Natural Enemies of *Atta vollenweideri* (Hymenoptera: Formicidae) Leaf-Cutter Ants Negatively Affected by Synthetic Pesticides, Chlorpyrifos and Fipronil

ANDREA C. GUILLADE[1] AND PATRICIA J. FOLGARAIT

Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal BXD1876, Buenos Aires, Argentina

J. Econ. Entomol. 107(1): 105–114 (2014); DOI: http://dx.doi.org/10.1603/EC12498

ABSTRACT In southern South America, *Atta vollenweideri* Forel (Hymenoptera: Formicidae) is a significant pest of several crops and forestry, also considered to reduce the carrying capacity of pastures. The most usual control method used in Latin America is the application of synthetic pesticides, mainly chlorpyrifos and fipronil. However, no studies have assessed the effects of these agrochemicals on natural enemies of ants. We aimed to evaluate the efficiency of these pesticides on leaf-cutter ants' control and to test their effect on phorid fly parasitoids. Chlorpyrifos failed to exert complete control over ant colonies in the field and was gravely detrimental to specific parasitoids, reducing their percentage of parasitism, pupal survivorship, and adult longevity. Fipronil, however, exerted complete control over the treated colonies. Laboratory tests using both pesticides, either on ants from foraging trails or on pupariae, showed that chlorpyrifos and fipronil decreased larval and pupal survivorship, as well as adult longevity of parasitoids, in comparison to controls. In conclusion, these pesticides will likely affect parasitoids with regard to their reproductive capacity, leading to the decreased levels of natural parasitism observed in the field after treatments. We discuss why neither pesticide should be taken into account for integrated pest management programs.

RESUMEN En el sur de Sudamérica, *Atta vollenweideri* Forel es una plaga importante en numerosos cultivos y forestaciones, considerándose además responsable de reducir la capacidad de carga en pasturas. El método de control más usualmente empleado en América Latina es la aplicación de plaguicidas sintéticos, principalmente clorpirifós y fipronil. Ningún estudio ha evaluado los efectos de estos agroquímicos sobre los enemigos naturales de las hormigas. Nos propusimos entonces evaluar la eficiencia de estos plaguicidas para el control de hormigas cortadoras de hojas, y probar sus efectos sobre sus fóridos parasitoides. El clorpirifós no ejerció un control completo sobre las colonias de hormigas en el campo, y fue gravemente perjudicial para los parasitoides específicos, reduciendo el parasitismo natural, la supervivencia pupal y la longevidad de los adultos. El fipronil, por otro lado, ejerció un control completo sobre las colonias tratadas. Ensayos de laboratorio usando ambos plaguicidas, ya sea sobre hormigas colectadas de caminos de forrajeo o sobre puparios, mostraron que tanto el clorpirifós como el fipronil disminuyeron la supervivencia larval y pupal y la longevidad adulta, en comparación con los controles. En conclusión, estos plaguicidas son altamente capaces de afectar a los parasitoides con respecto a su capacidad reproductiva, lo que resultaría en los niveles inferiores de parasitismo natural observados en el campo luego de su aplicación. Argumentamos entonces por qué ninguno de estos plaguicidas debería ser considerado para programas de manejo integrado de plagas.

KEY WORDS phoridae, leaf-cutter ant, chlorpyrifos, fipronil

Leaf-cutter ants in the genus *Atta* F. (Hymenoptera: Formicidae) are among the most important agricultural pests in Latin America (Cherret 1986, Hölldobler and Wilson 1990). *Atta vollenweideri* Forel is a significant pest of several crops and forestry (Cherret 1986), also causing economical losses to cattle ranchers through loss of carrying capacity of pastures (Vaccaro and Mousques 1997). However, systematic and insightful evaluations of losses are missing or are not published. The most usual method of control of leaf-cutter ants is the application of pesticides, mainly chlorpyrifos and fipronil (Della Lucia 1993, Link 1993, Boaretto and Forti 1997, Vaccaro and Mousques 1997, De Coll 1998, Filho and Dorval 2003, Zanetti et al.

[1] Corresponding author, e-mail: andreaguillade@gmail.com.

2003), although no long-term studies on the efficacy of these agrochemicals are available. Furthermore, few studies have assessed the effects these pesticides may have on beneficial insects in the agroecosystem (Smith and Lockwood 2003, Medina et al. 2007, Adán et al. 2011).

Chlorpyrifos, an organophosphate acting as a cholinesterase inhibitor, is a largely nonspecific pesticide with a growingly decreased efficacy against several pests (Rodríguez et al. 2010), and cases of pest resistance to this agrochemical have been widely reported (Rust and Reierson 1991, Nyrop and Hassney 2006, Ouyang et al. 2010, Rodríguez et al. 2010). In addition, growing evidence on its deleterious effects on human health have lead to substitution of chlorpyrifos with more modern pesticides (Eskenazi et al. 1999; Lee et al. 2004; Rauh et al. 2006, 2011), most notably fipronil, although small and mid-scale producers still use chlorpyrifos owing to its lower price, in an attempt to keep leaf-cutter ants under control. Fipronil is a phenylpyrazole that blocks $GABA_A$-gated chloride channels in the central nervous system, which results in excess neuronal stimulation and death of the target insect (Tingle et al. 2003). Despite the popularity of this pesticide (i.e., in Argentina, Cámara de Seguridad Agropecuaria y Forestal 2011), no long-term studies on its efficacy against leaf-cutter ants are available, and no assessments of its effect have been conducted for natural enemies of ants. However, fipronil has been reported to be highly toxic to other nontarget and beneficial organisms (Grout et al. 1997, Tingle et al. 2003, Williams et al. 2003, Gunasekara et al. 2007, Medina et al. 2007, Adán et al. 2011, Vidau et al. 2011), and several studies have linked this pesticide to human health issues (Hurley et al. 1998, Hodgson and Rose 2007, Sidiropoulou et al. 2011).

One of the alternatives to pesticides proposed for controlling leaf-cutter ant populations is biological control using natural enemies of ants. To our knowledge, only generalist entomopathogens and mycopathogens have been tried because they are available in the market (i.e., Attacebo, TrichoD-WP), although the only publications found refer to in vitro tests in the laboratory (Da Silva and Diehl-Fleig 1988, Diehl-Fleig et al. 1993, Silva et al. 2006, Lemus et al. 2008), and one to field tests on whole colonies (López and Orduz 2003). However, parasitoid flies in the genera *Apocephalus* Coquillet, *Myrmosicarius* Borgmeier, and *Eibesfeldtphora* Disney (Diptera: Phoridae) can be considered good candidates owing to the negative effect they exert on their hosts (Orr 1992, Feener and Brown 1993, Tonhasca Jr. 1996, Bragança et al. 1998, Erthal and Tonhasca 2000, Tonhasca et al. 2001, Elizalde and Folgarait 2010, Guillade and Folgarait 2011). A recent study on *Eibesfeldtphora trilobata* Disney (Guillade and Folgarait 2012) has shown that one female fly per foraging trail is enough to significantly reduce ant traffic, size of foragers on trails, and dry weight of plant material transported into the colony, thus affecting the colony's food intake. Furthermore, Elizalde and Folgarait (2012) have reported at least one phorid species attacking ants working at refuse piles, another essential task for the well-being of a colony. Therefore, an assemblage of phorids has a reasonable chance of keeping ant herbivory under control (Guillade and Folgarait 2011), and the chances of success are likely to increase if phorids are combined with other compatible agents of pest control.

