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CONTROL OF ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE) IN CITRUS USING METHOPRENE AND IMIDACLOPRID DELIVERED IN LIQUID BAIT STATIONS

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ABSTRACT

We conducted trials in a citrus grove infested with Argentine ants [*Linepithema humile* (Mayr); Hymenoptera: Formicidae] using bait stations (9.8 per ha) filled with bait consisting of either 0.001% imidacloprid as one treatment or 0.25% methoprene as a second treatment. The 2 treated areas and a control were widely separated in the grove. Within each area, trees were randomly selected to receive sucrose water monitors. Consumption of the sucrose water is a measurement of ant foraging activity that we used to compare treatments and the control. For all weekly samples except one, consumption by ants in the imidacloprid-treated area was significantly lower than in the controls. The methoprene treatments gave a more complex outcome: the consumption of the bait was significantly higher than in the controls during wk 5 and 8, but then rapidly descended below the consumption level of the control during wk 11 and 12. A post-experimental examination of the control and methoprene areas showed that queen numbers in the methoprene area were 93% lower than in the control area. A subsequent laboratory experiment comparing the methoprene bait in sucrose to sucrose only showed significant worker mortality after 9, 12, and 16 wk, while at the end of the experiment queen mortality was 24% higher than in the controls (ns). The initial rise in field ant numbers with the methoprene bait would pose a problem for its use by growers unless it would be used early in the season before hot weather would drive the annual increase in ant numbers.

Key Words: Argentine ant, *Linepithema humile*, bait stations, methoprene, imidacloprid, citrus

RESUMEN

Hemos llevado a cabo ensayos en una plantación de cítricos infestados con hormigas argentinas [*Linepithema humile* (Mayr); Hymenoptera: Formicidae] que utilizan las estaciones de cebo (9.8/ha) llenas de cebo que consiste en cualquiera de 0.001% imidacloprid como un tratamiento o un 0,25% metopreno como tratamiento de segunda. Las dos zonas tratadas y de control de uno estaban muy separados en la plantación. Dentro de cada área, los árboles fueron seleccionados al azar para recibir monitores de sacarosa de agua. El consumo de agua de la sacarosa es una medición de la actividad de las hormigas alimentación que se utilize para comparar los tratamientos y el control. Para todas las muestras semanales, except uno, el consumo de imidacloprid fue significativamente menor que en los controles. Los tratamientos metopreno dió un resultado más complejo: el consume del cebo fue significativamente mayor que en los controles de las semanas 5 y 8, pero luego descendieron rápidamente por debajo del consume de control durante las semanas 11 y 12. Un examen post-experimental de las áreas de control y metopreno mostró que el número de reinas en el área de metopreno fueron 93% menores que en el área de control. Un experimento de laboratorio posterior comparando el cebo metopreno en sacarosa a solo sacarosa sólo presenta la mortalidad trabajador significativa después de 9, 12 y 16 semanas, mientras que al final de la mortalidad reina experiment fue de 24% inferior que en los controles (ns). El aumento inicial en el número de hormigas de campo con el cebo metopreno supondría un problema para su uso por los agricultores, a menos que se podría utilizer a principios de la temporada antes de tiempo caluroso impulse el incremento annual en el número de hormigas.

Palabras Clave: Hormigas argentinas, *Linepithema humile*, estaciones de cebo, methoprene, imidacloprid, cítricos

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), is a substantial pest on citrus crops in California (Woglum 1942; Debach et al. 1951; Markin 1970). Foraging by *L. humile* interferes with the natural moderation of honeydew-producing hemipteran pests by disrupting predators and parasitoids (Borden 1923; Debach et al. 1951). Populations of *L. humile* can become extremely large. For instance, Markin (1967) estimated that in heavily infested orchards between 50,000 to 600,000 ant visits can occur daily in each tree to tend hemipterans. In California approximately 99% of the *L. humile* diet consists of honeydew and nectar (Markin 1967).

