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LIQUID BORATE BAIT FOR CONTROL OF THE ARGENTINE ANT, *LINEPITHEMA HUMILE*, IN ORGANIC CITRUS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

A liquid bait delivery system containing borate was evaluated for controlling the Argentine ant, *Linepithema humile* (Mayr), in an organic citrus orchard. Two concentrations of disodium octaborate tetrahydrate (1% and 0.5%) were tested in 500-mL capacity bait stations placed at the base of trees. Both concentrations significantly reduced ant activity over the 11-wk duration of the test when compared with controls. However, the 1% concentration of borate significantly reduced ant activity up to 76 m away from the treatment, whereas the 0.5% did not. Compared to ant control with contact insecticides, the bait delivery system uses less insecticide and is more target-specific, reducing environmental contamination.

Key Words: Citrus, Argentine ant, borate, liquid bait delivery system, organic, homopterous pests, bait station

RESUMEN

Un sistema de distribución de líquidos conteniendo boratos fue evaluado por el control de la hormiga argentina, *Linepithema humile* (Mayr), en una huerta de árboles cítricos. Dos concentraciones de disodium octaborate tetrahydrate (1% y 0.5%) fueron probadas en estaciones de comida puestas cerca de los troncos de los árboles. Comparado con los controles, las dos concentraciones redujeron significativamente la actividad de las hormigas durante las 11-semanas del experimento. No obstante, la concentración 1% del borato produjo una reducción significante en la actividad de las hormigas hasta una distancia de 76 metros, mientras que la solucion 0.5% de borato no tenia ese efecto. Comparado con el control de hormigas con insecticidas de contacto, nuestro sistema de distribución usa menos insecticida y es dirigido específicamente a la hormiga; y de esa manera reduce la contaminación ambiental.

Translation provided by the authors.

Argentine ants became a serious pest in citrus shortly after their introduction into the United States in the late 1800s, most likely offloaded from ships transporting coffee from Brazil into the port of New Orleans (Newell & Barber 1913). As early as 1918, a researcher in Louisiana reported trapping 1,307,222 Argentine ant queens and collecting 1,150 gallons of workers and brood over a one-year period in a 19-acre citrus grove (Horton 1918). In 1905 they were reported in southern California, and by 1908 they had spread through the citrus growing regions as far north as San Francisco (Vega & Rust 2001).

Colonies of Argentine ants have tremendous capacity for growth and expansion due to numerous queens (typically 15 to every 1000 workers) and their ability to undergo colony multiplication by fission (Aron 2001; Majer 1993). Colony fission or budding eventually creates a network of interrelated nests that form a cooperative unit, which sometimes extends over an entire habitat. The flow of food in these supercolonies is decentralized, moving in many directions depending on the needs of the individual colonies. This behavior is known as dispersed central place foraging (Holway & Case 2000; McIver 1991).

These large cooperative units channel their energy into foraging and colony growth, and by the sheer number of ants produced out-compete native species for limited resources. Their populations can reach astronomical proportions, as for example, in a citrus grove in San Diego County, California, it was estimated that from 50,000 to 600,000 ants ascended each tree daily in order to tend homopterans (Markin 1967).

Argentine ants tend a variety of homopterans in citrus including the citrus mealybug, *Planococcus citri* Risso, spirea aphid, *Aphis spiraecola* Patch, wooly whitefly, *Aleurothrixus floccossus* (Maskell), and brown and black soft scales, *Coccus hesperidum* L. and *Saissetia oleae* Olivier, respectively. These phloem-feeding homopterans excrete honeydew, which is the primary food of Argentine ants (Markin 1970). The ants guard this resource tenaciously by protecting the homopterans from parasites and predators and consequently interfere with biological control programs. The outcome of this trophobiotic association is an increase in populations of both ants and homopterans. (See reviews of this topic in Bartlett 1961; Flint et al. 1991; and Gulla 1997).