Phorid females use their ovipositors to insert an egg into the body of worker ants engaged in different tasks; as development progresses, the larvae feed from their hosts' tissues, eventually killing them at the time of pupation (Disney 1994). The fate of these pupae in the environment is not yet clear, but one possibility is that they might be carried out to the refuse dumps among the carcasses of dead ants (at least for ants that have external refuse dumps). Thus, they are likely to be affected by pesticide applications, particularly those with high capacity for horizontal transfer, such as fipronil (Soeprono and Rust 2004, Wiltz et al. 2009). Similarly, sprayed pesticides may affect forager ants that have been parasitized but are still alive, as can be the case for fipronil, a pesticide that is often sprayed over the perimeter of a plot to control leaf-cutter ants within. A more thorough understanding of the effects of pesticides, not only on the targeted pests, but also on nontarget beneficial insects such as phorid flies, is necessary to better establish the most adequate strategy when attempting to control leaf-cutter ant populations.

The main objective of this study was to determine the effect of applying synthetic pesticides for the control of leaf-cutter ants on these pests, as well as on the populations of their specialist natural enemies, the flies in the Phoridae family. For this purpose, we decided to assess the effectiveness of the two pesticides, chlorpyrifos and fipronil, most commonly used against leaf-cutter ants, on these pests as whole colonies in the field (nest scale), on individual ants (ant scale), and also on pupae of phorid flies (phorid pupa scale). Furthermore, we aimed to identify their effect on key life history traits of phorid flies, such as developmental times, pupal survivorship, and adult longevity.

Materials and Methods

We tested the two most commonly used pesticides, chlorpyrifos and fipronil, on *A. vollenweideri* ants and their phorids, organizing the experiments according to the scale at which organisms were affected.

Nest Scale: Field Application Over Workers at Foraging Trails. This step consisted in applying each pesticide to whole colonies (nests), using the standard method used by ranchers, as described below. Each pesticide was tested separately and on different sets of nests.

Chlorpyrifos tests were conducted between February 2009 and March 2010. Twenty nests of similar dimensions (diameter = 5.39 ± 0.97 m and height = 0.44 ± 0.13 m) were selected, and samples of foraging ants were taken from each of them, to attain baseline data on both ants and parasitoids (the latter obtained from parasitized ants using passive sampling). Dimen-

sions of nests were obtained using a measuring tape. We verified that no significant differences existed between control and treatment nests regarding size (diameter: $F = 1.17$; df = 9; $P = 0.41$; height: $F = 2.82$; df = 9; $P = 0.06$), ant activity as number of active trails (10.31 ± 3.01; $F = 2.01$; df = 9; $P = 0.15$), and traffic as number of ants passing a fixed point in the trail in a minute (115 ± 34.12), counted in the trail exhibiting the heaviest traffic in each nest, that is, ants per trail ($F = 1.02$; df = 9; $P = 0.49$), as well as parasitism by phorids ($2.83 \pm 3.24\%$ parasitized ants; $F = 2.27$; df = 9; $P = 0.11$). Nests were assigned to two groups, control and treatment, with a minimum 150 m distance between nests of the same group; the distance between the control group and the treatment group was of at least 200 m, to ensure that treatments with pesticide did not affect control nests. After checking that no ant trails crossed the other patch, a first treatment with pesticide (Hormifav) chlorpyrifos 2.5 mg/g wettable powder (F. A.V. Esperanza, Santa Fe, Argentina) was applied to the 10 nests in the treatment group using a measuring cup (Colombraro, Buenos Aires, Argentina), at a rate of 17 g per foraging trail in all trails of the nest, which is the dose suggested in the pesticide's packaging, whereas the other 10 nests remained untreated (controls). We measured number of active trails and traffic as number of ants passing a fixed point in the trail per minute, counted in the trail exhibiting the heaviest traffic in each nest; we then took samples (between 300 and 400 individuals) of foragers every 3 mo from both treated and control nests. Pesticide application was repeated on treated nests after each sampling, following the standard application regime used by ranchers when using this pesticide, as treated nests continued to exhibit ant activity. Phorids were obtained by means of passive sampling (Elizalde and Folgarait 2010), which consists in collecting forager ants from trails and feeding them sucrose solution *ad libitum* until death, at which point pupae from parasitized ants can be reared in environmentally controlled chambers ($24 \pm 1°C$, $80 \pm 5\%$ relative humidity [RH], and a photoperiod of 12:12 [L:D] h) to determine natural percent parasitism, plus the following traits of phorid parasitoid life cycles: pupal survivorship, developmental times, adult longevity, size of parasitized ants (width of head capsule below the eyes), and size of adult phorids (width of mesonotum), following Guillade and Folgarait (2011).

Fipronil tests were conducted following the same design on another set of 20 nests with no significant differences regarding diameter (5.46 ± 0.85 m; $F = 1.97$; df = 9; $P = 0.16$), height (0.49 ± 0.16 m; $F = 1.04$; df = 9; $P = 0.47$), number of active trails (12.13 ± 2.14; $F = 1.27$; df = 9; $P = 0.36$), traffic of ants (112.33 ± 28.59 ants per nest; $F = 1.40$; df = 9; $P = 0.31$), and parasitism ($12.62 \pm 7.65\%$ parasitized ants; $F = 1.38$; df = 9; $P = 0.32$); experiments took place from May 2010 to April 2011. Nests were assigned to two groups, control and treatment, with a 200 m distance between both groups, to avoid interactions between control and treated nests. Pesticide (Fipronil 20 g 100/cc Clap, Bayer CropScience, Argentina) was applied at a rate of 100 ml per foraging trail, at a dose of 0.5 ml/liter, using a backpack pesticide sprayer (Giber, Buenos Aires, Argentina). Control nests were sprayed with 100 ml distilled water per foraging trail, using a handheld sprayer (Colombraro, Buenos Aires, Argentina) to avoid contamination with residual pesticides in the backpack sprayer.

Data regarding active trails, ant traffic, and natural percent parasitism, both for chlorpyrifos and fipronil assays, were analyzed by means of Repeated Measurements ANOVA using Statview for Windows 5.0 software (SAS Institute 1998), as data were normally distributed and showed homoscedasticity. Whenever data did not meet the assumptions of sphericity (ant traffic, pupal survivorship, and longevity of adults), a corrected F was reported. Data regarding phorid life cycles was not normally distributed; therefore, we used nonparametric statistics, Kruskal–Wallis nonparametric ANOVA for comparing more than two groups, and Mann–Whitney test for two groups comparisons; *P* values for all contrasts were adjusted by Bonferroni correction (Sokal and Rohlf 1995). We used Statistics for Windows 2.0 (Analytical Software 1998).

