavior of pink lady beetle, *Coleomegilla maculata* DeGeer (Smith and Krischik 1999) and green lacewing, *Chrysoperla carnea* Stephens (Rogers et al. 2007).

KEY WORDS *Anagyrus pseudococci*, systemic, imidacloprid, nectar feeding, biological control

Integrated pest management (IPM) programs use biological control, biorational insecticides, and conventional insecticides as tactics to control pests. The side effects section of the website of Koppert (2005) provides information on the compatibility of insecticides and biological control organisms. It advises that foliar imidacloprid is not compatible with biological control, whereas systemic imidacloprid is considered more compatible. Foliar applications of imidacloprid killed foraging predators and parasitoids when they come in contact with residue on foliage (Mizell and Sconyers 1992, Boyd and Boethel 1998, Sclar et al. 1998). The predatory bugs *Dicyphus tamaninii* (Wagner), *Macrolophus caliginosus* (Wagner), *Orius laevigatus* (Fieber), and *Podisus maculiventris* (Say) were placed on leaves 1, 3, 8, 21, and 30 d after foliar imidacloprid was applied, and toxicity was generally higher on 1- and 3-d residues (Figuls et al. 1999). Foliar applications of imidacloprid to apple trees were highly toxic to the wooly apple aphid parasitoid, *Aphelinus mali* (Haldeman) (Cohen et al. 1996). *Encarsia nigricephala* (Dozier), *E. pergandiella* (Howard), and *Eretmocerus* sp. exposed to imidacloprid-treated plants were killed (Simmons and Jackson 2000).

However, few studies evaluated if soil-applied imidacloprid was translocated to nectar and affected foraging predators and parasitoids used for biological control. *Coleomegilla maculata* had reduced mobility and survivorship when fed flowers from sunflower and dandelion that were treated with soil-applied imidacloprid (Smith and Krischik 1999). Systemic applications of imidacloprid reduced survivorship of *Orius insidiosus* (Say), perhaps because of their behavior of feeding on plant sap (Sclar et al. 1998, Al-Deeb et al. 2001). Soil applications of imidacloprid reduced the number of parasitoids that emerged from euonymus scale, *Unaspis euonymi* (Comstock), although the mechanism was not evaluated (Rebek and Sadof 2003). The parasitoid *Microplitis croceipes* Cresson fed extrafloral nectar from cotton treated with systemic imidacloprid had significantly reduced longevity and foraging ability (Stapel et al. 2000).

Because imidacloprid is widely used in landscape, greenhouse, and field crops, foraging parasitoids may use nectar from imidacloprid-treated plants. Most, if not all, adult parasitoids feed on sources of sugar, such as nectar or honeydew (Hagen 1986, Jervis and Kidd 1986, Jervis et al. 1993, Heimpel and Collier 1996,

[1] Corresponding author: 1980 Folwell Ave. #219, St. Paul, MN 55108 (e-mail: krisc001@umn.edu).

0046-225X/07/1238–1245$04.00/0

Heimpel et al. 1997). Nectar feeding increases parasitoid longevity (Syme 1975, Wäckers and Swaans 1993, Laetemia et al. 1995, Dyer and Landis 1996), improves fecundity (Syme 1975, Laetemia et al. 1995, Olson and Andow 1998), and increases the amount of time females search for hosts (Wäckers 1994, Takasu and Lewis 1995, Jervis et al. 1996). Cover crops such as buckwheat, *F. esculentum*, and mustard, *Brassica junceab* L. Czern., strips of nectary plants, nectar stations, and the practice of spraying solutions of sugars and yeast on plants are used to increase parasitoid survivorship and improve host searching (van Emden 1962, 1965, Altieri and Whitcomb 1979, Powell 1986).

There is little published research on the effects of soil-applied imidacloprid on nectar-feeding parasitoids. However, there are many research papers on the effects of the seed treatment Gaucho (40.7% active ingredient; Bayer CropScience, Research Triangle Park, NC) on nectar-feeding *A. mellifera*, and bumble bees, *Bombus* sp. When Gaucho is used as a seed protectant, the concentration of imidacloprid and its metabolites are lowered as it spreads throughout the growing plant as the plant increases biomass. We argue that in greenhouse and landscape, imidacloprid is often applied to the soil at flowering, and it can move directly into nectar and affect nectar-feeding parasitoids and predators.