Moreno et al. (1987) demonstrated that by controlling Argentine ants in citrus, the wooly whiteflies and citrus mealybugs were reduced in number by their natural enemies. They applied residual insecticides (chlorpyrifos or diazinon) as a barrier on the trunk or on the ground around skirt-pruned trees. Recently, however, growers have reduced their use of broad spectrum insecticides, and as a consequence ant populations have increased, and there is a growing demand for selective pesticide baits (Martinez-Ferrer et al. 2003).

In previous research in commercial citrus groves, Klotz et al. (2003, 2004) obtained significant reductions of Argentine ant populations using liquid baits (25% sucrose-water) with ultra-low concentrations (1×10^{-4}%) of fipronil or thiamethoxam.The purpose of this study was to test borate in a sugary solution for Argentine ant control in citrus. If effective, then organic growers would have a means of reducing Argentine ant populations in citrus. This is especially significant considering the limited options for ant control available to organic growers.

MATERIALS AND METHODS

Test Site and Experimental Design

An organic citrus grower in Fallbrook (Rainbow Valley Orchids, San Diego County, California) provided us with an orange grove, which we partitioned into 21 plots, each consisting of 3 rows by 5 trees, and measuring 12.2×15.2 m. The rows were 6.1 m apart and trees within rows 3.0 m apart. Each plot was a minimum of 20 m from adjacent plots. This buffer zone was set up in order to mitigate any treatment effects from neighboring plots due to the movement of toxicant through the ant population.

A randomized block design was used consisting of 7 blocks of 3 treatments. Each block consisted of plots with similar ant activity based on a pretreatment survey (see monitoring below) in order to reduce variability due to differences in the initial ant activity.

Monitoring Plots

To estimate ant activity in each plot, we monitored 3 trees in the center row of each plot with sucrose-water monitors. Monitored trees were never on the edge of the plot. Due to missing trees in some plots, several plots had less than 3 trees to monitor. The monitors consisted of 50-mL plastic centrifuge tubes (Fisher Scientific, Pittsburgh, PA) filled with 25% sucrose-water. The cap on the monitor had a 2-cm hole drilled in its center and was screwed down over a 6-cm square piece of Weedblock (Easy Gardener, Waco, TX), a perforated plastic material with many tiny holes. The monitors were inverted and taped to tree trunks so that trailing ants could feed on the sucrose-water. To correct for evaporative water loss in monitors, a 50-mL tube was filled with 25% sucrose-water, inverted, and suspended on a string from a tree branch in the grove. The string was coated with Stikem Special (Seabright, Emeryville, CA) to prevent ants from feeding on this tube. The tube and monitors were left on the trees for 24 h and consumption of sucrose-water by the ants was obtained by correcting for evaporative water loss. Consumption of sucrose-water from these monitors indicated the number of ant visits, with each mL consumed corresponding to about 3300 ant-visits (Reierson et al. 1998). Estimates of ant activity were made in all plots before treatments and on a weekly basis for 11 wk after treatment (wk 6 and 10 were skipped).

Monitoring Transects

At the end of the 11-wk study we also monitored sucrose-water consumption along a series of transects in order to determine how far the toxic baits were having an effect. Each transect extended ≈76 m out from a baited plot into surrounding untreated areas (i.e., some treatment plots were adjacent to parts of the grove that we did not use for plots). Beginning in the middle of the treated plot, monitors were placed in trees at ≈6 m intervals along the transect. Monitors were left out for 24 h, and then collected to measure the consumption of sucrose-water by the ants. As described in the procedure for monitoring plots, a tube was also used to correct for evaporative water loss.