Ant Scale: Laboratory Application of Pesticides Over Workers Gathered From Foraging Trails. This stage consisted in applying each pesticide directly on ants collected during September 2011 from foraging trails of untreated nests, to better assess the effect of each agrochemical on individual ants and on parasitoids developing inside them, particularly for fipronil, as its application on nests resulted in complete inactivity of all colonies after the first treatment, and therefore it was not possible to collect foragers from treated nests. Three samples of ≈300 worker ants each were collected per nest from 10 nests and kept in separate plastic containers (28 by 14 by 8 cm, length, width, height) with a window covered with fine wire mesh to allow ventilation; one remained untreated as control, chlorpyrifos wettable powder was applied to the second sample, at a rate of 34 g per container, and the third sample was treated with 200 ml fipronil (0.5 ml/liter). Rates were twice those of the nest scale tests, as in that instance the pesticide was applied for a minute and traffic at those nests was ≈150 ants/min. Because our samples for this assay test were twice that number of ants, we doubled the rate, though maintaining the dose, to equal the number of ants directly exposed to the pesticide during the field application. All samples were maintained in the containers and fed sucrose solution *ad libitum* until all individuals were dead, to calculate mortality curves. Dead ants were counted and removed from containers every 48 h, then searched for phorid pupae, which were in turn reared as described previously. We measured percentage of parasitized ants, pupal survivorship, developmental times, adult longevity, and size of pupae and adults for control and both treatments. Developmental times were quantified to determine whether pesticides affected them in any way.

Survivorship curves were obtained through Kaplan–Meier nonparametric estimations using the Man-

tel test to assess whether there were significant differences between or among colonies or treatments; we carried out these analyses using Systat 13 for Windows (SYSTAT Inc. 2009). The rest of the data were analyzed using Kruskall–Wallis and Mann–Whitney nonparametric tests, as data were not normally distributed. We made Bonferroni adjustments to the alpha level whenever multiple contrasts were performed.

Phorid Pupa Scale: Laboratory Application of Pesticides Over Ant Heads With Pupae. This final test consisted in applying each pesticide directly to ant head capsules containing pupae of *E. trilobata*, obtained by passively sampling untreated nests during November 2011 and rearing those ants. We divided ant head capsules in such a way that there were no significant differences in size of ant head capsules among treatments ($H = 1.16$; $P = 0.55$), as a significant correlation has been shown to exist between size of ant head capsules and the size of adults emerging from them (Guillade and Folgarait 2011). We treated 50 heads with pupae of *E. trilobata* with 5.5 g of chlorpyrifos wettable powder, sprinkled through a fine wire mesh, and other 50 with 30 ml fipronil (0.5 ml/liter) applied with a hand-held sprayer; in this case dosages were one third of the field dosage, as we had one third of the amount of ants directly exposed during the field assay. A third batch of 50 untreated pupae, sprayed with distilled water, served as control. After treatment with pesticides, pupae were reared to determine pupal survivorship, developmental times, adult longevity, and size of parasitized ants and adult phorids for control and both treatments. Tests were conducted only on *E. trilobata* because, owing to the draft affecting our study area, this was the only species available in sufficient numbers for testing.

Because data were not normally distributed, we used nonparametric statistics with Bonferroni corrections as described in the previous scale, using Statistics for Windows 2.0.

Results

Nest Scale: Field Application Over Workers at Foraging Trails. Treatment with chlorpyrifos significantly reduced both the number of active trails per nest (treatment: $F = 91.81$, df = 18, $P < 0.0001$; time: $F = 2.15$, df = 4, $P = 0.08$; interaction: $F = 4.59$, df = 4, $P = 0.0023$) and the ant traffic on the trails (treatment: $F_C = 6.17$, df = 18, $P < 0.0001$; time: $F = 6.71$, df = 4, $P = 0.0001$; interaction: $F = 6.39$, df = 4, $P = 0.0002$), although the pesticide failed to exert complete control over the nests after a year of repeated treatment (Fig. 1A). Natural percent parasitism was also reduced when compared with control nests (treatment: $F = 17.39$, df = 18, $P = 0.0006$; time: $F = 1.15$, df = 4, $P = 0.3371$; interaction: $F = 1.50$, df = 4, $P = 0.2$) when taking into account all four phorid species present. Of these species, only *Apocephalus setitarsus* Brown could be statistically analyzed separately, as it was the only one present in sufficient numbers for statistical analyses throughout the study. Pupal survivorship for this species decreased as the assay progressed throughout the year (treatment: $F_C = 26.71$, df = 16, $P < 0.0001$; time: $F = 2.67$, df = 3, $P = 0.0387$; interaction: $F = 0.41$, df = 3, $P = 0.128$). The treatment exerted a detrimental effect on the longevity of adults (treatment: $F_C = 3.42$, df = 20, $P < 0.0001$; time: $F = 0.211$, df = 4, $P = 0.93$; interaction: $F = 0.231$, df = 4, $P = 0.22$). However, we found no significant differences between control and treatment for size of adults or developmental times (larval, pupal, and total) (Table 1).

Fipronil, however, completely halted activity on all treated nests after the first application (Fig. 1B), so that it was not possible to collect ants from foraging trails of treated nests from the second sampling date onward, whereas activity in control nests only fluctuated owing to seasonal patterns.

Ant Scale: Laboratory Application of Pesticides Over Workers Gathered From Foraging Trails. The effects of both pesticides on ant survivorship are shown in Fig. 2. We found a great variability among colonies, for control as well as both pesticide treatments. Nonetheless, when comparing the three treatments, there were significant differences between both pesticides and the control, with chlorpyrifos being the fastest in killing all ants (median: 2 d), whereas fipronil-treated ants had a median survivorship of 4 d ($\chi^2 = 7{,}590.44$; $P < 0.001$). It is important to point out that *A. vollenweideri* workers separated from their colonies tend to live an average of 10–12 d (A. C. G., unpublished data), so part of the mortality observed in our experiment is likely owing to natural senescence, as evidenced by the mortality recorded in the controls. However, both pesticides achieved 100% mortality significantly earlier than in control colonies. In addition, a colony by colony analysis on the effect of treatments on survivorship showed differences among the control, chlorpyrifos, and fipronil treatments (each $P < 0.05$).

