Effects of Spring Imidacloprid Application for White Grub Control on Parasitism of Japanese beetle (Coleoptera: Scarabaeidae) by *Tiphia vernalis* (Hymenoptera: Tiphiidae)

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ABSTRACT Imidacloprid, a relatively long residual neonicotinoid soil insecticide, is often applied to lawns and golf courses in spring for preventive control of root-feeding white grubs. We evaluated effects of such applications on spring parasitism of the overwintered third-instar Japanese beetle, *Popillia japonica* Newman, by *Tiphia vernalis* Rohwer, an introduced solitary ectoparasitoid. Natural rates of parasitism on a golf course rough were significantly lower in plots treated with full or one-half label rates of imidacloprid in early May compared with untreated turf. Parasitism also was reduced when female *T. vernalis* were exposed to imidacloprid residues on turf cores in the laboratory. Such exposures did not affect wasp mortality, longevity, survival, or developmental period of *Tiphia* larvae feeding on hosts in treated turf. They did, however, reduce wasps' ability to parasitize hosts in nontreated soil for at least 1–2 wk postexposure. In Y-trail choice tests, wasps that previously had been exposed to treated turf failed to respond normally to host frass trails in the soil. Female wasps did not avoid imidacloprid residues, imidacloprid-treated host frass, or host grubs that had previously been exposed to treated soil. This study indicates that applying imidacloprid in early spring can interfere with biological control by *T. vernalis*, whereas postponing preventive grub treatments until June or July, after the wasps' flight period, will help to conserve *T. vernalis* populations.

KEY WORDS *Popillia japonica*, imidacloprid, sublethal effects, host location, conservation biological control

ROOT-FEEDING SCARABAEID larvae, commonly called white grubs, are the most damaging insect pests of turfgrass throughout most of the United States (Potter 1998). Because white grubs have traditionally been controlled with soil insecticides, relatively little attention has been paid to the biology or conservation of their natural enemies, especially parasitoids. Recent years, however, have seen increased restrictions on pesticide usage in urban and suburban areas, loss of insecticide registrations, and growing awareness of the benefits of environmental stewardship by the golf and landscape industries. These trends make it increasingly important to seek ways that integrate indigenous natural enemies with chemical controls.

Solitary wasps in the genus *Tiphia* are the predominant parasitoids of scarabaeid larvae (Clausen 1940). Despite the presence of >80 species of *Tiphia* in North America (Krombein et al. 1979), before our recent studies (Rogers and Potter 2002, Rogers et al. 2003), little was known about the biology of these wasps and the interactions with their host grubs. *Tiphia vernalis* Rohwer, which is native to Japan, Korea, and China, was first released in the United States in the 1920s for biological control of the Japanese beetle, *Popillia japonica* Newman (Clausen and King 1927, Fleming 1968). Although data concerning its present distribution are limited, the wasp seems to be established across much of the range of *P. japonica* in the eastern United States (Fleming 1968). In Kentucky, adults of *T. vernalis* are active from early May through early June, ovipositing on overwintered third-instars. We have found parasitism rates of *P. japonica* by *T. vernalis* to range from 15 to 50% at golf courses in central Kentucky (unpublished data).

Insofar as is known, most *Tiphia* are host specific, although some species may attack several congeneric host species (Jaynes and Gardner 1924). Female *Tiphia* wasps burrow into the soil and locate their soil-dwelling hosts using species-specific kairomones present in grub body odor trails and frass (Rogers and Potter 2002). Once a host is located, the wasp stings it, causing temporary paralysis. An egg is then attached to the grub in a location that is specific for each particular *Tiphia* species (Clausen and King 1927). The *Tiphia* larva feeds externally during its first four instars by piercing the grub's integument and imbibing the host's body fluids. During the fifth instar, it devours all but the sclerotized portions of the grub and then spins a silken cocoon in which it overwinters and from which it emerges the following year.

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Imidacloprid, a chloronicotinyl insecticide that acts on the nicotinic acetylcholine receptors of the insect nervous system (Elbert et al. 1991), is widely used for control of scarabaeid grubs, curculionid larvae, and mole crickets in turf. It is broad spectrum, systemic in plants, and has several months of residual effectiveness in the soil. Imidacloprid can be directly toxic to beneficial insects (Mizell and Sconyers 1992, Gels et al. 2002), but adverse sublethal effects also may occur. For predatory and parasitic insects, such effects may include decreased longevity and reduced mobility leading to a decline in foraging and prey consumption (Smith and Krischik 1999, Stapel et al. 2000, Elzen 2001, Kunkel et al. 2001).