Research on Gaucho used in maize, sunflower, and canola showed that imidacloprid was translocated to nectar and pollen, and in some studies, altered behavior and reduced survivorship of *A. mellifera* and *Bombus* sp. Imidacloprid reduced the orientation of *A. mellifera* at 25 ppb (Lambin et al. 2001). Foraging bees reduced their visits to syrup feeders that had concentrations of imidacloprid at 6 (Colin et al. 2004) and 50 ppb (Kirchner 1999). Reduction in recruitment was postulated as a result of decrease in effectiveness of dances at the hive to recruit bees (Kirchner 1999). Researchers showed that there was no effect on *A. mellifera* at <20 ppb (Schmuck 1999, Schmuck et al. 2001), whereas at levels >20 ppb, behavior was changed, as measured by a reduction in recruitment to food sources (Schmuck 1999). Oral toxicity was identified at a LD_{50} of 50 ppb (Suchail et al. 2000). In another study, oral toxicity to *A. mellifera* was 370 ppb at 72 h. The olefin metabolite was more toxic (290 ppb) and the hydroxy metabolite less toxic (2,060 ppb) compared with imidacloprid (Suchail et al. 2001). Chronic feeding tests revealed that imidacloprid at 48–96 ppb was lethal to caged worker bees (Decourtye et al. 2003). A review paper concluded that honey bees were exposed to lethal and sublethal doses in fields that regularly used imidacloprid (Rortais et al. 2005). However, others concluded that field exposure was negligible (Maus et al. 2003).

This research examined the effects of systemic imidacloprid on survivorship and behavior of the parasitoid *Anagyrus pseudococci* exposed to flowers from *Fagopyrum esculentum* treated with soil applications of imidacloprid. Imidacloprid is widely used in interiorscape, greenhouse, and landscapes on flowering plants to control pest insects (Mullins 1993). The cold anthrone test was done to verify that parasitoids were feeding on nectar and not dying from starvation caused by repellency of nectar. Also, residue analysis was performed on nectar to determine concentrations of imidacloprid and its two primary metabolites, olefin and hydroxy.

Materials and Methods

Experimental Organisms. *Anagyrus pseudococci* was selected for this research because it nectar feeds, males and females can be easily distinguished, it lives for 30 d, and it is readily available from insectaries. *A. pseudococci* are often released in greenhouses and conservatories to control citrus mealybug [*Planococcus citri* (Risso)] (Steiner and Elliot 1987, Islam et al. 1997, Stauffer and Rose 1997). *A. pseudococci* can live for 30 d if a sugar source is available (Avidov et al. 1967). Parasitoids were obtained from Foothill Agricultural Research (FAR) Insectary (Corona, CA) and provisioned with water and honey for at least 1 wk before the bioassay. Males and females were kept together in 100 by 60-cm plastic cages at a photoperiod of 12:12 (L:D) h, 25°C, and 70–75% RH. Males were removed for the bioassay. Females are easy to distinguish because they are larger, have brown bodies compared with black bodies of males, and have differently colored antennae.

Fagopyrum esculentum is used in the United States as a nectar crop for beneficial insects, as a green manure crop, or a smother crop to control weeds (Allotey et al. 1995, Tominaga and Uezu 1995). Beneficial insects are attracted to feed on nectar and pollen in *F. esculentum* flowers because of the open flowers with easily accessible nectar (Versehora 1962, McGregor 1976). For a continuous supply of *F. esculentum* flowers for the duration of the experiment, five flats per treatment were planted each week. Each flat contained 10 pots (10.5 cm^2) filled with Premier Pro-Mix BX sterile potting soil (Premier Horticulture, Red Hill, PA) and six to eight *F. esculentum* seeds (Johnny's Select Seeds, Winslow, ME).

Experiment 1: Bioassays with Soil-Applied Imidacloprid. Three replicate experiments were performed with eight treatments, each with 10 bioassay containers with 10 female parasitoids. Bioassay containers were 10 by 2 cm (diameter) Aquapic (Syndicate Sales, Kokomo, IN) water tubes. In the plastic cap of each water tube, a 0.5-ml centrifuge tube with a hinged plastic cap and pointed end was inserted. A hole was made in the pointed end to accommodate a flower or leaf stalk. The water or sugar water was placed inside the centrifuge tube to keep the flower or leaf hydrated. Flowers were changed daily to ensure nectar availability. All bioassay chambers were kept in laboratory incubators and maintained under a photoperiod of 12:12 (L: D) h, 25°C, and 70–75% RH.