Although no parasitized ants were recovered from the chlorpyrifos treatment, we were able to determine that application of fipronil reduced the percentage of parasitized ants to a third of the percentage in control ants ($U = 72$; $P = 0.01$; Table 2). Of the pupae recovered from fipronil-treated ants, only those of *E. trilobata* were available in sufficient numbers for statistical analysis (Table 2). Pupal survivorship for this species was 50% lower when treated with fipronil ($U = 67.50$; $P = 0.03$). Developmental times, both pupal ($U = 47.50$; $P = 0.000$) and total ($U = 36$; $P = 0.000$), were longer for phorids recovered from fipronil-treated ants, and adult longevity was reduced from 3.18 d for control phorids to 0.54 d for those emerging from treated ants ($U = 1344$; $P < 0.001$). Although there was no significant difference between the size of adults from control versus treatment ($U = 466$; $P = 0.18$), the pupae recovered from treated ants were a little larger, although significantly, than those from control ants ($U = 2574.5$; $P = 0.0001$).

Phorid Pupa Scale: Laboratory Application of Pesticides Over Ant Heads With Pupae. Pupal survivorship was lower for pupariae treated with fipronil ($U = 120$; $P = 0.0061$) and chlorpyrifos ($U = 123$; $P = 0.0035$) in comparison to the control. Furthermore, application of pesticides resulted in a significant de-

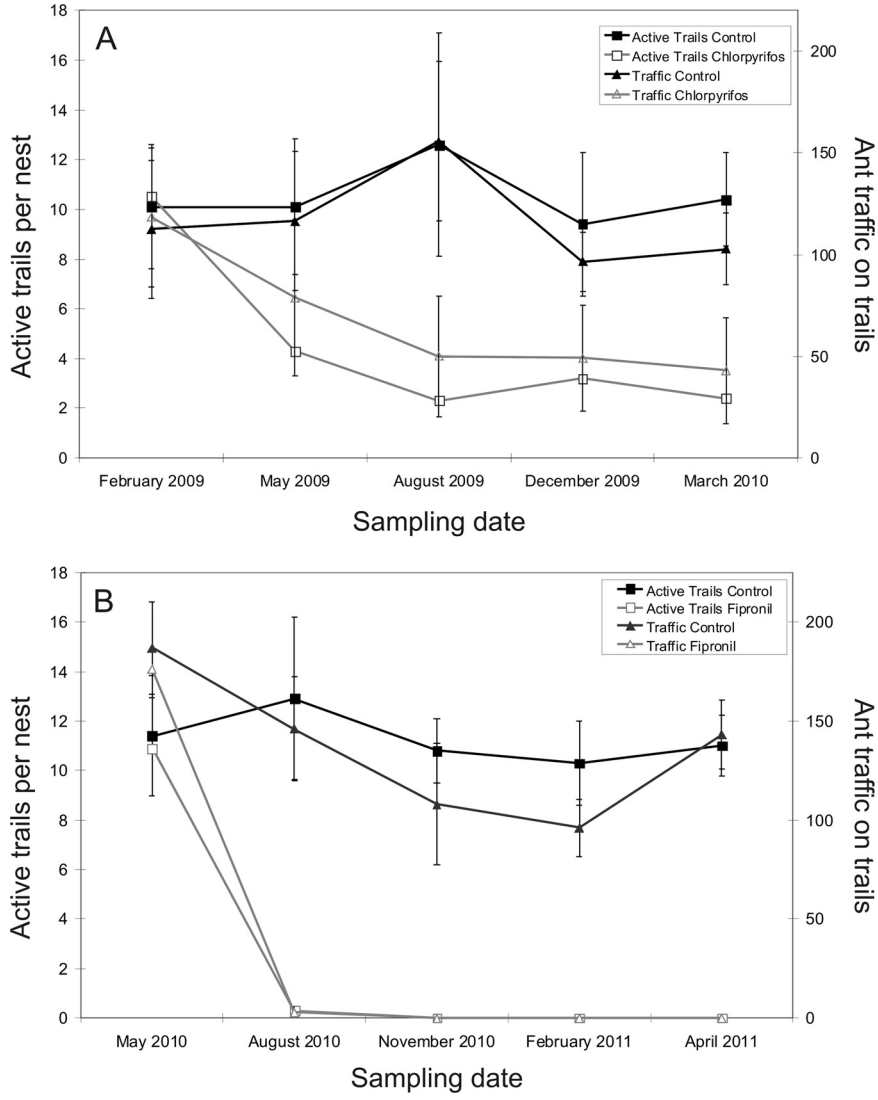

Fig. 1. Effect of pesticides on ants in nest scale experiments. (A) Mean number and standard deviations of active trails in nests and traffic of ants (workers per trail per minute) treated with chlorpyrifos versus control nests throughout the sampling dates. (B) Mean number and standard deviations of active trails in nests and traffic of ants (workers per trail per minute) in nests treated with fipronil versus control nests throughout the sampling dates.

Table 1. Pupal survivorship (%) and adult longevity (d) of *Ap. setitarsus* from control versus chlorpyrifos nests for the nest scale assays

Life history trait	Treatment	Feb. 2009	May 2009	Aug. 2009	Dec. 2009	Mar. 2010
Pupal survivorship (%)	Control	85 ± 32	89 ± 13	85 ± 22	88 ± 18	84 ± 18
		10	*10*	*10*	*10*	*10*
	Chlorpyrifos	84 ± 13	57 ± 26	49 ± 33	47 ± 33	43 ± 31
		10	*10*	*10*	*10*	*10*
Adult longevity (d)	Control	3 (3–3)	3 (2–4)	3.5 (3–4)	3 (3–4)	3 (3–4)
		23	*49*	*24*	*28*	*19*
	Chlorpyrifos	3 (3–2)	2 (2–3)	2 (1–3)	2 (1–3)	1.50 (1–2)
		28	*17*	*6*	*15*	

Results are shown as means with SD for pupal survivorship, and medians with 25% and 75% quartiles for all other measurements. Sample sizes for each sampling period are shown in italics; for pupal survivorship, this is the number of nests from which pupae were obtained.

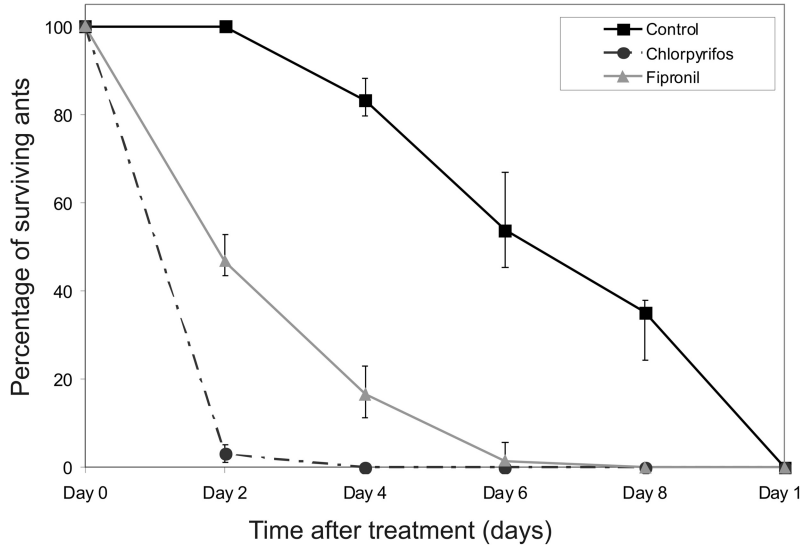

Fig. 2. Effect of pesticides on the survivorship of ants (median with 25% and 75% quartiles) in ant scale experiments.

crease in adult longevity, from 3.21 d in controls, to 0.75 d in adults from the fipronil treatment (U = 1218; $P = 0.0000$) and to 0.66 d with the chlorpyrifos treatment (U = 1134; $P = 0.0000$).

