Combined Effect of Hemipteran Control and Liquid Bait on Argentine Ant Populations

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J. Econ. Entomol. 103(5): 1790–1796 (2010); DOI: 10.1603/EC10150

ABSTRACT The invasive Argentine ant, *Linepithema humile* (Mayr), has become a worldwide problem capable of inflicting significant ecological and economic injury on urban, agricultural, and natural environments. The mobility of this pest ant has long been noted, rapidly moving nests to new food resources and then away as resources are depleted. This ant, like many pest ant species, has a special affinity for honeydew excreted by phloem-feeding Hemiptera. We investigated the effect of various hemipteran control strategies on terrapin scale densities and measured their indirect effect on local Argentine ant densities and foraging effort. We then determined whether this indirect treatment strategy improved the performance of an ant bait. We predicted that Argentine ants would move nests away from trees treated for Hemiptera and then move nests back when a liquid bait was offered, followed by a decline in ant numbers due to intake of the toxicant. A horticultural oil spray and soil application of the systemic insecticide, imidacloprid, had no effect on terrapin scale numbers. However, trunk-injected dicrotophos caused a reduction in scale and a decline in local Argentine ant nest density and canopy foraging effort. We also recorded a reduction in local Argentine ant ground foraging when large amounts of liquid bait were applied, and we found no evidence that combining dicrotophos with liquid ant bait performed better than each treatment alone. We suggest that a strategy of combined hemipteran control plus application of liquid ant bait can reduce local Argentine ant densities, when both components of this system are highly efficacious.

KEY WORDS Coccidae, *Linepithema humile*, *Mesolecanium nigrofasciatum*, pest management, red maple

The Argentine ant, *Linepithema humile* (Mayr), has become a major international pest capable of inflicting significant ecological and economic injury in urban, agricultural, and natural environments (Silverman and Brightwell 2008). It is thought that several dominant ants, including many invasive species, achieve their numerical superiority through the dominance of carbohydrate resources, such as hemipteran honeydew (Davidson et al. 2003). The Argentine ant, a dominant species, is well known for its attraction to honeydew (Newell and Barber 1913, Holway et al. 2002). Liquid food composes the greatest proportion of retrieved food for Argentine ant colonies (Markin 1970, Abril et al. 2007), with honeydew serving as an important source of colony carbohydrate (Flanders 1951, Holway et al. 2002, Ness and Bronstein 2004).

Limiting honeydew resources to reduce local Argentine ant densities has been suggested as an indirect method of controlling Argentine ant infestations (Rust et al. 2003, Silverman and Brightwell 2008, Brightwell and Silverman 2009). The dispersed central-place foraging strategy used by Argentine ants ensures that nests relocate near new food resources and then away when those food sources are depleted or reduced (Newell and Barber 1913, Holway and Case 2000, Silverman and Nsimba 2000). Carbohydrate-rich liquids, such as honeydew, are the preferred food for many pest ant species, including the Argentine ant (Newell and Barber 1913, Baker et al. 1985) and baits mimicking honeydew are preferred by Argentine ant workers (Silverman and Roulston 2001). This offers the possibility that pest ants may be encouraged to consume more of a liquid carbohydrate-based toxic bait if alternate sources of carbohydrate, such as honeydew, are reduced (Silverman and Brightwell 2008).

We investigated the possibility for improving liquid bait performance by reducing honeydew availability through the reduction of phloem-sucking hemipterans. Recently, Brightwell and Silverman (2009) demonstrated that excluding Argentine ant foragers from the native terrapin scale, *Mesolecanium nigrofasciatum* (Pergande), lead to a dramatic decline in the number of Argentine ant nests at the base of the host red maple, *Acer rubrum* L. The decline in Argentine ant nests around these banded trees was probably due to relocation of the nests after exclusion from honeydew resources within the host tree. Banding ornamental