At 14 d after emergence, before flower stalk initiation, *F. esculentum* plants were treated with a soil application of Marathon 1% G [1% (AI); Olympic Horticultural Products, Mainland, PA] at label rate (1×) of 1.4 g per pot and twice label rate (2×) of 2.8 g

per pot. Imidacloprid is a chloronicotinyl insecticide that binds to the nicotinic acetylcholine receptor in the postsynaptic membrane in insects and causes trembling, paralysis, and eventual death (Boyd and Boethel 1998, Lind et al. 1998a, 1998b). At 1 d before bioassays, Azatin XL [3% (AI); Olympic Horticultural Products] was sprayed onto plants at label rate (1.3 ml/liter). Azadirachtin is a limonoid insect growth regulator (IGR) derived from the neem tree, *Azadirachta indica* (A. Juss.), and is used to control a broad spectrum of greenhouse and nursery insects by interfering with the insect molting hormone ecdysone, causing insects to die during the molting process. If Azatin XL did not kill the parasitoid, it could be used in IPM programs instead of imidacloprid (Hoddle et al. 2003).

Treatments were label rate (1×) and twice label rate (2×) imidacloprid, which were done to determine whether imidacloprid was translocated to nectar and affected parasitoid survivorship and behavior. A control treatment, untreated flowers (UF), had no soil-applied imidacloprid. A sugar water treatment (S) was used to determine how long parasitoids survived when feed on a constant source of 12% sugar and not exposed to imidacloprid. A treatment of untreated flowers placed in 12% sugar water (UFS) was used to determine if water dilutes nectar. A starvation treatment (N) was used to determine how long parasitoids could live without feeding. A leaves-only treatment (LVS with sugar water wick) treatment was used to determine if imidacloprid killed parasitoids when they walked on leaves from 1× imidacloprid-treated plants. Finally, Azatin XL (AZ) at label rate was studied as a biorational that controlled pests and conserved parasitoids.

Survivorship and trembling were measured every 12 h for the first 2 d and then every 24 h until 7 d. Trembling rendered wasps immobile. Survivorship data were analyzed by PROC GLM for treatment, replicate, and treatment by replicate interactions. If the replicate term was significant, each replicate was analyzed independently with PROC GLM, Levene test for homogeneity (transformed if necessary) and Tukey-Kramer honestly significant difference (HSD) multiple range test (SAS Institute 2003). To meet the assumptions of homogeneity on day 7, treatments with no survivorship were not included in the analysis.

Experiment 2: Cold Anthrone Test. Two replicate experiments, each containing 20 insects per treatment, were performed. After 24 h of starvation, insects were placed in bioassay containers, permitted to feed for 1 h on 1×, 2×, and UF treatments, and frozen. Each frozen parasitoid was placed on a slide, drenched with 25 μl of the anthrone solution, covered with a coverslip, and subjected to moderate pressure to empty the contents of the insect's gut into the anthrone. After 1 h, a positive color change from yellow to blue or green indicated fructose in the gut. The cold anthrone test was used to independently verify if parasitoids fed. It was developed by van Handel (1967, 1968) and van Handel et al. (1972) as a test for visual color change in anthrone in the presence of fructose in nectar, either alone or as a component of the disaccharide sucrose (Percival 1961, van Handel et al. 1972, Olson et al. 2000, Fadamiro and Heimpel 2001, Lee and Heimpel 2003, Heimpel et al. 2004, Fadamiro et al. 2005). An anthrone solution was prepared by combining 990 μl of anthrone reagent (van Handel et al. 1972) with 10 μl 1:1 chloroform: methanol to remove cuticular wax. Data from the cold anthrone test was analyzed by a χ^2 test (SAS Institute 2005).

Experiment 3: Determination of Imidacloprid, Hydroxy, and Olefin Residue in Nectar. Flowers on plants that were used in bioassays were harvested for residue analysis. Five replicate experiments were performed, each containing two samples of 425 flowers collected from 40 plants for each of the three treatments (1×, 2×, and UF). Flowers were frozen in an ultralow freezer and shipped frozen to Enviro-Test (Edmonton, Canada) for determination of levels of imidacloprid, hydroxy, and olefin metabolites in flower nectar.