Linepithema humile is highly polygynous with as many as 16.3 queens per 1000 workers (Keller et al. 1989) and produces new colonies through fission (Aron 2001). This method of reproduction, along with the large number of queens, gives *L. humile* a tremendous capacity to produce new colonies. Fission or budding creates a network of interrelated colonies in which resources are shared in a decentralized fashion and are distributed according to the need of individual colonies (Markin 1968; Holway and Case 2000).

The conventional method to control *L. humile* consists of tree trunk and ground applications of Lorsban® (chlorpyrifos). In response to an increased demand by growers to reduce the use of broad spectrum insecticides such as Lorsban, research has focused on improving the efficacy of liquid, carbohydrate-based baits (Martinez-Ferrer et al. 2003). Klotz et al. (2003, 2004) significantly reduced populations of *L. humile* in a commercial citrus orchard using ultra-low concentrations (1×10^{-4} %) of fipronil or thiamethoxam formulated in 25% sucrose water placed in modified PVC pipes that were used as bait stations. In an organic orchard, Greenberg et al. (2006) showed that a bait station approach using Gourmet Liquid Ant Bait (1% disodium octaborate tetrahydrate, DSOBTH, Innovative Pest Control, Boca Raton, Florida) could be effective in reducing Argentine ant numbers.

Although liquid bait such as DSOBTH is effective against *L. humile* in citrus orchards, the number of stations needed per ha has not been determined. Bait stations at 262 per ha, containing 1 or 0.5% DSOBTH, effectively reduced orchard populations (Greenberg et al. 2006). Currently there is only 1 liquid bait registered in California for use in citrus against *L. humile*, Gourmet Ant Bait. Two other products, Vitis (0.001% imidacloprid) (Bayer CropSciences, Research Triangle Park, North Carolina), and Tango (4.9% S-methoprene) (Wellmark International, Schaumburg, Illinois) are no longer registered. Ant bait formulated with the neonicotinoid, imidacloprid, affects control by killing workers, larvae, and ultimately the queen or queens (Rust et al. 2004). In contrast, insect growth regulators in general do not kill workers or the queen. If colony death occurs, it takes weeks to months (Banks et al. 1983) resulting from the inability to replace workers (Banks & Lofgren 1991).

Methoprene, an insect growth regulator (IGR), causes the cessation of egg production, the deformation of pupae, and a shift from the production of workers to reproductives in laboratory colonies of the red imported fire ant, *Solenopsis invicta* Buren (Vinson & Robeau 1974). Investigations of Glancey & Banks (1988) showed that IGRs caused the degeneration of *S. invicta* queen ovaries. In large-plot field trials using an experimental IGR applied twice at a 6 month interval at 11.85 g ai/ha, 89.5% of active colonies were eliminated after 12 mo (Banks et al. 1983).

The majority of research investigating the impact of IGRs on ant pests has focused on imported fire ants and to a much lesser degree on other important ant pests. The big headed ant, *Pheidole megacephala* (Fabricius), for example, is a pest in pineapple plantations and IGRs affect it similarly as *S. invicta*. Pheidole colonies treated with 2% pyriproxyfen or fenoxycarb ceased egg production in 5 to 7 wk after treatment (Reimer et al. 1991). These IGRs caused significant reduction of brood in 5 wk, principally due to the mortality of larvae (Reimer et al. 1991).

IGRs also negatively impact the pharaoh ant, *Monomorium pharaonis* (L.), a common urban pest in homes, office buildings, and hospitals throughout the United States (Williams & Vail 1993; Vail & Williams 1995; Oi et al. 2000; Edwards 1985). This species is highly polygynous (Buczkowski & Bennett 2009) and also responds to IGRs similarly as *S. invicta*. Vail &Williams (1995) evaluated bait formulated with 0.25, 0.5 or 1% fenoxycarb and found that egg production by queens decreased and pupae died in approximately 3 wk after treatment.

This study evaluated the effectiveness of liquid baits formulated with imidacloprid and methoprene under field conditions. Also, laboratory experiments were conducted to assess the effect of methoprene on mortality of adult Argentine ants.

Materials and Methods

In 2007 we conducted experiments at the Historic State Citrus Park located in Riverside County, California. The orchard was approximately 15-yr-old and under conventional management practices. Tree spacing measured approximately 4.9 m within rows and 6.1 m between rows.