Imidacloprid may also have beneficial sublethal effects. At sublethal dosages, it can stimulate egg production in predatory mites (James 1997) or have antifeedant effects on targeted pests (Nauen et al. 1998, Devine et al. 1996). It also may alter the behavior of certain pests so as to make them more susceptible to biological control agents (Ramakrishnan et al. 1999, Koppenhöfer et al. 2000, Roditakis et al. 2000, Furlong and Groden 2001). Generalizations on the compatibility of imidacloprid with natural enemies cannot be made without first examining both the lethal and sublethal effects on the insect in question.

Because of its long residual activity, imidacloprid can be applied any time from May to July for preventive control of univoltine grub species (e.g., Japanese beetle, masked chafers) that normally eclose in July and August. Homeowners and lawn care companies often apply it in May for "season-long" grub control, and golf course superintendents may also do so when attempting to control the earlier-hatching black turfgrass ataenius, *Ataenius spretulus* (Haldeman), with the same application (Niemczyk and Shetlar 2000). Such application timing coincides with the activity of *T. vernalis* during the spring. We therefore examined the compatibility of an early May application of imidacloprid with parasitism of *P. japonica* by *T. vernalis.*

Materials and Methods

Collection and Handling of Parasitoids and Grubs. Female *T. vernalis*, which feed on honeydew secretions of aphids, scale insects, and other homopterans (Clausen and King 1927), were collected from golf courses in central Kentucky in late April and May. The wasps were attracted by spraying a 10% sugar water solution on the foliage of trees bordering areas of turf. A hand-held vacuum (BioQuip, Gardena, CA) was used to capture them. Female wasps were placed individually into covered 118-ml plastic cups (Solo, Highland Park, IL) half-filled with moist autoclaved soil. A film canister lid containing a piece of dental wick soaked in 10% sugar water was placed on the soil surface as food. Cups were held at room temperature and a light regimen of 14:10 h (L:D) until wasps were used in experiments during the following 14 d. Overwintered third instar *P. japonica* were collected from beneath turf at a central Kentucky sod farm in late April and early May. Grubs were held in plastic containers (26.5 by 19.5 by 10 cm) containing a 1:1 mixture of autoclaved soil and peat moss at room temperature (22–24°C) until used.

Effects of Imidacloprid on Grub Parasitism Rates and Wasp Fitness. Twenty-four PVC rings (39.0 cm diameter by 10.2 cm height), arranged in a 3 by 8 randomized block design, were driven into the rough, consisting primarily of Kentucky bluegrass (*Poa pratensis* L.), at a golf course in Pendleton County, KY. Parasitized grubs had been found at the site the previous year, and *T. vernalis* adults also were observed visiting sugar-sprayed leaves of nearby trees. On 1 May, 30 third-instar *P. japonica* were placed onto the surface of the turf in each ring and allowed to burrow into the soil. After 1 h, the turf within each ring was treated with imidacloprid (Merit 75 WP [wettable powder]) at full label rate (0.45 kg [AI]/ha), one-half label rate (0.225 kg [AI]/ha), or left untreated. Applications were made using a nozzle from a hand-operated spray bottle that was inserted into a graduated cylinder to spray out an exact quantity of insecticide. All rings were then irrigated with 1.5 cm of water using a clean graduated cylinder and spray nozzle. After 21 d, the turf within each ring was excavated down to 20 cm, and all *P. japonica* grubs, with or without an attached *Tiphia* larva, and *Tiphia* cocoons were collected. Number of grubs per enclosure and total number of grubs that had been parasitized (including *Tiphia* cocoons) were compared among the treatments by one-way analysis of variance (ANOVA) (Analytical Software 2000), followed by Dunnett's test to compare treatment means against controls (Steel and Torrie 1960). In addition, a χ^2 test for heterogeneity (Steel and Torrie 1960) was used to compare the total proportion of parasitized versus nonparasitized grubs between each rate of imidacloprid and the control.