Each sample of 0.5 g (425 flowers) was placed in 15 ml of water in a 50-ml culture tube, placed in an ultrasonic bath for 2 min, and placed on a wrist shaker for 2 h, filtered, partitioned with dichloromethane, filtered, and evaporate to dryness. The residue was dissolved in 20% acetonitile/0.1% acetic acid and brought to 1 ml, frozen, and extracted with acetonitrile and concentrated with a rotovaporator. The samples were analyzed by Liquid Chromatography-Electronspray Ionization Mass Spectrometry LC/MC/MC (PE Sciex API III system) with variant solvent delivery system, water chromatography syringe pump, and Rainin Dynamax Automatice Sample Injector with Scix Turbo-Ionspray source (Woburn, MA). The operating conditions were a Phenomenex C8 column, 5 μm particle size, 20°C, mobile phase A 0.1% acetic acid in water and mobile phase B 0.1% acetic acid in acetonitrile, flow rate 1.0 ml/min, and injection volume 20 μl. Gradient was 0 min 50% A, 50% B: 3 min 20% A, 80% B; 5 min 20% A, 80% B; 6 min 50% A, 50% B.

The standards were received from Bayer CropScience (imidacloprid lot no. 93R-008–140, purity 98.4%; hydroxy lot no. 98r83–144 purity 99.3%; olefin lot no. M11453, purity 98.6%). The spiking standards were prepared in 20% acetonitrile/0.1% acetic acid. Controls with extracted nectar were fortified with imidacloprid, hydroxy, and olefin at 0.05, 0.10, and 15 ppm. Retention time was 2.31 min for imidacloprid (mass transition, 256.6–175.0), 2.06 for hydroxy (mass transition, 272.5–190.7) and 1.59 min olefin (mass transition, 254.5–205). The limit of quantification for imidacloprid, hydroxy, and olefin was 0.025 ppm based on a 1.0-g sample and final volume of 1.0 ml. The average recovery of imidacloprid, hydroxy, and olefin was 91, 84, and 91%, respectively, at 0.05, 0.10, and 15 ppm. Chemical residue data were analyzed by PROC GLM for treatment, replicate and treatment by replicate interactions. If the replicate term was significant, each replicate was analyzed independently with PROC GLM, Levene test for homogeneity (transformed if necessary) and a Tukey-Kramer HSD multiple range test (SAS Institute 2003).

Table 1. Percentage survivorship (mean ± SE) of *A. pseudococci* at 1 and 7 d after feeding on flowers from control plants (UF), plants treated with 1× label and 2× label rate of soil-applied imidacloprid (Marathon 1% G), and other treatments

Treatment	Percent survivorship ± SE							
	All replicates		Replicate 1		Replicate 2		Replicate 3	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
2×	17 ± 4.2[a]	0	11 ± 2.8[a]	0	25 ± 3.7[a]	0	15 ± 4.0[a]	0
1×	38 ± 6.7[a]	0	45 ± 4.8[a]	0	25 ± 4.0[a]	0	45 ± 7.5[a]	0
UF	98 ± 1.2	57 ± 13.7	96 ± 1.6	47 ± 6.8	100	84 ± 5.6	98 ± 1.3	40 ± 3.3
S	99 ± 0.7	81 ± 10.5	98 ± 1.3	84 ± 2.7	100	97 ± 1.5	98 ± 1.3	61 ± 8.5
UFS	98 ± 1.7	44 ± 20.6	95 ± 2.2	10 ± 4.7[a]	100	81 ± 3.5	100	41 ± 4.1
N	86 ± 8.9	0	69 ± 4.1[a]	0	99 ± 1.0	0	90 ± 3.3	0
LVS	94 ± 3.5	42 ± 11.8	87 ± 5.0	21 ± 6.7[a]	97 ± 2.1	62 ± 7.9[a]	98 ± 1.3	43 ± 6.8
AZ	99 ± 0.3	46 ± 8.9	99 ± 1.0	29 ± 4.6[a]	100	59 ± 5.3[a]	99 ± 1.0	50 ± 4.5
F value	52.92	1.37	99.26	29.38	266.64	9.35	92.81	2.30
df	7, 239	4, 149	7, 79	4, 49	7, 79	4, 49	7, 79	4, 49
P	0.0001	0.31	0.0001	0.0001	0.0001	0.0001	0.0001	0.07

Treatments are described in text.

[a] Means within a column are statistically different ($\alpha = 0.01$). Data were analyzed by PROC GLM and Tukey-Kramer HSD multiple range test.