The treatment with chlorpyrifos shortened both the larval and the pupal time, thus resulting in a significantly shorter total developmental time (U = 894; $P = 0.0001$). Specifically, *E. trilobata*'s larval time was significantly shorter under the fipronil treatment (U = 752.5; $P = 0.0006$) than the control, but although it was also shorter for chlorpyrifos-treated pupariae than the control, this difference was not significant (U = 1577; $P = 0.02$). Pupal time, however, was significantly shorter for both treatments when compared with the control (fipronil vs. control: U = 845, $P = 0.0059$; chlorpyrifos vs. control: U = 827, $P = 0.0014$). When considering total developmental time, treatment with fipronil did not result in significant differences (U = 448; $P = 0.06$), as larval time was longer but pupal time shorter than the control. There was no significant difference in size between the adults emerged from pupae treated (both with fipronil and chlorpyrifos) and the control.

Discussion

Nest scale tests showed that chlorpyrifos, although significantly decreasing ant activity, could not effectively control *A. vollenweideri* colonies, as treated nests continued to exhibit active trails. Fipronil, however, completely stopped ant activity for at least 1 yr in all treated nests after the first application. Conversely, treatments on worker ants collected from foraging trails showed that application of chlorpyrifos resulted in complete mortality of treated ants within 24 h, whereas workers treated with fipronil lived between 48 and 72 h after treatment. Although there could be some overestimation of the mortality induced by the agrochemicals, given that application in containers did not allow the ants to escape the pesti-

Table 2. Pupal survivorship (%), adult longevity (d), size of hosts (head width, millimeters), and developmental times (d) for *E. trilobata* at the ant scale and phorid pupae scale experiments

Assay scale	Treatment	Pupal survivorship (%)	Adult longevity (d)	Size of hosts (mm)	Larval time (d)	Pupal time (d)	Total time (d)
Ant scale[a]	Control	87 ± 16	3 (3–3.75)	2.02 (1.92–2.16)	10 (9–10)	22 (21–23)	31.5 (31–32)
		10	*112*	*158*	*158*	*112*	*112*
	Fipronil	36 ± 21	0.50 (0.50–0.50)	2.16 (2.07–2.28)	10 (9–10)	27 (25–28)	37 (34.25–38)
		10	*12*	*52*	*52*	*12*	*12*
Pupae scale	Control	88 ± 19	3 (3–4)	2.41 (2.19–2.49)	6 (3–10)	21 (19–22)	26 (24.75–30.25)
		12	*42*	*50*	*50*	*42*	*42*
	Chlorpyrifos	41 ± 12	0.50 (0.50–1)	2.19 (2.07–2.40)	5 (4–6)	17 (17–24)	23 (22–27)
		12	*27*	*50*	*50*	*27*	*27*
	Fipronil	43 ± 18	0.50 (0.50–1.25)	2.19 (2.07–2.40)	7 (10–13)	20 (15–21)	29 (27–31.50)
		12	*29*	*50*	*50*	*29*	*29*

Data are shown as means with SD for pupal survivorship, and median with 25% and 75% quartiles for all other parameters. Sample sizes are shown in italics; for pupal survivorship, this is the number of nests from which pupae were obtained. See text for statistical comparisons.

[a] Data using chlorpyrifos are not available, as no parasitized ants were recovered from the chlorpyrifos treatment.

cide, we consider this a valid result, as our field observations show that ants directly sprinkled with pesticides die minutes after coming into contact with these substances, which results in great numbers of dead ants lining the foraging trails and nest entrances. Studies on termites (Ibrahim et al. 2003, Bagnères et al. 2009), Argentine ants (Soeprono and Rust 2004), and ghost ants (Ulloa-Chacón and Jaramillo 2003), show significant horizontal transfer from treated to untreated workers in fipronil assays, which would indicate that, although fipronil application does not result in immediate death of all workers treated, the high transfer rate between nestmates ensures the eventual death of the colony. Chlorpyrifos, however, effectively killed all ants directly exposed, but it evidently failed to reach the majority of the workers in the colony, thus reducing the number of ants in the trails but not halting their activity.

The effect of both pesticides on phorid flies is remarkably harmful. During the year of the chlorpyrifos field assay, only one species, *Ap. setitarsus*, could be reared in enough numbers for statistical analyses, and flies obtained from ants collected in foraging trails of treated nests had significantly greater pupal mortality and shorter adult lives than those reared from control nests. Our results from the ant scale tests indicate that *E. trilobata* larval survivorship is even more affected, as no parasitized pupae were recovered in this instance from chlorpyrifos assays, whereas we obtained them in the control group. It is noteworthy that these parasitoids exhibit marked seasonality in their abundance in the field, and that abundance is prone to change following environmental shifts in temperature, humidity, and precipitation (Elizalde 2009). During the 2 yr of our study, *E. trilobata* had a low natural percent parasitism throughout 2009, but the population peaked in autumn–winter of 2010 (March–July) and remained in high numbers during spring–summer of 2011 (September 2010–March 2011). *Ap. setitarsus*, however, was more abundant during autumn 2009, when *E. trilobata* was more scarce, but the populations began decreasing in autumn 2010 (March) and exhibited low abundance in winter–spring of 2010 (May–October). This population had a slight recovery in summer of 2010–2011 (December 2010–March 2011), and then plummeted again in autumn 2011 (April). This was the reason why we were forced to switch focal species from our nest scale assays in 2009 to our ant and phorid pupa scale assays in 2010–2011. Fortunately, our results from the control groups gave similar results on developmental parameters as previous ones gathered in the same area for the two parasitoid species (Guillade and Folgarait 2011), indicating that the negative effect detected on the phorids was owing to the pesticides and not to changes in circannual activities.