Treatments

Gourmet Liquid Ant Bait (Innovative Pest Control Products, Boca Raton, FL) containing 1% disodium octaborate tetrahydrate (DSOBTH) was used. One of the treatments consisted of bait applied at full strength (1% DSOBTH) and the other diluted with deionized water to half strength (0.5% DSOBTH). The liquid bait was delivered in 500 mL capacity KM AntPro Stations (KM AntPro, LLC, Nokomis, FL). Stations were placed on the ground at the base of every other tree in the treatment plots, staggering the placement between rows of trees. In case of a missing tree, the bait station was placed where the tree should have been. Thus, there were 7 or 8 stations per plot, making a total of 105 stations used in the study. Stations were checked weekly and refilled when necessary. During the monitoring procedure the stations were closed to prevent ants from feeding on them and potentially attracting them away from the monitors, thereby reducing our estimate of ant numbers at the monitors. In addition to the

2 bait treatments we had a third treatment consisting of control plots, which were not baited.

Statistical Analyses

Examination of the plot data with histograms and probability plots to assess normality showed that a square root function, rather than a logarithmic transformation, more closely approximated normality. Therefore, to compare the treatments and controls over time we did a repeated measures ANOVA (Systat 2004) on the square root (X + 1) transformation of sucrose-water consumption for the 11 post-treatment wk that we monitored. In this analysis we were interested in the Between Subjects (Treatments) effects, which is equivalent to comparing the grand means of the treatment profiles over the 11 wk. Each monitor is compared with itself over time, giving a mean value for each monitor and a grand mean for each treatment. We also did separate ANOVAs for each data period with the transformed data. For all the ANOVAs the blocking variable was used to remove variability due to differences in initial ant numbers in the plots and the remainder, or MSE, was used for tests of significance.

For the transect data originating in baited plots, consumption of sucrose-water was plotted against distance and pooled for each treatment. A linear regression analysis was performed on these pooled data for each treatment (Systat 2004).

Results

Table 1 shows a summary of the results. One wk post-treatments, ant visits to the monitors in treatment plots were significantly less than in the controls, with reductions of 54 and 47%, respectively, for the 0.5% and 1% DSOBTH. In the second wk the respective reductions were 68% and 70%. However, consumption in the control plots also began to decline in the second wk and was not now significantly different from the treatments. From wk 7 through 11 the consumption of sucrose-water in the 1% DSOBTH was again significantly lower than in the control. Consumption of sucrose-water in the 0.5% DSOBTH treatments was significantly lower than controls only in wk 1 and 8.

The grand means of the mean consumption of sucrose-water for each treatment, ignoring the pretreatment values, were obtained by finding the mean of each monitor over the 11 post-treatment wk and averaging these means within each treatment. These grand means showed overall reductions from pre-treatment values in sucrose consumption by 76, 52, and 48%, respectively, for the 1% DSOBTH, 0.5% DSOBTH, and the controls. The differences between the grand means were tested for significance by looking at the Between Subjects (Treatments) part of a repeated measures ANOVA (Systat 2004) for the 11 post-treatment wk on square root (X + 1) transformed grand means. The treatment (df = 2, 48; F = 12.5) and blocking (df = 6, 48; F = 4.5) effects were both significant (P < 0.001). A follow-up comparison of the grand means with Tukey's HSD test showed that sucrose-water consumption for both the 1% and 0.5% DSOBTH bait treatments were lower than the controls (P < 0.001 and P < 0.01, respectively), but not different from one another (P > 0.25).

Eleven transects of sucrose-water consumption vs. distance were completed. Five of these received the 0.5%, and 6 the 1.0%, DSOBTH treatments. The regression analysis of the 1% DSOBTH bait transects (Fig. 1a) was highly significant (P < 0.001), whereas it was not significant for transects from plots treated with 0.5% DSOBTH bait (P > 0.25, Fig. 1b).

TABLE 1. MEAN[1] CONSUMPTION OF SUCROSE-WATER (G) AS A MEASURE OF ANT ABUNDANCE.