Results

Experiment 1: Bioassays with Soil-Applied Imidacloprid. Feeding on flowers from imidacloprid-treated plants affected parasitoid behavior and survivorship by causing them to tremble and die. Trembling was 88% on 1× and 94% on 2× treatments, but 0% on the other treatments (UF, S, UFS, N, LVS, and AZ). At 1 d, survivorship of parasitoids confined to the flowers of the 1× and 2× treatments was significantly lower than survivorship on untreated flowers and all other treatments (Table 1): 38% in 1×, 17% in 2×, 98% in UF, 99% in S, 98% in UFS, 86%. in N, 94% in LVS, and 99% in AZ treatments.

At 7 d, mortality was 100% for 1×, 2×, and N treatments (Table 1). None of the other treatments were significantly different when 1×, 2×, and N treatments were excluded from the analysis (because of 0% survivorship).

Experiment 2: Cold Anthrone Test. The cold anthrone test showed that parasitoids ingested nectar from both untreated and imidacloprid-treated *F. esculentum* flowers. In replicate experiment 1, 100% of parasitoids confined to untreated flowers tested positive for nectar feeding, whereas parasitoids confined to 1× and 2× imidacloprid-treated flowers showed 50 and 65% positive results for nectar feeding ($\chi^2 = 12.75$, df = 2, $P = 0.002$), respectively. In replicate experiment 2, 95% of parasitoids confined to untreated flowers tested positive for nectar feeding, whereas those confined with 1× and 2× imidacloprid-treated flowers showed 65% positive results for nectar feeding in both treatments ($\chi^2 = 6.29$, df = 2, $P = 0.04$). We observed that 100% of parasitoids that fed on imidacloprid-treated flowers fell to the base of the flowers and trembled. We speculate that in 1× and 2× treatments, parasitoids died before ingesting enough fructose to be identified by the cold anthrone reagent.

Experiment 3: Determination of Imidacloprid, Hydroxy, and Olefin Residue in Flowers. For all five replicates, the UF treatment had no residues, whereas 1× and 2× flowers contained imidacloprid, hydroxy, and olefin metabolites (Table 2). Each sample consisted of nectar extracted from 425 flowers (adjusted concentration per flower). Imidacloprid concentra-

Table 2. Imidacloprid, hydroxy, and olefin residues in nectar of flowers of *F. esculentum* (ppm mean ± SE) from control plants (UF) and plants treated with 1× label and 2× label rate of soil-applied imidacloprid (Marathon 1% G)

Replicate	Residues in nectar of flowers of *F. esculentum*								
	Imidacloprid ppm (mean ± SE)			Hydroxy ppm (mean ± SE)			Olefin ppm (mean ± SE)		
	UF	1×	2×	UF	1×	2×	UF	1×	2×
1	0.0 ± 0.0a	3.55 ± 0.05b	4.70 ± 0.10c	0.0 ± 0.0a	0.75 ± 0.050b	0.92 ± 0.190b	0.0 ± 0.0a	0.24 ± 0.01b	0.30 ± 0.01c
	$F = 1440.60$, df (2,5), $P < 0.0001$			$F = 19.4275$, df (2,5), $P < 0.0192$			$F = 1458.5$, df (2,29), $P < 0.0001$		
2	0.0 ± 0.0a	6.65 ± 0.35b	5.20 ± 0.70b	0.0 ± 0.0a	1.40 ± 0.01b	2.15 ± 0.35b	0.0 ± 0.0a	0.47 ± 0.07ab	0.74 ± 0.20b
	$F = 59.89$, df (2,5), $P < 0.0038$			$F = 29.16$, df (2,5), $P < 0.0108$			$F = 9.37$, df (2,29), $P < 0.0513$		
3	0.0 ± 0.0a	7.50 ± 0.343b	25.15 ± 2.50b	0.0 ± 0.0a	1.51 ± 0.09a	3.25 ± 0.45b	0.0 ± 0.0a	00 ± 00a	0.86 ± 0.03b
	$F = 88.77$, df (2,5), $P < 0.0021$			$F = 38.55$, df (2,5), $P < 0.00072$			$F = 821.78$, df (2,29), $P < 0.0001$		
4	0.0 ± 0.0a	11.45 ± 1.25b	17.40 ± 3.00b	0.0 ± 0.0a	1.49 ± 0.32ab	2.75 ± 0.32b	0.0 ± 0.0a	0.30 ± 0.30a	0.67 ± 0.20a
	$F = 22.21$, df (2,5), $P < 0.0159$			$F = 28.55$, df (2,5), $P < 0.0112$			$F = 2.66$, df (2,29), $P < 0.2163$		
5	0.0 ± 0.0a	3.59 ± 0.45ab	7.50 ± 0.34b	0.0 ± 0.0a	3.59 ± 0.45a	8.92 ± 2.29a	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	$F = 11.140$, df (2,5), $P < 0.00409$			$F = 6.90$, df (2,5), $P < 0.0756$					
All	0.0 ± 0.0a	6.55 ± 1.00b	12.27 ± 2.70c	0.0 ± 0.0a	1.08 ± 0.20b	1.94 ± 0.40c	0.0 ± 0.0a	0.20 ± 0.10b	0.51 ± 0.10b
	$F = 13.55$, df (2,29), $P < 0.0001$			$F = 18.16$, df (2,29), $P < 0.0001$			$F = 10.70$, df (2,29), $P < 0.0004$		