Measurement of Argentine Ant Activity

To estimate ant activity we used monitoring tubes consisting of 50-mL centrifuge tubes filled with 25% sucrose water. The monitoring tubes were constructed by cutting a 2 cm diam hole in the cap and then placing a 6 cm^2 piece of Weedblock landscape fabric (Easy Gardener, Waco, Texas) over the tube opening and screwing on the cap. Usually a single tube was inverted and affixed to a tree trunk, using tape or surgical tubing, approximately 0.25 m above the soil surface. When ant populations were very high, 2 tubes were affixed side by side on the tree. The holes in the Weedblock fabric are 1 mm in diam and allow the ants to feed while being resistant to dripping when the tube is inverted. We corrected for evaporative water loss by suspending an inverted monitoring tube filled with 25% sucrose water on a string from a tree branch in the orchard. The string was coated with Stikem Special (Seabright, Emeryville, California) to prevent ants from feeding. In each treated area 20 randomly selected trees were used for monitoring ant numbers. The same trees were used for every sample period.

Sucrose-water consumption provided an estimation of the number of ant visits; each mL consumed corresponds to about 3,300 ant-visits (Reierson et al. 1998). We estimated ant activity by determining sucrose-water consumption corrected for evaporative water loss. Trees were monitored for 24 h periodically from 12-VII to 11-X-2007. Before each monitoring period, bait stations were closed in order to avoid competition with the monitoring tubes.

Bait Stations

We used KM AntPro® Bait Stations (AntPro LLC, Nokomis, Florida) for testing the liquid baits. Each bait station was placed at the base of a navel orange tree where it would be shaded and protected from the sun. Argentine ants move up the trunk to tend aphids, scale insects, and mealybugs that are feeding on the leaves. Large nests of the ants are frequently at the base of these trees. When the bait stations are open there is a small space for ants, but very little else, to enter the station. Each bait station has a 500 mL reservoir. Early in the project many of these stations had to be re-filled each wk. Bait stations were closed when monitoring for ants.

Evaluation of Liquid Ant Baits

Bait stations were placed in each of the 2 treated areas (each approx. 0.61 ha in size) in a 30.5 m square grid pattern (9.8 stations per ha). In one of these areas the bait stations were filled with Vitis™ Liquid Ant Bait (0.001% imidacloprid, Bayer CropScience LP, Research Triangle Park, North Carolina). For the other treated area, Tango™ (4.9% (S) methoprene, Wellmark International, Schaumburg Illinois) was diluted to 0.25% (S) methoprene in 25% sucrose water before filling the bait stations. A control area of similar size did not have bait stations but was monitored for ant activity simultaneously. Bait stations remained in the field for 116 days, from 16 Jun to 10 Oct and were refilled as needed.

Statistical Analysis

A repeated measures analysis using the ant population at each tree as the 'subject' was conducted with PROC GLM (SAS). In this experimental design, each tree and associated ant population is considered a replicate and becomes its own control over time. This design may seem unusual for ant studies (see Discussion section) that usually have "plots" or "houses" as replicates. Between treatment analysis at each evaluation period was conducted using Tukey's HSD test. A follow-up ANOVA, using all weekly data after the pretreatment, was done to compare the grand means of treatments and controls (the between treatments analysis of the repeated measures ANOVA).

Examination of Ants in the Methoprene Study Area

After removing all the bait stations, we returned to the control and methoprene-treated plots to collect ants for examination in the laboratory. Ant samples were collected during 3 separate outings between 25 Oct and 1 Nov 2007. Samples from 10 different locations within each area were collected by digging up the nests. For transporting to a laboratory chamber, nest samples were placed in a plastic 18.9-L bucket coated with fluoropolymer resin (Teflon®, product type 30, DuPont,Washington, West Virginia). At the laboratory, ants were separated from the soil by thinly spreading the soil within a large wooden collection box that had its sides coated with fluoropolymer resin to prevent escape. Moist plaster of Paris disks were stacked in the center of the collection box. As the soil dried, the ants moved into the plaster disks and then were tapped into a large plastic box without soil. Worker ants were aspirated from the box, weighed, and the queens counted.