Both in ant and phorid pupa scale experiments, chlorpyrifos significantly reduced pupal survivorship and adult longevity in *E. trilobata*, two life cycle traits that are key to the survival of a phorid population, as a reduced adult longevity means greatly diminished chances for reproduction, especially for such short-lived flies. In other words, chlorpyrifos was in fact much more detrimental to natural enemies than to its intended target, the leaf-cutter ant *A. vollenweideri*. Given the relatively short life cycle of phorid flies, repeated applications of this organophosphate are likely to lead to local extinction of populations, as the half-life of the agrochemical and its high dispersal potential ensure its presence in the environment for up to 1.5 yr after the first treatment (Baskaran et al. 1999). To the environmental costs of using chlorpyrifos, we must add the cost to human health. Chlorpyrifos has been considered responsible for increased incidence of cancer, particularly in pesticide applicators (Lee et al. 2004), and it has been linked to several motor and mental disorders in children (Rauh et al. 2006, 2011). This organophosphate is slow to dissipate from soil, having a half-life of 116–1,576 d, and has been detected in fat and other tissues of slaughtered cattle (Ivey et al. 1978) and sheep (Ivey and Palmer 1981). It has also been linked to decreased organic matter decomposition (De Silva et al. 2010), suggesting that continued applications might lead to poorer soil quality for pastures, which would in turn force ranchers to provide cattle with dietary supplements, thus increasing production costs. Resistance to this pesticide has been reported for several organisms, such as German cockroaches (Rust and Reierson 1991), mosquitoes (Liu et al. 2005), greenbugs (Archer et al. 1994), citricola scale (Ouyang et al. 2010), and coddling moth (Rodríguez et al. 2010), among others. The high environmental and human health risks posed by this pesticide, along with its low effectiveness against leaf-cutter ants, and its detrimental effects on their natural parasitoids, should be arguments enough to cease applications of chlorpyrifos to attempt to control *A. vollenweideri*.

Fipronil was extremely effective against ants, but also highly detrimental to *E. trilobata* phorid flies. The pesticide reduced both larval and pupal survivorship, and it also decreased adult longevity, both in ant and phorid pupa scale assays. As discussed above for chlorpyrifos assays, fipronil is likely to affect the populations of parasitoids by reducing the adults' chances for reproduction and decreasing the availability of hosts. Because phorid flies have a rather low natural percent parasitism (Guillade and Folgarait 2011), a further reduction of their presence in the agroecosystem is likely to have fatal consequences for their populations if treated with this agrochemical. Fipronil has been detected in stored pollen loads in France and Spain (Chauzat et al. 2006, Bernal et al. 2010), and has been suspected to increase honey bee mortality (Vidau et al. 2011), as well as being shown to affect their olfactory memory processes (El Hassani et al. 2005). This pesticide has also been proven deleterious to a wide variety of natural enemies such as the predators *Euseius addoensis* and *Euseius citri* (Grout et al. 1997), and *Orius insidius* (Al-Deeb et al. 2001), the parasitoid *Anaphes iole* (Williams et al. 2003), and the endoparasitoids *Hyposoter didymator* (Medina et al. 2007) and *Psyttalia concolor* (Adán et al. 2011). Serious concerns have been raised regarding its effects

to human health and the environment (Tingle et al. 2003, Das et al. 2006), and it has been banned for applications in forestry (Forest Stewardship Council 2007, 2012). The long half-life in soil, both of the parent compound (111–350 d) and its highly toxic secondary product, fipronil-desulfinyl (1,479–7,159 d; Gunasekara et al. 2007), added to the many nontarget organisms affected, lead us to propose that the environmental costs of relying on this pesticide for the control of leaf-cutter ants may well exceed the benefits. Repeated applications of this pesticide are likely to produce a great loss of biodiversity, whereas many ecosystem services, which are poorly known and rarely quantified, might be lost in turn. Moreover, ants have been considered ecosystem engineers because of their role in nutrient cycling, decomposition of organic matter, and soil structuring and bioturbation (Folgarait 1998). The detrimental effects of suppressing them from the environment must be therefore taken into account when making cost–benefit evaluations regarding their control, particularly in situations where estimating crop loss to *Atta* herbivory is not straightforward, as is the case for forestry, where the plants have the potential to recover from ant herbivory, and thus defoliation is not immediately translated to yield loss.

In conclusion, our results show that the use of both chlorpyrifos and fipronil is incompatible with control programs using phorid flies against leaf-cutter ants, owing to the high mortality rates in both larval and pupal stages, and the reduced adult longevity induced by these agrochemicals on *Ap. setitarsus* and *E. trilobata.* In fact, we suggest from all the implications discussed here that both pesticides are incompatible for any integrated pest management program or strategy that incorporates not only phorid flies as biocontrollers, but any other type of insect or invertebrate. We propose that it is time to consider integrated pest management programs using several types of biological controllers (Drees et al. 2013) plus cultural and mechanical practices, and to start avoiding the use of synthetic pesticides. This should especially be the case for social insects that are so difficult to control.

Acknowledgments

The authors thank Familia Capovilla for allowing us to work in their ranch, and Familia Juncos for their logistical support. P.J.F. and A.C.G. thank CONICET. This research was funded by grants to P.J.F. by the National Council for Scientific and Technological Research, CONICET (PIP #5444 and E-643/10), the National Agency for the Promotion of Science and Technology, ANPCyT (PICT #20924), and Quilmes National University (PUNQ #0395/07).

References Cited

Adán, A., E. Viñuela, P. Bengochea, F. Budia, P. Del Estal, P. Aguado, and P. Medina. 2011. Lethal and sublethal toxicity of fipronil and imidacloprid on *Psyttalia concolor* (Hymenoptera: Braconidae). J. Econ. Entomol. 104: 1541–1549.

Al-Deeb, M. A., G. E. Wilde, and K. Y. Zhu. 2001. Effect of insecticides used in corn, sorghum, and alfalfa on the predator *Orius insidiosus* (Hemiptera: Anthocoridae). J. Econ. Entomol. 94: 1353–1360.

Analytical Software. 1998. Statistix for windows user's manual. Analytical Software, Tallahassee, FL.

Archer, T. L., E. D. Bynum, and F. W. Plapp, Jr. 1994. Chlorpyrifos resistance in greenbugs (Homoptera: Aphididae): cross-resistance and synergism. J. Econ. Entomol. 87: 1437–1440.

Bagnères, A. G., A. Pichon, J. Hope, R. W. Davis, and J. L. Clèment. 2009. Contact versus feeding intoxication by fipronil in *Reticulitermes* termites (Isoptera: Rhinotermitidae): laboratory evaluation of toxicity, uptake, clearance, and transfer among individuals. J. Econ. Entomol. 102: 347–356.

Baskaran, S., R. S. Kookana, and R. Naidu. 1999. Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. Pestic. Sci. 55: 1222–1228.

Bernal, J., E. Garrido-Bailón, M. J. Del Nozal, A. V. González-Porto, R. Martín-Hernández, J. C. Diego, J. J. Jiménez, J. L. Bernal, and M. Higes. 2010. Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. J. Econ. Entomol. 103: 1964–1971.

Boaretto, M.A.C., and L. C. Forti. 1997. Perspectivas no controle de formigas cortadeiras. Serie Técnica IPEF 11: 31–46.