	Pretreat.	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 7	Wk 8	Wk 9	Wk 11
0.5% DSOBTH	25.0 (2.48) a	11.6 (1.77) b	8.0 (1.28) a	7.5 (1.20) a	3.2 (0.73) a	6.9 (1.27) a	16.4 (3.23) a	6.8 (0.76) b	3.4 (0.60) ab	16.4 (1.60) a
% reduction	—	53.5	67.8	70.0	87.1	72.2	34.3	72.9	86.5	34.4
1.0% DSOBTH	29.3 (4.17) a	13.8 (2.95) b	8.7 (1.97) a	7.3 (1.51) a	5.1 (1.07) a	3.7 (0.84) b	6.7 (1.16) b	5.6 (0.39) b	2.5 (0.65) b	6.9 (1.11) b
% reduction	—	46.8	70.4	75.1	82.5	87.5	77.0	80.8	91.5	76.6
CONTROL	29.4 (3.94) a	27.8 (5.87) a	16.0 (3.21) a	11.4 (2.21) a	6.6 (1.60) a	5.4 (0.90) ab	19.0 (1.82) a	9.7 (0.96) a	6.4 (1.53) a	15.2 (2.74) a
% reduction	—	9.3	45.4	61.3	77.7	81.4	35.2	67.0	78.1	48.2

[1]Means (± SE). In each column values followed by the same letter are not significantly different (P > 0.05), with Tukey's HSD test performed on square root (X + 1) transformed data; untransformed means are shown above. Blocking variable was used in the ANOVAs to reduce the error variability, thereby increasing the power of the treatment statistics. DSOBTH = disodium octaborate tetrahydrate. n = 19 for all treatments, except for Wk 1, where n = 16 for the Control and the 1% DSOBTH. % reduction = % reduction from pretreatment values.

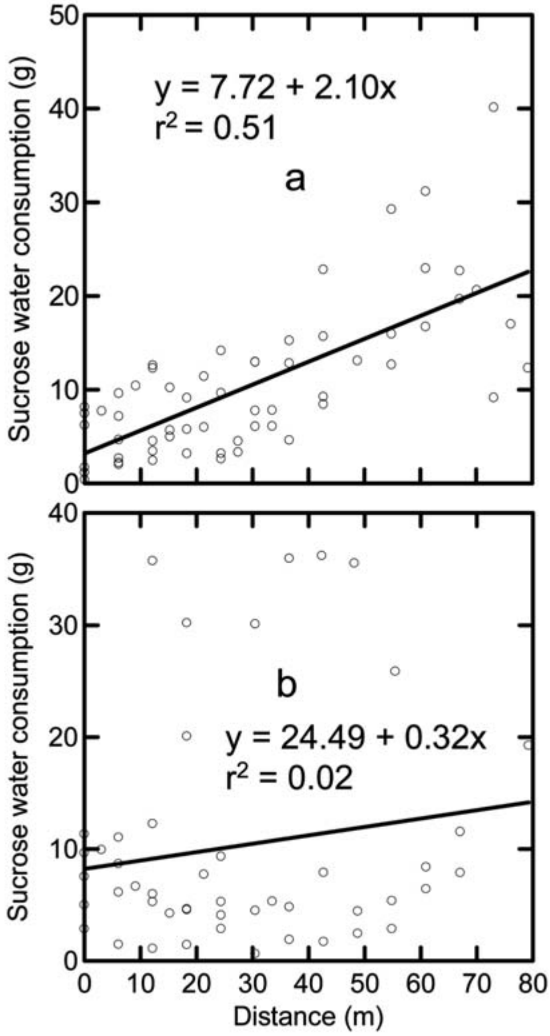

Fig. 1. Pooled data of regressions of sucrose-water consumption (g) vs. distance (m) from treated plots. (a) 1% DSOBTH (6 transects, n = 60), and (b) 0.5% DSOBTH (5 transects, n = 48).

Discussion

As described above, ant numbers in control plots began to decline 2 wk after treatments. To test the hypothesis that the treatments could influence control plots, we sampled ants along transects starting at a treatment plot and going into untreated parts of the citrus grove. For the treatments with the 1% DSOBTH the significant regression analysis shows an effect at least 70 m away from the treatment. In spite of the control plots being within the active space of the treatment plots, we were still able to show overall differences between treatments and controls. The regressions suggest that these differences would be higher if the control plots were further from the treatments.