Each mean represents nectar extracted from a combined sample of 425 flowers. Means within a row under a chemical name followed by different letters are statistically different ($\alpha = 0.05$). Data were analyzed by Proc GLM and Tukey-Kramer HSD multiple range test.

tion was 0 ppm in untreated flowers, 6.6 ppm (16 ppb/flower) in 1× flowers, and 12.3 (29 ppb/flower) ppm in 2× flowers. Hydroxy concentration was 0 ppm in untreated flowers, 1.1 ppm (2.4 ppb/flower) in 1× flowers, and 1.9 ppm (4.4 ppb/flower) in 2× flowers. Olefin concentration was 0 ppm in untreated flowers, 0.2 ppm (0.5 ppb/flower) in 1× flowers, and 0.5 ppm (1.1 ppb/flower) in 2× flowers.

Discussion

Imidacloprid is a widely used insecticide with many formulations for different commodities and sites. It replaced organophosphates in ready-to-use products for homeowners (Bayer Advanced All-in-One Rose and Flower Care and Bayer Advanced Season Long Grub Control), as well as landscape, greenhouse, and nursery insecticides (Merit, Marathon, and Imicide) and agricultural products (Admire, Provado, and Gaucho). It is applied as a foliar spray, soil drench, soil granular, seed treatment, injected into irrigation systems, or injected directly into trees. Imidacloprid is a broad-spectrum insecticide that kills most insect species (Lind et al. 1998a, 1998b) As a systemic insecticide, imidacloprid's affect on behavior and mortality of beneficial insects is not well researched.

These data showed that imidacloprid applied as a soil granular is translocated to nectar in flowers and causes parasitoids to tremble and die. Imidacloprid induces overstimulation of the synapses, which results in hyperexcitation, convulsions, paralysis, and death (Nagata et al. 1997, Bloomquist 2001). A reduction in general mobility affects prey-finding abilities of natural enemies (Croft 1990) or increases mortality factors such as predation and desiccation (Ffrench-Constant and Vickerman 1985). The cold anthrone test showed that nectar was found in the guts of parasitoids feeding on UF, 1×, and 2× treatments, which confirmed that they were dying from imidacloprid treatments and not starvation. Furthermore, walking on the leaves did not kill parasitoids in LVS treatments. The AZ treatment did not kill parasitoids, indicating that it is a good choice for conserving parasitoids in IPM programs (Hoddle et al. 2003).

Residue analysis showed that imidacloprid and its olefin and hydroxy metabolites were present in *F. esculentum* nectar. Although few published papers were found on parasitoids, much research evaluated whether seed applications of imidacloprid, Gaucho, were translocated to nectar and affected behavior and mortality of *A. mellifera* and *Bombus* sp. Other studies investigated the translocation of imidacloprid to plant parts and nectar. When Gaucho is used in canola, maize, and sunflower, the large accumulation of biomass from seed to flowering will decrease the amount of imidacloprid present in plant parts. When imidacloprid is used at the time of flowering in potted or landscape plants, imidacloprid and its metabolites are translocated directly to leaves and nectar, probably in higher concentrations than when used as a seed treatment.