A similar experiment was conducted in the laboratory in early May to examine the effects of imidacloprid on grub parasitism rates, as well as mortality, longevity, and oviposition success of adult *T. vernalis.* Using a golf course cup cutter, 30 turf cores (10.5 cm diameter) were taken from a stand of perennial ryegrass, *Lolium perenne* L., at the University of Kentucky research farm. Each turf core was placed into a plastic container (11 cm diameter by 14 cm height). A cork borer was used to make 10 holes by removing plugs (12 mm diameter by 25 mm depth) from each core. One third-instar *P. japonica* was placed into each hole, and the plugs were replaced. Using the nozzle from a hand-held spray bottle inserted into a graduated cylinder to deliver an exact quantity of insecticide, 10 turf cores were treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha, 10 were treated at one-half label rate (0.225 kg [AI]/ha), and 10 turf cores were left untreated. All turf cores were then irrigated with 1.5 cm of water as before. One female *T. vernalis* was then confined on each turf core by inverting a clear plastic container (11 cm diameter by 14 cm height) over the container with the soil core. The bottom had been removed from the inverted containers and replaced with fine mesh screen for ventilation. The edges of the two containers were sealed using tape. Before sealing, a plastic film canister lid containing a piece of dental wick moistened with 10% sugar water solution was placed on the surface of the turf core as a food source for the wasp. After 10 d, each turf core was broken apart, and the grubs were examined for parasitism.

Wasps were recovered from each core to compare mortality among treatments. Wasps still alive were then held individually to determine if prior exposure to imidacloprid affected their longevity and oviposition rate. The exact age of the wasps used in this experiment is unknown because they were field collected. However, all wasps were collected on the same date early in their seasonal flight period, and thus, it is likely that there was little variation in age. These females were placed into separate 118-ml plastic cups (Solo) half-filled with moist autoclaved soil. Food (10% sugar water) was provided as before. One third-instar *P. japonica* was placed into the cup, and a lid was added to prevent the wasp from escaping. Containers were held at room temperature and a light regimen of 14:10 h (L:D). Each day, all grubs were checked for parasitism, and parasitized grubs were replaced with new grubs as needed. Longevity of wasps previously confined to imidacloprid treated and untreated turf cores was compared using the Log rank test for survival analysis (Analytical Software 2000). Oviposition (number of grubs on which an egg was laid) was compared by one-way ANOVA (Analytical Software 2000) followed by Dunnett's test (Steel and Torrie 1960) to compare the treatment means against controls. Data were analyzed separately within three intervals: 1–8, 9–16, and 17–24 d after removal of wasps from the turf cores. The total number of grubs parasitized over the 24-d period also was compared.

Imidacloprid Effects on Host Location and Recognition. *Tiphia* spp. exploit host species-specific kairomones present in grub body odor trails and frass to locate their hosts while below ground (Rogers and Potter 2002). We therefore examined whether sublethal exposure to imidacloprid affects the normal ability of *T. vernalis* to follow frass trails to their hosts. Thirty *L. perenne* turf cores were harvested, treated with imidacloprid at 0.45 kg ([AI])/ha and irrigated with 1.5 cm of water as previously described. A second set of thirty turf cores received only 1.5 cm of irrigation. One female *T. vernalis* was confined on each of these cores, as previously described, for 24 h. Choice tests were conducted in a soil-filled observation chamber as described in Rogers and Potter (2002). Briefly, a Y-shaped trail (0.5 cm wide) was made by dragging a dental wick in the soil. The base of the Y was 8 cm long with each arm extending at a 45° angle for 8 cm. *P. japonica* frass (0.3 g) was sprinkled along one arm of the Y-trail, while the other arm remained empty. Each wasp was then introduced into the observation chamber through a tube at the base of the Y-trail and observed as it moved along the trail and encountered the two arms of the Y. A choice was recorded when the wasp had traveled at least 6 cm down one arm of the Y-trail. Between runs with individual wasps, the observation chamber was disassembled and cleaned, fresh soil was added, and a new Y-trail was made with the treatments rotated to the opposite arms. Wasp trail choice was analyzed by χ^2 test against the null hypothesis of equal trail choice (Steel and Torrie 1960).