Bragança, M.A.L., A. Tonhasca, Jr., and T.M.C. Della Lucia. 1998. Reduction in the foraging activity of the leaf-cutting ant *Atta sexdens* caused by the phorid *Neodohrniphora* sp. Entomol. Exp. Appl. 89: 305–311.

Cámara de Seguridad Agropecuaria y Forestal. 2011. Mercado Argentino 2011 de productos fitosanitarios. (http: www.casafe.org/infomercadofitosanitarios2011.pdf).

Chauzat, M.- P., J.- P. Faucon, A.- C. Martel, J. Lachaize, N. Cougoule, and M. Aubert. 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. J. Econ. Entomol. 99: 253–262.

Cherret, J. M. 1986. The economic importance and control of leaf-cutting ants, pp 165–192. *In* S. B. Vinson (ed.), Economic impact and control of social insects. Praeger Publishers, New York.

Da Silva, M. E., and E. Diehl-Fleig. 1988. Avaliaçao de diferentes linhagens de fungos entomopatogênicos para control da formiga *Atta sexdens piriventris* (Santschi 1919) (Hymenoptera: Formicidae). An. Soc. Entomol. Bras. 17: 263: 269.

Das, P. C., Y. Cao, N. Cherrington, E. Hodgson, and S.R.L. Rose. 2006. Fipronil induces CYP isoforms and citotoxicity in human hepatocytes. Chem. Biol. Interact 164: 200–214.

De Coll, O. R. 1998. Ensayo de control de *Acromyrmex lobicornis* Emery (Formicidae) con cebo fluramim (sulfluramida). Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina.

Della Lucia, T.M.C. (ed.). 1993. As formigas cortadeiras. Bib. Cent. Univ. Fed. Viçosa, Brasil.

De Silva, C. S., A. Pathiratne, N. M. van Straalen, and C.A.M. van Gestel. 2010. Chlorpyrifos causes decreased organic matter decomposition by suppressing earthworm and termite communities in tropical soil. Environ. Pollut. 158: 3041–3047.

Diehl-Fleig, E., M. E. Da Silva, A. Specht, and M. E. Valim-Labres. 1993. Efficiency of *Beauveria bassiana* for *Acromyrmex* spp. control (Hymenoptera: Formicidae). An. Soc. Entomol. Bras. 22: 281–285.

Disney R. H. L. 1994. Scuttle flies: the Phoridae. Chapman & Hall, London.

Drees, B. M., A. A. Calixto, and P. R. Nester. 2013. Integrated pest management concepts for red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). Insect Sci. 20: 429–438.

El Hassani, A. K., M. Dacher, M. Gauthier, and C. Armengaud. 2005. Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*). Pharmacol. Biochem. Behav. 82: 30–39.

Elizalde, L. 2009. Biogeografía y comunidades de fóridos parasitoides de hormigas cortadoras de hojas. Diversidad del sistema y partición del recurso hospedador. Ph.D thesis. Universidad Nacional de Quilmes. Buenos Aires, Argentina.

Elizalde, L., and P. J. Folgarait. 2010. Host diversity and environmental variables as determinants of leafcutter phorid parasitoid's species richness. J. Biogeogr. 37: 2305–2316.

Elizalde, E., and P. J. Folgarait. 2012. Behavioral strategies of phorid parasitoids and responses of their hosts, the leafcutter ants. J. Insect Sci. 12: 135.

Erthal, M., Jr., and A. Tonhasca, Jr. 2000. Biology and oviposition behavior of the phorid *Apocephalus attophilus* and the response of its host, the leaf-cutting ant *Atta laevigata.* Entomol. Exp. Appl. 95: 71–75.

Eskenazi, B., A. Bradman, and R. Castorina. 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ. Health Perspect. 107: 409–419.

Feener, D. H., Jr., and B. V. Brown. 1993. Oviposition behavior of an ant-parasitizing fly, *Neodohrniphora curvinervis* (Diptera: Phoridae), and defense behavior by its leaf-cutting ant host *Atta cephalotes* (Hymenoptera: Formicidae). J. Insect Behav. 6: 675–688.

Filho, O. P., and A. Dorval. 2003. Efeito de formulaçôes granuladas de diferentes produtos químicos e à base de folhas e de sementes de gergelim, Sesamum indicum, no controle de formigueiros de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae). Ciência Florestal. 13: 67–70.

Folgarait, P. J. 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers. Conserv. 7: 1221–1244.

Forest Stewardship Council. 2007. FSC guidance document. FSC Pesticides policy: guidance on implementation. (http://ebookbrowse.com/fsc-pol-30-001-en-fsc-pesticides-policy-2005-pdf-d342630382).

Forest Stewardship Council. 2012. FSC pesticides policy guidance addendum: list of approved derogations for use of 'highly hazardous' pesticides. (http://www.fsc.org.download.fsc-gui-30-001a-v1-0-en.113.htm).

Grout, T. G., G. I. Richards, and P. R. Stephen. 1997. Further non-target effects of citrus pesticides on *Euseius addoensis* and *Euseius citri* (Acari: Phytoseiidae). Exp. Appl. Acarol. 21: 171–177.

Guillade, A. C., and P. J. Folgarait. 2011. Life history traits and parasitism rates of four phorid species (Diptera: Phoridae), parasitoids of *Atta vollenweideri* (Hymenoptera: Formicidae) in Argentina. J. Econ. Entomol. 104: 32–40.

Guillade A. C., and P. J. Folgarait. 2012. Efectos de la acción de parasitoides específicos (Diptera: Phoridae) a distintas densidades sobre caminos de forrajeo de *Atta vollenweideri* (Hymenoptera: Formicidae), pp. 276. *In* Proceedings VIII Congreso Argentino de Entomología, 17–20 April 2012, Bariloche, Argentina. Sociedad Entomológica Argentina, Buenos Aires.

Gunasekara, A. S., T. Truong, K. S. Goh, F. Spurlock, and R. S. Tjeerdema. 2007. Environmental fate and toxicology of fipronil. J. Pestic. Sci. 32: 189–199.

Hodgson, E., and R. L. Rose. 2007. Human metabolic interactions of environmental chemicals. J. Biochem. Mol. Toxicol. 21: 182–186.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Cambridge University Press, New York.

Hurley, P. M., R. N. Hill, and R. J. Whiting. 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ. Health Perspect. 106: 437–445.

Ibrahim, S. A., G. Henderson, and F. Huixin. 2003. Toxicity, repellency, and horizontal transmission of fipronil in the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96: 461–467.

Ivey, M. C., and J. S. Palmer. 1981. Chlorpyrifos and 3,5,6-trichloro-2-pyridinol: residues in the body tissues of sheep treated with chlorpyrifos for sheep ked control. J. Econ. Entomol. 74: 136–137.

Ivey, M. C., J. S. Palmer, and E. C. Hooten. 1978. Chlorpyrifos and 3,5,6-trichloro-2-pyridinol: residues in the body tissues of cattle wearing chlorpyrifos-impregnated plastic ear bands. J. Econ. Entomol. 71: 697–700.