Research evaluated whether complaints by growers that imidacloprid was killing honey bees was justified. French bee keepers believe that honey bee mortality since 1995 and especially in 1997 was caused by Gaucho used in sunflower, maize, and canola. Gaucho was banned as a seed treatment in France in 1999 for 2 yr, in 2001 for 2 yr, and in 2004 for 3 yr (Apiservices 2005, Bonizzoni et al. 2006). Since 2000, a similar controversy occurred in Prince Edward Island (PEI) and New Brunswick, Canada. Admire is used on potatoes and was linked by beekeepers to massive losses of honey bees needed for blueberry pollination. Mortality of bees in PEI and New Brunswick was usually 5–10% annually, but recently mortality rates of 30–50% were found. Bees do not feed on potato flowers, but bee keepers place hives in fields to feed on clover, a common rotation crop. Field samples were collected from sites in PEI and New Brunswick and residue analysis found levels of imidacloprid at 38 ppb in soil, but residues were not detectable in clover flowers or nectar, or pollen collected by honey bees (Rogers and Kemp 2003). In New Zealand, substantial unexplained bee losses caused growers to remove bees used as pollinators from squash fields where imidacloprid was used. Also, imidacloprid-treated clover was planted and may be another cause of mortality (Gregory 2005). Some research supports that imidacloprid previously in soils can be translocated to growing plants and pollen (Bonmatin et al. 2005b).

Data showed that imidacloprid in sugar solutions can alter behavior and kill *A. mellifera* and *Bombus* sp. After ingesting imidacloprid for 8 d, mortality was 50% at levels between 0.1 and 10 ppb (Suchail et al. 2001). In another study, imidacloprid presented to *A. mellifera* at 5 ppb in syrup for 13 d caused changes in behavior, such as higher frequency of pollen carrying and larger number of capped brood cells, which was reversed when contaminated syrup was no longer provided (Faucon et al. 2005). Reports from a Bayer researcher argued that levels below the 20-ppb level do not affect *A. mellifera* behavior (Schmuck 1999, Schmuck et al. 2001), whereas above 20 ppb, a change in behavior was observed as a reduction in recruitment to food sources (Schmuck 1999). Bumble bees, *Bombus impatiens* Cresson and *B. occidentalis* Greene, exposed to 7 ppb imidacloprid showed no change in foraging rate, whereas bees exposed to 30 ppb had slower foraging rates and longer handling time (Morandin and Winston 2003). Ten weeks after seed treatment with Gaucho, flowers of *Phacelia tanacetifolia* Bentham contained 3–10 ppb of imidacloprid in nectar, which had no effect on behavior or survivorship of *A. mellifera* (Wallner et al. 1999).

Residue analysis from samples collected through France from 2000 to 2003 showed that imidacloprid was found in leaves, pollen, and nectar after Gaucho treatments (Bonmatin et al. 2005a). In maize pollen, Gaucho treatments resulted in 0.1–18 ppb (mean, 2 ppb) imidacloprid (Bonmatin et al. 2005a). In sunflower pollen, Gaucho treatments resulted in 3 (Bonmatin et al. 2005a) and 13 ppb imidacloprid at 1.3× label rate (Laurent and Rathahao 2003). In canola

pollen, Gaucho treatments resulted in 4.4–7.6 ppb imidacloprid (Scott-Dupree and Spivak 2001). Other research showed that sunflower and maize pollen contained 3.3 ppb imidacloprid (Schmuck et al. 2001). Gaucho treatments resulted in 1.9 ppb imidacloprid in sunflower nectar (Schmuck et al. 2001) and 0.6–0.8 ppb in canola nectar (Scott-Dupree and Spivak 2001). In this experiment, 16 ppb imidacloprid was found in *F. esculentum* nectar, which is higher than reported in the previous two studies.

Conservation biological control advocates the use of nectar plants for beneficial insect conservation (Landis et al. 2000). Soil-applied imidacloprid is used on plants grown in the greenhouse that are installed in the landscape. Also, homeowners and professionals use imidacloprid to protect flowering herbaceous plants, trees, and shrubs from insects. Predators, such as *C. maculata* (Smith and Krischik 1999), *Chrysoperla carnea* (Rogers et al. 2007), and in this study, a parasitoid had altered behavior and mortality after nectar-feeding on plants treated with soil-applied imidacloprid. The use of soil-applied systemic insecticide does not appear to be compatible with the use of nectar-feeding insects for biocontrol.

Acknowledgments

This research was supported by AURI PRO-807, Agricultural Utilization Research Institute, AURI, University of Minnesota, 2001.

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Received for publication 29 December 2006; accepted 5 July 2007.