Imidacloprid Avoidance Tests. Three experiments were conducted to determine if *T. vernalis* avoids imidacloprid-treated turf. In the first experiment, 10 pairs of *L. perenne* turf cores (10.5 cm diameter), placed in plastic containers (11 cm diameter by 14 cm height), were provisioned with 10 third-instar *P. japonica* per core as previously described. One turf core from each pair was treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha), whereas the other core was left untreated. Both cores in each pair were irrigated with 1.5 cm of water. Each pair of turf cores was then placed into a larger plastic container (33 by 26.5 by 10.5 cm) with another container of equal size inverted over the first and the edges sealed with tape. Four holes (4 cm diameter) had been cut in the top of the inverted container and covered with a fine mesh screen for ventilation. This created an enclosure within which the wasps could move freely between the cores but from which they could not escape. A dental wick soaked in 10% sugar water was placed in the bottom of the enclosure for food, and one female wasp was placed into each enclosure. After 10 d, each set of cores was broken apart, and grubs were examined for parasitism. The total number of parasitized grubs was compared between enclosures containing two untreated cores and enclosures containing one imidacloprid treated and one untreated turf core by one-way ANOVA (Analytical Software 2000). Paired *t*-tests were used to compare grub parasitism among the pots within each treatment.

In a second experiment, two trials were conducted using the soil Y-trail test described earlier to determine if *Tiphia* avoided imidacloprid residues in the soil. In the first trial, one arm of the Y-trail remained empty, whereas the other arm contained 0.3 g of frass from *P. japonica.* The frass was treated with a solution of imidacloprid (0.45 kg [AI]/ha) using an eyedropper. Water was applied to the opposite empty arm of the Y-trail to provide similar soil moisture conditions. In a second trial, both arms of the Y-trail were provisioned with 0.3 g of host frass. One frass trail was treated with imidacloprid (0.45 kg [AI]/ha), whereas the other was treated with water only. Choice of 30 female *T. vernalis* was tested in each trial. Between runs with individual wasps, the observation chamber was disassembled, cleaned, fresh soil was added, and a new Y-trail made as before. Treatments were rotated to opposite arms of the Y-trail before each run. The criterion for trail choice and data analyses were as described before.

To test if female *T. vernalis* avoid hosts contaminated with imidacloprid, choice tests were conducted where wasps were provided with grubs previously confined on either imidacloprid-treated or untreated turf cores. Fifty third-instar *P. japonica* were placed into turfgrass cores as previously described. The label rate of imidacloprid (0.45 kg [AI]/ha) was then applied to the cores containing grubs, followed by 1.5 cm of water. An additional 50 third-instars were placed into untreated turf cores that were then irrigated with 1.5 cm of water. Grubs remained in the cores 7 d, after which five imidacloprid-treated and five untreated grubs were placed into each of 10 plastic containers (15 cm diameter by 6.5 cm height) filled with autoclaved soil. To distinguish between grubs from the two treatments, the left antenna was removed from those exposed to imidacloprid, whereas the right antenna was removed from the controls. A film canister lid containing dental wick moistened with 10% sugar water was placed onto the soil surface, and one female wasp was then added to each container. After 5 d, grubs were examined for parasitism. In each container, the number of parasitized grubs from each treatment was compared using a paired *t*-test (Analytical Software 2000).

Imidacloprid Effects on *Tiphia* Larva Survival and Development. Ability of *T. vernalis* larvae to develop on hosts in imidacloprid-treated soil was examined. Sixty *L. perenne* turf cores were placed into plastic containers (11 cm diameter by 14 cm height). Five third-instar *P. japonica* bearing an egg of *T. vernalis* were placed into each core as previously described. Twenty cores were treated with imidacloprid at label rate (0.45 kg [AI]/ha), 20 cores were treated at one-half label rate (0.225 kg [AI]/ha), and 20 cores were left untreated. All cores were irrigated with 1.5 cm of water after treatment. Five turf cores from each treatment were destructively sampled at 7, 14, 21, and 28 d after grubs were placed into the cores. Survival of *Tiphia* larvae on each sampling date was compared between treatments using the Log rank test for survival analysis (Analytical Software 2000). The instar distribution of *Tiphia* present on the grubs at each sample date was compared between treatments using one-way ANOVA (Analytical Software 2000).