Laboratory Methoprene Study

Fresh ants were collected from the control plot in the manner described above. Ants from a single large field nest were subdivided into replicates for a laboratory study. These ants were maintained in plastic boxes (26.5 × 30 × 10 cm, Tristate Plastics, Dixon, Kentucky) coated with a thin film of the fluoropolymer resin on the inner walls. Each box of ants had a nest consisting of a plastic Petri dish (9 cm diam × 0.5 cm) filled with plaster of

Paris with the center 4 cm hollowed out to serve as a nest and a 25-mL water vial plugged with cotton for moisture. Two g of workers (approximately 5,000 ants) and 15 queens from the field-collected nest were put into each of 6 boxes on 13-XI-2006. Sucrose water was provided ad libitum from polystyrene weighing dishes (3.8 × 2.5 cm, Fisher Scientific, Pittsburgh, Pennsylvania) holding a small ball of cotton. Three of the nest boxes received 25% sucrose water and cut-up American cockroaches, *Periplaneta americana* (L.). The other 3 received 0.25% methoprene in 25% sucrose water and the same cut-up cockroaches. Dead workers in each box were counted periodically over 26 wk from 27-XI-2006 to 12-V-2007. At the end of the experiment, the numbers of live workers and queens were determined.

Statistical Analysis

ANOVAs and Kruskal-Wallis tests comparing the mortality of methoprene-treated ants and the controls were done at each time period.

RESULTS

Evaluation of Liquid Ant Baits

Fig. 1 shows the results of mean consumption of sucrose-water (control), sucrose-water + imidacloprid, and sucrose-water + methoprene during 12 wk in the citrus grove. The grand mean ± SEM of sugar water consumed over time in the imidacloprid trial (17.0 ± 1.55 mL) was significantly lower than that observed in the control plots (28.0 ± 1.74 mL), while the amounts consumed over time in the methoprene-treated areas (30.9 ± 1.24) were not significantly different than the control area.

One wk post-treatment all areas showed increased sugar-water consumption by the ants, but the imidacloprid- and methoprene-treated areas each had significantly lower consumption than the control area ($P < 0.05$). At wk 2, post-treatment consumption in each of the 2 treated areas was again lower than the controls ($P < 0.001$). Starting with wk 2 post-treatment, consumption in the imidacloprid-treated areas was significantly lower than the controls for all wk except wk 5. In contrast, consumption in the methoprene-treated areas sharply increased between wk 5 and 8. In that same treatment between wk 8 and 11 there were sharp reductions in sucrose consumption. For wk 11 and 12, mean sugar water consumption in the methoprene-treated plots decreased to levels statistically below those observed in the control plots, $P < 0.01$ and $P < 0.001$, respectively.

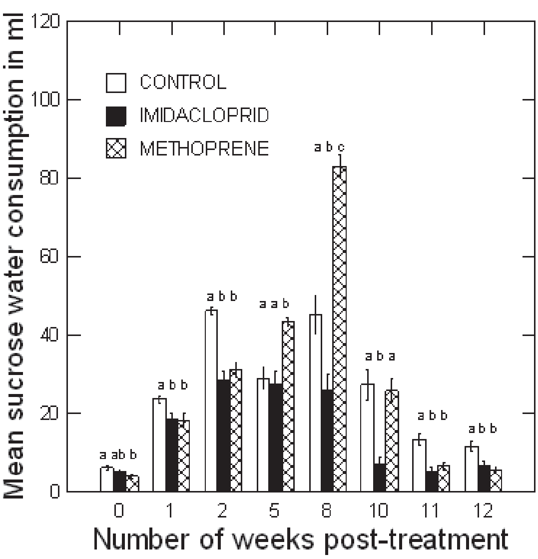

Fig. 1. Mean ± SE consumption of sucrose-water during a 24 h monitoring period for sugar water (control), sugar water + imidacloprid, and sugar water + methoprene-treated plots. For each sample period, groups that are not significantly different have the same letter.