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trees with a sticky substance has been used to directly control flightless pests such as ants (Phillips et al. 1987), gypsy moths (Thorpe et al. 1993), and flightless weevils (e.g., Magarey et al. 1992). Indirectly controlling phloem-feeding Hemiptera via ant exclusion is not often applied commercially and is mainly limited to scientific study (Phillips et al. 1987, Karhu 1998, Nagy et al. 2007, Brightwell and Silverman 2010). Conventional hemipteran control strategies directly target the Hemiptera through horticultural oil sprays (e.g., Fernandez et al. 2005), foliar sprays (Kanwar et al. 2009), and systemic insecticides are the usual methods used (Hubbard and Potter 2006, Grafton-Cardwell et al. 2008, Raupp et al. 2008). We tested the efficacy of horticultural oil spray, a systemic ground drench, and a systemic trunk-injected insecticide in controlling terrapin scale and their indirect effects on local Argentine ant densities.

Brightwell and Silverman (2009) predicted that Argentine ants excluded from hemipteran honeydew would suffer greater mortality from exposure to liquid baits than ants that had access to their natural food source. However, no bait effect was recorded, possibly because Argentine ant workers may have had limited access to the bait (Brightwell and Silverman 2009). In this study, we aimed to address this earlier potential limitation by providing much larger volumes of liquid bait in stations with a greatly increased surface area, thus accommodating many workers simultaneously. We predicted that terrapin scale numbers would decline after hemipteran treatment, with subsequent declines in the numbers of Argentine ants foraging into the canopy, and occupying nests at the base of trees treated for Hemiptera. We also predicted that ants would reestablish nests at the base of trees treated for Hemiptera and receiving liquid bait, with a decline in both ant nests and ground foraging workers after toxicant intake.

Methods and Materials

We conducted our experiments at a commercial center in Research Triangle Park, NC, which has a large population of Argentine ants covering ≈42 ha. Trails of Argentine ant workers are commonly observed between the soil and the canopy of many ornamental trees and bushes within the site. Red maple is extensively used as an ornamental tree within the site, with many of these red maples situated within the area infested by Argentine ants. The maples are heavily infested with the native terrapin scale (Brightwell and Silverman 2009, Brightwell and Silverman 2010), a coccid known to excrete copious amounts of honeydew (Simanton 1916).

Comparisons of Three Terrapin Scale Control Methods. We evaluated three methods aimed at reducing the numbers of terrapin scale on red maple: a horticultural oil spray, a ground drench of imidacloprid, and a trunk injection of dicrotophos. We randomly chose 30 red maple trees from across the commercial center that were infested with terrapin scale for the horticultural oil trial. Soluble paraffinic oil (Southern Agricultural Insecticides, Inc., Hendersonville NC) was sprayed onto 15 of the 30 experimental trees at the label rate for dormant soft scales of 23 ml of oil per liter of water. The remaining 15 trees remained untreated as controls. The application was completed in February 2009 by a commercial horticultural contractor, before budbreak in red maple. All treatment plants were sprayed to runoff from soil to the top of the tree from two directions to ensure complete coverage.

We arbitrarily selected 24 red maple trees within the commercial center and allocated 12 trees to the systemic insecticide ground drench treatment, and 12 trees to the control group. All trees were infested with terrapin scale and had Argentine ants trailing into the canopy before selection. Fifteen liters of Merit solution (Bayer Environmental Science, Research Triangle Park, NC. Active ingredient 75% imidacloprid) was applied as a soil drench in late August 2008, at the label rate of 0.028 g Al per liter. Any mulch and loose soil or debris was removed from around the drip line of the treatment trees and a shallow ditch (≈50 mm deep by 300 mm wide) was dug along the drip line. The liquid insecticide was applied into the ditch only and then the ditch was filled with soil and mulch. This protocol was adopted to prevent any ants foraging within the mulch or on the tree direct exposure to imidacloprid.

We arbitrarily chose 44 red maples across the site with 22 trees randomly assigned to an injected systemic treatment. The systemic treatment consisted of injecting dicrotophos (INJECT-A-CIDE B, J. J. Mauget Co. Arcadia, CA) into the base of the tree trunk by a professional horticultural contractor as per the label rate of one 2-ml capsule per 5 cm (2 in.) of tree diameter at breast height (1.64 g [AI] per capsule). Each tree was watered with 150 liters of water to facilitate toxicant uptake. We treated these trees in early July 2009.