Results

Effects of Imidacloprid on Grub Parasitism Rates and Wasp Fitness. In the field, there was no difference in total number of healthy and parasitized grubs, plus *Tiphia* cocoons, recovered from imidacloprid-treated versus untreated turf ($F = 1.76$; df = 2, 21; $P = 0.19$), indicating that the treatments themselves did not significantly reduce the host population. Mean number of grubs surviving in each plot were 20.5 ± 3.2, 15.1 ± 2.9, and 13.4 ± 2.2 for controls, one-half and full label rates, respectively. However, significantly fewer parasitized grubs were found in turf treated with imidacloprid at label and one-half label rates compared with the untreated control ($F = 6.87$; df = 2, 21; $P < 0.05$; Dunnett's test; $P < 0.05$; Fig. 1). The overall percentage of grubs parasitized was 13.1, 35.5, and 43.9% for full rate, one-half rate, and control plots, respectively. Compared with the controls, the proportion of available grubs parasitized was significantly reduced by the full rate ($\chi^2 = 27.0$, $P < 0.001$) but not by the one-half rate ($\chi^2 = 1.69$, $P > 0.05$).

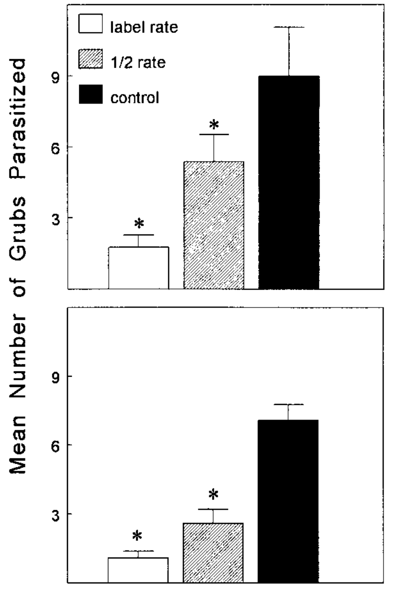

Fig. 1. Number (mean ± SE) of *P. japonica* parasitized by *T. vernalis* in field plots (top graph) and *L. perenne* turf cores in the laboratory (bottom graph) treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha), one-half label rate (0.225 kg [AI])/ha), or left untreated. Field plots and turf cores initially were infested with 30 or 10 grubs, respectively. Means marked with an asterisk are significantly different from the control (Dunnett test, $P < 0.05$).

Similarly, when wasps were confined for 10 d on pots of turf treated with imidacloprid at label or one-half label rates, significantly fewer grubs were parasitized in both imidacloprid treatments compared with control pots ($F = 25.1$; df = 2, 27; $P < 0.001$; Dunnett's test; $P < 0.05$; Fig. 1). Despite the reduction in grub parasitism, confinement on imidacloprid-treated cores did not affect wasp survival. Nearly all of them survived, with only 1 of 30 wasps dead after confinement for 10 d on imidacloprid-treated or -untreated cores.

After removal from turf cores, there was no difference in survival of wasps among treatments (Log rank test; $\chi^2 = 3.18$, df = 2, $P > 0.05$). Wasps previously confined on untreated turf cores or cores treated with one-half or full label rate of imidacloprid survived an average of 18.6 ± 2.3, 18.1 ± 2.3, and 16.8 ± 2.3 d, respectively. Despite having no effect on their mortality and longevity, prior exposure to the full label rate of imidacloprid did significantly reduce subsequent parasitism (one-way ANOVA; $F = 9.26$; df = 2, 69; $P < 0.001$; Fig. 2). Wasps previously exposed to turf treated with the label rate of imidacloprid laid significantly fewer eggs compared with wasps confined on untreated turf cores during the first 16 d after confinement (Dunnett's test; $P < 0.05$; Fig. 2). This effect eventually waned, with no significant difference in fecundity during the last 8 d of the experiment. Overall, the combined parasitism rate from all three 8 d observation periods was significantly reduced in imi-

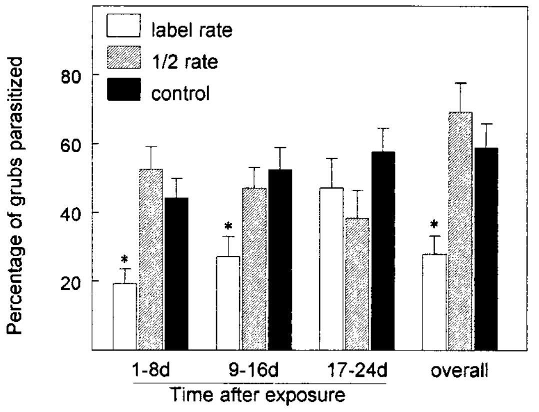

Fig. 2. Percentage of *P. japonica* grubs parasitized by *T. vernalis* previously confined for 10 d on *L. perenne* turf cores treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha), one-half label rate (0.225 kg [AI]/ha), or left untreated. Wasps were provided with one host grub per day. Means marked with an asterisk are significantly different from the control (Dunnett test, $P < 0.05$).