Examination of Ants in the Methoprene Study Area

Table 1 shows the results of the field-collected ants from methoprene treated and untreated (control) areas. The total weights of workers collected from the control and methoprene-treated areas were nearly equal. However, only 10 queens were recovered from the latter, while in the control samples there were 142 queens. This represents a 93% reduction in the number of queens in the methoprene-treated area compared to the control area. Although the reduction is large, there were not enough experimental replicates to show significance (with $n = 3$, $P > 0.1$, Wilcoxon test).

Laboratory Methoprene Study

The results of the laboratory study to determine mortality of worker ants receiving methoprene in the sucrose-water solution vs. those receiving no methoprene in the sucrose-water solution are shown in Fig. 2. Between wk 9 and 16 worker mortality in the methoprene boxes was significantly greater than in the controls; significance levels for wk 9, 12 and 16 were 0.002, 0.006, and 0.030, respectively. The number of live queens at the end of the experiment was 12.7 ± 1.86 for the control in contrast to 9.7 ± 1.67 for the methoprene trials (a reduction of 24%). These means were not significantly different ($P > 0.1$, Kruskal Wallis test).

TABLE 1. WEIGHTS OF WORKERS COLLECTED AND NUMBERS OF QUEENS WITH WORKERS IN THE CONTROL AND METHOPRENE TREATED AREAS. EACH COLLECTION REPRESENTS A POOLED SAMPLE OF 10 NESTS. COLLECTIONS WERE MADE DURING 3 SEPARATE OUTINGS BETWEEN 25 OCT AND 1 NOV 2007.

Treatment	Weight of workers (g)	Number of queens	Queens/g of workers
First Collection			
Controls	8.19	27	3.30
Methoprene	3.02	3	0.99
Second collection			
Controls	4.94	2	0.40
Methoprene	1.99	0	0
Third collection			
Controls	14.55	113	7.77
Methoprene	20.32	7	0.34
Totals			
Controls	27.68	142	5.13
Methoprene	25.33	10	0.40

DISCUSSION

Our experimental design for the field study may seem unusual to ant researchers accustomed to using 'plots' as experimental units. In each of the 0.61 ha fields that were chosen for this experiment, 20 trees were randomly selected for measuring ant numbers. In citrus orchards *L. humile* nests occur near the base of trees within the tree drip-line, because of their preference for nesting in moist soil and near tree hemipterans that provide honeydew. This close association between nests and trees helps to assure that the ants are likely to feed each time on the monitor closest to their nest during the 24 h that the monitor is on the tree. Given this association we believe that the randomly selected trees and their ant populations in the study are statistically independent and can therefore be considered replicates. We also believe that the use of large 0.61-ha treated areas allowed us to evaluate bait station placement under actual field conditions rather than employing several smaller plots for each treatment.

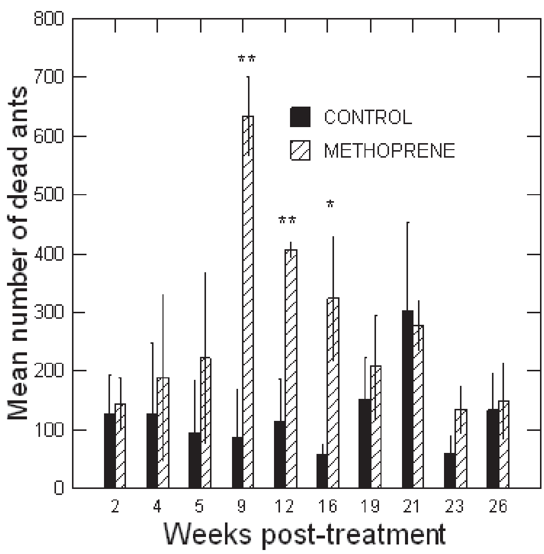

Fig. 2. Mortality of worker ants (mean ± SE) receiving methoprene in the sucrose-water solution vs. those receiving no methoprene in the sucrose-water solution over 26 wk in the laboratory. Asterisks over bars indicate significant differences between controls and treatments, where * = P < 0.05, and ** = P < 0.01.