Lee, W. J., A. Blair, J. A. Hoppin, J. H. Lubin, J. A. Rusiecki, D. P. Sandler, M. Dosemeci, and M.C.R. Alavanja. 2004. Cancer incidence among pesticide applicators exposed to chlorpyrifos in the agricultural health study. J. Natl. Cancer Inst. 96: 1781–1789.

Lemus, Y. A., G. M. Rodríguez, R. A. Cuervo, V.J.A. Durán, C. L. Zuluaga, and G. Rodríguez. 2008. Determinación de la factibilidad del hongo *Metarhizium anisopliae* para ser usado como control biológico de la hormiga arriera (*Atta cephalotes*). Rev. Científica Guillermo de Ockham. 6: 31–40.

Link, D. 1997. Hormigas cortadoras, hábitos y su control. *Actas XII Jornadas Forestales de Entre Ríos.* Instituto Nacional de Tecnología Agropecuaria, Concordia, Argentina.

Liu, H., Q. Xu, L. Zhang, and N. Liu. 2005. Chlorpyrifos resistance in mosquito *Culex quinquefasciatus.* J. Med. Entomol. 42: 815–820.

López, E., and S. Orduz. 2003. *Metarhizium anisopliae* and *Trichoderma viridae* for control of nests of the fungus-growing ant, *Atta cephalotes.* Biol. Control 27: 194–200.

Medina, P., J. J. Morales, F. Budia, A. Adán, P. Del Estal, and E. Viñuela. 2007. Compatibility of endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) protected stages with five selected insecticidas. J. Econ. Entomol. 100: 1789–1796.

Nyrop, J. P., and M. L. Hassney. 2006. Onion maggot (Diptera: Anthomiidae) resistance to chlorpyrifos in New York onion fields. J. Econ. Entomol. 99: 1375–1380.

Orr, M. R. 1992. Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 30: 395–402.

Ouyang, Y., P. Chueca, S. J. Scott, G. H. Montez, and E. E. Grafton-Cardwell. 2010. Chlorpyrifos bioassay and resistance monitoring of San Joaquin Valley California citricola scale populations. J. Econ. Entomol. 103: 1400–1404.

Rauh, V. A., R. Garfinkel, F. P. Perera, H. F. Andrews, L. Hoepner, D. B. Barr, R. Whitehead, D. Tang, and R. W. Whyatt. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118: 1845–1859.

Rauh, V. A., S. Arunajadai, M. Horton, F. Perera, L. Hoepner, D. B. Barr, and R. Whyatt. 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ. Health Perspect. 119: 1196–1201.

Rodríguez, M. A., D. Bosch, B. Sauphanor, and J. Avilla. 2010. Susceptibility to organophosphate insecticides and activity of detoxifying enzymes in Spanish populations of *Cydia pomonella* (Lepidoptera, Tortricidae). J. Econ. Entomol. 103: 482–491.

Rust, M. K., and D. A. Reierson. 1991. Chlorpyrifos resistance in German cockroaches (Didtyoptera: Blatellidae) from restaurants. J. Econ. Entomol. 84: 736–740.

SAS Institute. 1998. Statview user's manual, version 5.0. SAS Institute, Cary, NC.

Sidiropoulou, E., M. Sahana, J. Flaskos, W. Harris, A. J. Hargreaves, and Z. Woldehiwet. 2011. Fipronil interferes with the differentiation of mouse N2a neuroblastoma cells. Toxicol. Lett. 201: 86–91.

Silva, A., A. Rodrígues, M. Bacci, Jr., F. C. Pagnocca, and O. C. Buerro. 2006. Susceptibility of the ant-cultivated fungus *Leucoagaricus gongylophorus* (Agaricales: Basidiomycota) towards microfungi. Mycopathologia 162: 115–119.

Smith, D. I., and J. A. Lockwood. 2003. Horizontal and trophic transfer of diflubenzuron and fipronil among grasshoppers (*Megaloplus sanguinipes*) and between grasshoppers and darkling beetles (Tenebrionidae). Arch. Environ. Contam. Toxicol. 44: 377–382.

Soeprono, A. M., and M. K. Rust. 2004. Effect of horizontal transfer of barrier insecticides to control Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97: 1675–1681.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry; the principles and practice of statistics in biological research, 3rd ed. W.H. Freeman, New York.

SYSTAT Inc. 2009. SYSTAT 13 for Windows user's manual. Systat Software Inc., Chicago, IL.

Tingle, C. C., J. A. Rother, C. F. Dewhurst, S. Lauer, and W. J. King. 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Rev. Environ. Contam. Toxicol. 176: 1–66.

Tonhasca, A., Jr. 1996. Interactions between a parasitic fly, *Neodhorniphora declinata* (Diptera: Phoridae) and its host, the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Ecotropica 2: 157–164.

Tonhasca, A., Jr., M.A.L. Bragança, and M. Erthal, Jr. 2001. Parasitism and biology of *Myrmosicarius grandicornis* (Diptera: Phoridae) in relationship to its host, the leaf-cutting ant *Atta sexdens* (Hymenoptera: Formicidae). Insectes Soc. 48: 154–158.

Ulloa-Chacón, P., and G. I. Jaramillo. 2003. Effects of boric acid, fipronil, hydramethylnon, and diflubenzuron baits on colonies of ghost ants (Hymenoptera: Formicidae). J. Econ. Entomol. 96: 856–862.

Vaccaro, N. C., and J. A. Mousques. 1997. Hormigas cortadoras (géneros *Atta* y *Acromyrmex*) y tacurúes en Entre Ríos. Actas XII Jornadas Forestales de Entre Ríos. Concordia, Entre Ríos, Argentina.

Vidau, C., M. Diogon, J. Aufauver, R. Fontbonne, B. Viguès, J-L. Brunet, C. Texler, D. G. Biron, N. Blot, H. El Alaoui, et al. 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS ONE 6: e21550. doi: 10.1371/journal.pone.0021550.

Williams, L., III, L. D. Price, and V. Manrique. 2003. Toxicity of field-weathered insecticide residues to *Anaphes iole* (Hymenoptera: Mymaridae), an egg parasitoid of *Lygus lineolaris* (Heteroptera: Miridae), and implications for inundative biological control in cotton. Biol. Control 26: 217–223.

Wiltz, B. A., D. R. Suiter, and W. A. Gardner. 2009. Activity of bifenthrin, chlorfenapyr, fipronil and thiamethoxam against Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 102: 2279–2288.

Zanetti, R., J, C. Zanuncio, A. J. Mayhé-Nunes, A. G. Barros Medeiros, and A. Souza-Silva. 2003. Combate sistemático de formigas cortadeiras com iscas granuladas, em eucaliptais com cultivo mínimo. Rev. Árvore. 27: 387–932.

Received 12 December 2012; accepted 26 October 2013.