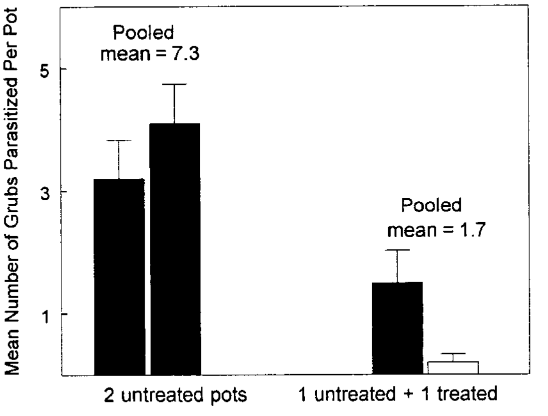

Fig. 3. Number (mean ± SE) of grubs parasitized by *T. vernalis* when the wasps were confined with two pots of untreated *L. perenne*, when one pot was treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha) and the other pot was left untreated. There were initially 10 *P. japonica* grubs per pot.

dacloprid treated plots compared with controls (Dunnett's test; $P < 0.05$; Fig. 2). Prior exposure to the one-half rate of imidacloprid did not affect parasitism by *T. vernalis* (Dunnett's test; $P > 0.05$; Fig. 2).

Imidacloprid Effects on Host Location and Recognition. Wasps previously confined on untreated turf cores responded normally (Rogers and Potter 2002) in Y-trail choice tests, following the trails containing host frass (Table 1). Of the 30 wasps exposed to cores treated with imidacloprid, only two chose the trail containing frass from *P. japonica* while the rest were nonresponsive. On reaching the juncture of the Y-trail, the nonresponding wasps briefly antennated the frass and then slowly backed down the trail, stopping to groom their antennae and antennate the surrounding soil. This process lasted several minutes with no attempt made by wasps to proceed along either arm of the Y-trail.

Imidacloprid Avoidance Tests. Wasps confined with two pots of untreated turf parasitized significantly more grubs compared with wasps confined with an imidacloprid-treated and -untreated turf core (one-way ANOVA; $F = 61.1$; df = 1, 18; $P < 0.001$; Fig. 3). Within the treatments, there were more grubs parasitized in untreated turf cores than in treated turf cores ($t = 2.51$, df = 9, $P = 0.03$), but no difference in parasitism rate among pairs of untreated cores ($t = 0.76$, df = 9, $P = 0.47$).

In Y-trail choice tests, wasps chose trails containing imidacloprid-treated frass over empty trails (Table 2). When both arms of the Y trail were provisioned with frass and the frass on one arm was treated with imidacloprid, wasps showed no significant difference in trail choice (Table 2).

In the choice tests wherein *T. vernalis* females were confined in containers of soil with 10 *P. japonica* grubs, one-half of which had previously been held in turf cores treated with imidacloprid, wasps showed no preference in ovipositing on grubs from either treatment (paired *t*-test, $P > 0.05$). Mean (±SE) numbers that were parasitized were 2.7 ± 0.4 versus 4.7 ± 0.8 for imidacloprid-exposed versus control grubs, respectively ($t = 1.75$, df = 9, $P = 0.11$).

Imidacloprid Effects on *Tiphia* Larva Survival and Development. There was no difference in survival of *Tiphia* larvae on grubs in untreated turf cores or those

Table 1. Response of female *T. vernalis* to frass from their host (*P. japonica*) after wasps were confined on untreated turf cores or cores treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha)

Treatment	n	Trail selected[a]		χ^2
		P. japonica frass	Empty trail	
Wasps confined on untreated cores	40	26	4	16.1[b]
Wasps confined on imidacloprid treated cores	40	2	0	NR[c]

[a] Response of wasps when given a choice between two trails.
[b] $P < 0.001$.
[c] Lack of response precluded statistical analysis.