Sucrose-water consumption by ants is a reliable indicator of ant foraging activity and indirectly ant numbers. For many years we have monitored consumption by Argentine ants from May through Sep in both urban settings and in citrus groves (Greenberg et al. 2006; Klotz et al. 2009; Klotz et al. 2010). Typically there is a large increase in foraging activity in Jun or Jul, which lasts through early Oct. During this study pretreatment ant consumption started low because of cool weather (mean consumption of 6.2 mL in the controls, corresponding to over 20,000 ant visits) and reached its maximum at wk 2 (mean consumption of 46.1 mL, corresponding to over 152,000 ant visits).

Treated areas also showed an initial increase in ant visits, although less than the increase in the controls (Fig. 1). By wk 12 (Oct 9), the controls had declined to a mean consumption of 11.65 mL (approx. 38,000 ant visits). Consumption in the treated areas also declined at this time, although consumption in both treatments was significantly lower than the controls (Fig. 1).

In the imidacloprid-treated areas the sucrose consumption was significantly lower than the controls for most time periods (Fig. 1). However, the results in the methoprene-treated areas were more complex. At wk 5 and 8 sucrose consumption in the methoprene area was significantly higher than that of either the imidacloprid or control areas. Methoprene and other IGRs are known to be slow-acting and reduce insect populations by preventing brood maturation and egg production, leading to a reduction in numbers over time as worker ants die and are not replaced (Troisis & Riddiford 1974; Vinson & Robeau 1974; Banks et al. 1983; Glancey & Banks 1988; Reimer et al. 1991; Williams & Vail 1993). In our study methoprene was mixed with 25% sucrose water bait, the preferred food of Argentine ants and a source of energy for the colony. It is likely that the consumption of this bait ad libitum led to the explosion of ant numbers seen in wk 5-8. At wk 10-12 in the methoprene area there was a large decrease in sucrose water consumption and at wk 11-12 consumption in the methoprene areas had become significantly lower than in the controls. Drees & Barr (1998) reported that methoprene treatment of fire ant colonies took 8-10 wk before worker attrition was seen, which is consistent with our finding with Argentine ants. Aubuchon et al. (2006) used methoprene bait (Extinguish®) against fire ants and found that mound abundance was reduced after 16 wk.

Under field conditions methoprene-treated worker foraging showed a sharp decrease between wk 8 and 10 (Fig. 1). Interestingly, mortality among workers ants in the laboratory colonies treated with methoprene was significantly higher than in control colonies at 9-10 wk post-treatment. Mortality in the laboratory controls did not increase at this time, which suggests that the methoprene caused worker mortality in addition to death by attrition. Worker mortality may also explain the field results. Furthermore, queen numbers in methoprene-treated field colonies were 93% lower than those in the control area, while the reduction in the laboratory colonies was smaller (24%). Keller et al. (1989) and Reuter et al. (2001) describe the odd phenomenon in Argentine ants by which workers kill off most of their overwintering queens before development of new queens in the spring. We do not know whether the methoprene in our study led to worker killing of queens or whether it killed queens directly. Further studies are necessary to see how methoprene affects queen number in these ants. Although we did not presample queen numbers in the methoprene plot, it is highly unlikely that worker numbers at wk 8 could be twice as high as in the control area if over 90% of the queens had died or were about to die in the methoprene area.

In this study both the imidacloprid and the methoprene gave significant reductions in ant numbers as judged by consumption of sucrose water, although it took longer in the methoprene plot. The initial rise in ant numbers with the methoprene could discourage its use in citrus groves because high ant numbers lead to high densities of citrus pests, especially scale insects, mealybugs, and aphids. Growers would not tolerate these conditions for long. However, it may be possible to avoid this problem by early spring or fall application of the methoprene so that it enters the ant population before the growing season (Collins et al. 1992). It is encouraging that for both imidacloprid and methoprene the low density of bait stations used here (9.8/ha) significantly lowered ant foraging activity after 10 wk. Perhaps growers will consider the use of bait stations for ant control, especially when considering that they eliminate environmental contamination while controlling ant populations.

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