Various non-chemical and chemical methods have been developed for Argentine ant control in citrus (Vega & Rust 2001). In the early 1900s in Louisiana, traps consisting of wooden boxes containing decaying vegetable matter were set out in groves to attract colonies of ants during the winter (Newell & Barber 1913). The warmth of the decomposing organic matter was thought to attract the ants, which moved into the boxes where they were treated with an insecticide such as carbon bisulfide. Another early method involved flooding orchards in order to force the ants into a concentrated area where they were treated with scalding water or kerosene (Newell & Barber 1913). Tree banding with a mixture of sulfur and sticky material was also a recommended treatment (Woglum & Neuls 1917). More modern banding techniques incorporate Stikem + repellents such as farnesol (Shorey et al. 1992), or controlled-release chlorpyrifos (James et al. 1998). Although effective, these methods have generally not been adopted by growers because they are labor intensive (Rust et al. 2003).

A more practical means of control is the application of broad-spectrum residual insecticides. Chlordane, for example, was the standard treatment for ant control in citrus in the mid twentieth century, until its use was prohibited by the Environmental Protection Agency (EPA) in 1980 (Moreno et al. 1987). Organophosphates, such as chlorpyrifos and diazinon, replaced the chlorinated hydrocarbons and are still being used today for ant control. However, their use is being phased out in urban environments, and growers are also reducing their applications of these chemicals for ant control (Martinez-Ferrer et al. 2003).

Baits offer several advantages over residual insecticides. First, with regard to efficacy, baits exploit the recruitment and food-sharing behaviors of ants to spread a toxicant throughout the colony. In the case of Argentine ants, baits have the added benefit of being spread among nests due to transfer of foods and movement of ants in this unicolonial species. For example, Markin (1968) estimated that >50% of the worker population was exchanged among neighboring nests in 5 d. In contrast to baits, residual insecticides kill ants on contact, mostly the aboveground foragers, which are readily replaced with colony reserves.

Second, in comparison to residual insecticides there is far less active ingredient in baits and particularly when contained, as in bait stations, there is reduced environmental contamination. Indeed, the degree of environmental protection provided by bait stations convinced EPA that certain expensive data requirements could be waived, making future registration of these innovative technologies much more likely (Klotz et al. 2004).

In previous tests in urban settings, we reduced Argentine ant populations by 80% using 0.5% boric acid in 25% sucrose-water (Klotz et al.

1998). Adopting our techniques, Daane et al. (2006) used the same bait in grape vineyards and significantly reduced Argentine ant populations at one of two sites where it was tested. Over the course of their 3-year study Daane et al. developed better dispensers and more effective deployment patterns for liquid baits leading to more consistent reduction in ants, and significantly less mealybugs and crop damage. In comparison, the standard treatment with chlorpyrifos for ants in vineyards had little or no long-term impact on the ant densities (Daane et al. 2006).

We believe that monitoring transects as was done in this study may provide valuable information for determining rates of application as well as concentration of active ingredient. For example, after 11 wk of exposure to the 1.0% DSOBTH bait there was significant reduction of ants up to 76 m away from the treated plots. On the other hand, the 0.5% DSOBTH bait did not have an effect over this same distance. A likely cause for this difference in efficacy is due to dilution of the bait toxicant by trophallaxis. Rust et al. (2004) showed that in the case of borates there is a relatively narrow range of concentrations that are effective, and that trophallaxis can readily dilute a toxicant to a sublethal dose. This dilution effect is magnified in the high population densities of Argentine ants that are found in some citrus groves. In lighter infestations as in the urban setting mentioned above, 0.5% boric acid bait was sufficient.

Based on previous research in commercial settings (Klotz et al. 2004), a baiting program for a heavy infestation of Argentine ants in organic citrus might start with 55 bait stations per hectare and 1% borate solution. Only half the number of bait stations would be used the following year, since there is significant carry-over of reduced populations from one season to the next (Klotz & Rust 2002). The number of stations might even be further reduced, but the amount is yet to be determined by future research.

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