Table 2. Response of female *T. vernalis* to host (*P. japonica*) frass treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha)

n	Trail selected[a]		χ^2
	Frass trail treated with imidacloprid	Empty trail	
30	26	4	16.1[b]
	Frass trail treated with imidacloprid	Frass trail without imidacloprid	
30	18	12	1.2

[a] Response of wasps when given a choice between two trails.
[b] $P < 0.001$.

Table 3. Percentage survival and instar distribution of *T. vernalis* on third instar *P. japonica* in pots of perennial ryegrass treated with imidacloprid (Merit 75 WP) at label rate (0.45 kg [AI]/ha), one-half label rate, or left untreated (n = 25 for each date)

Treatment	7 d	14 d			21 d				28 d		
	Percent survival	2nd instar	3rd instar	Percent survival	4th instar	5th instar	cocoon	Percent survival	5th instar	cocoon	Percent survival
Control	88	6	17	92	2	9	5	64	0	14	56
One-half rate	92	5	15	80	1	12	2	60	2	12	48
Label rate	84	3	16	96	0	9	2	44	1	11	48

treated with imidacloprid applied at label or one-half label rate (Logrank test; $\chi^2 = 0.75$, df = 2, $P > 0.05$; Table 3). Likewise, the instar distribution of *Tiphia* did not differ on hosts in imidacloprid-treated or -untreated turf cores ($F = 0.14$; df = 2, 205; $P > 0.05$; Table 3).

Discussion

Our study shows that parasitism of *P. japonica* by *T. vernalis* is reduced in imidacloprid-treated turf. Reduced parasitism was a result of sublethal effects of imidacloprid on foraging and oviposition behavior of adult females and not because of acute mortality or reduced longevity of the wasps or their larvae. This is not surprising because other studies have shown imidacloprid to have intoxicating effects on natural enemies that can reduce their foraging ability. For example, Kunkel et al. (2001) demonstrated that imidacloprid applied to turf caused paralysis and impaired the walking ability of the predatory ground beetle, *Harpalus pennsylvanicus* DeGeer. Such effects may have accounted for a temporary decline in predation on *Agrotis ipsilon* (Hufnagel) larvae after imidacloprid application (Kunkel et al. 1999). Imidacloprid has also been shown to reduce mobility of predatory coccinellids, likely resulting in reduced foraging success (Smith and Krischik 1999, Vincent et al. 2000).

Stapel et al. (2000) showed that, after feeding on extrafloral nectaries of cotton plants treated with imidacloprid, the parasitoid *Microplitis croceipes* Cresson did not show the typical flight response to host olfactory cues. Similarly, our Y-trail choice tests suggested that prior exposure to imidacloprid impairs the ability of female *T. vernalis* to recognize contact cues (e.g., grub frass) used in host location. Impaired locomotory behavior likely does not account for the reduction in grub parasitism because we never observed any reduction in activity of wasps previously exposed to imidacloprid. Such wasps appeared to move normally down the base of the Y-trail, but on reaching the split where a choice was to be made between a trail containing host frass versus one containing no host cues, the wasps began to behave abnormally. Nonimpaired wasps typically antennated the host frass and moved aggressively down the trail, rapidly antennating the frass and surrounding soil (Rogers and Potter 2002). In contrast, wasps previously exposed to imidacloprid only briefly antennated the frass and then immediately began grooming their antennae. After several minutes of antennal grooming, they eventually moved back down the base of the Y-trail in the direction from which they had previously entered.

One hypothesis that might account for this altered behavior is that imidacloprid interferes with chemosensory receptors on the wasps' antennae, thereby inhibiting host recognition. Grooming behavior is a reflex action (Eisner 1961) that probably is initiated by irritants on an insect's chemo- or mechanoreceptors. When chemical irritants were applied to the abdomen of *Periplaneta americana* L., a complex grooming behavior of the abdomen was begun (Reingold and Camhi 1978). These grooming or cleaning behaviors remove contaminates from irritated areas (Gratwick 1957). Golenda and Forgash (1986), for example, demonstrated that topical application of fenvalerate to *Musca domestica* L. evoked immediate grooming that removed 13% of the contaminant. When *Tiphia* spp. enter the soil searching for a host, the entire body is exposed to any contaminants that may be present. Because wasps groomed their antennae and no other body parts, it is possible that imidacloprid interfered with the antennal receptors and was the cause of wasps' nonresponsiveness to host cues and extensive grooming of their antennae.

Repellency of insecticides to beneficial insects can influence their compatibility with biological control (Longley and Jepson 1996). Because imidacloprid seems to have prolonged negative effects on host searching behavior of *T. vernalis,* it would be desirable for the wasps to avoid imidacloprid-treated turf. Repellency of imidacloprid has been reported for some insects, but many beneficials show no avoidance of imidacloprid-treated plants (Lagadic and Bernard 1993, Boiteau and Osborn 1997, Kunkel et al. 2001, Gels et al. 2002). Our results show that female *T. vernalis* do not avoid imidacloprid-treated turf. In Y-trail choice tests with imidacloprid-treated *P. japonica* frass versus an empty trail, wasps having no prior exposure to imidacloprid chose the trail containing the treated frass over the empty trail. Based on this experiment alone, it was possible that presence of frass was a stronger factor in trail choice than presence of imidacloprid. However, when both trails in the choice test contained frass, and the frass on one trail was treated with imidacloprid, wasps chose both trails with equal probability. Similarly, *T. vernalis* showed no preference between control grubs and those previously held in turf cores treated with imidacloprid.

Nonavoidance of imidacloprid by *T. vernalis* was further confirmed in the two-pot choice tests. Wasps parasitized significantly fewer total grubs when one of the two pots of grub-infested turf was treated with imidacloprid than when both were untreated. Hosts were not a limiting factor because there were a sufficient number of grubs for oviposition in the untreated turf core. There are two possible explanations for reduced parasitism in this experiment. First, as occurred after prolonged exposure (Fig. 2) and in the Y-trail choice tests (Table 1), even brief exposure to imidacloprid may impair wasps' host location behavior for an extended period thereafter. In the two-pot choice test, there was usually only one grub parasitized in the imidacloprid-treated turf core. Because successful oviposition by *T. vernalis* on a host grub takes ≈1 h (unpublished data), it is likely that once a wasp had entered the treated turf core and parasitized one grub, it had received sufficient exposure to imidacloprid to cause lasting impairment of host location behavior. An alternative explanation may be that the wasps recovered relatively quickly from brief exposure to imidacloprid but did not exhibit behavioral avoidance, even after repeated exposure. Regardless of which interpretation is correct, these results show that applying imidacloprid in May, when *T. vernalis* adults are active, may have detrimental effects on grub parasitism rates in nearby untreated turf as well as in treated areas.

Exposure to imidacloprid in soil had no observable effect on development time or survival of *Tiphia* larvae nor did it cause significant mortality of their post-overwintered third-instar hosts. Once grubs were parasitized, the external-feeding wasp larvae developing on *P. japonica* in turf cores treated with imidacloprid progressed through five larval instars to cocoon formation in the same amount of time as those larvae developing on hosts in untreated turf cores. We cannot determine from these results what effect, if any, imidacloprid may have on summer survival or overwintering success of *Tiphia* once they have formed cocoons. For example, exposure to nicotinoid insecticides may contribute to increased winter mortality of scarab larvae by inhibiting their normal downward movement in late autumn (Grewal et al. 2001).

This study underscores the importance of examining the interaction of insecticides and natural enemies for both lethal and sublethal effects. While survival, longevity, and locomotion of *T. vernalis* were unaffected by exposure to imidacloprid, their effectiveness in controlling white grubs was compromised by inhibition of host location. Proper timing of imidacloprid applications for grub control is therefore important for conserving natural *Tiphia* populations. Relatively long-residual nicotinoid soil insecticides allow preventive treatments to be applied before white grubs are present and are not intended to control overwintered third-instar *P. japonica* that are not as susceptible to imidacloprid as are neonates present in mid- to late summer. Additionally, these early season grub treatments may also be used to simultaneously control black turfgrass ataenius early in the year (Niemczyk and Shetlar 2000). However, if black turfgrass ataenius populations typically do not reach damaging levels at a site, postponing preventive grub applications until the flight period of *T. vernalis* has ended (about mid-June in Kentucky) will conserve the parasitoid population and likely result in greater natural control of *P. japonica* both in treated and adjacent untreated areas. Such timing will then provide good control of early instars eclosing in July and August when *T. vernalis* is not active.

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