We surveyed terrapin scale densities by clipping four branches from each experimental red maple. The canopy height was quartered, by eye, and one branch was arbitrarily selected from each quarter. The selected branches were cut to include all new growth to ensure all settled terrapin scales were captured (Simanton 1916). The branches from each tree were marked, and the settled terrapin scales were counted under a magnifying lamp. For the horticultural oil trials, pretreatment terrapin scale densities were assessed 14 d before oil treatment and then 8 wk after treatment (after leafbud) and 15 wk after treatment (after terrapin scale crawler emergence). Terrapin scale assessment for the imidacloprid trials was performed 7 wk before the application of the soil drench. Terrapin scale densities were then assessed 20, 33, and 40 wk after imidacloprid treatment. Terrapin scale densities for the dicrotophos trials were assessed 4 wk before and 6 wk after the application of dicrotophos, 24 d before the addition of toxic bait.

Augmentation of Dicrotophos Treatment With Liquid Bait. Dicrotophos treatment trials included augmentation with a liquid bait to directly control local Argentine ant densities. We had predicted that local

Argentine ant densities would increase once large amounts of carbohydrate-based liquid bait were added to the system. We randomly assigned one half of the trees receiving dicrotophos (systemic treatment trees) and one half of the trees not receiving dicrotophos (systemic control trees) to an additional treatment with liquid bait, introduced 10 wk after dicrotophos was applied. The liquid bait consisted of 0.5% (wt:vol) boric acid in 20% (wt:vol) sucrose water. Klotz et al. (1996) determined that low concentrations of boric acid were both nonrepellant to and effective against the Argentine ant, and Baker et al. (1985) found 25% sucrose or honey water highly attractive to Argentine ants. To minimize sucrose crystallization in the field, we used a 20% sucrose solution.

Liquid bait stations were placed under the loose pine (*Pinus* spp.) needle mulch at the base of trees assigned to the liquid bait treatment. Liquid bait (≈250 ml) was dispensed in a white polyvinyl chloride (PVC) tube (300 by 32 mm i.d.) (Charlotte Pipe and Foundry Co., Charlotte, NC) with three 13-mm-diameter holes drilled in line equally spaced along the length of the pipe. The PVC tube was capped at both ends with a leak-proof PVC cap (Lasco Fittings Inc., Brownville, TN). This tube was then placed inside a larger diameter white PVC tube (380 by 76 mm i.d.) to reduce evaporation. Fiberglass filtration media (310 by 128 mm) (Flanders, Washington, NC) was inserted into the inner PVC pipe and provided a matrix for Argentine ant workers to access the liquid bait without drowning. Both the fiberglass matrix and liquid bait were replaced weekly until the end of the experiment.

Surveying Local Argentine Ant Densities. We estimated ant activity by three methods. We recorded the number of Argentine ant nests and the number of ants foraging into the canopy on all experimental trees and Argentine ant ground foraging activity before and after dicrotophos treatment and after the introduction of the liquid bait stations. Nest surveys and canopy foraging counts were undertaken before dicrotophos treatment and 6, 14, and 18 wk after dicrotophos treatment. Argentine ant ground foraging activity was assessed before the introduction of liquid bait stations and weekly thereafter for 5 wk. The liquid bait stations were introduced 12 wk after dicrotophos treatment. We counted the number of nests (defined as a concentration of brood) around experimental trees by peeling back the mulch layer at the base of the tree and exposing the nests. When the mulch is peeled back carefully, Argentine ant nests will remain in the inspected area with this procedure (Silverman et al. 2006, Brightwell and Silverman 2009). All trees selected for the experiment had visible foraging trails on their trunk. Argentine ant canopy foraging effort was measured by counting the number of ants passing a predetermined point on a foraging trail, approximately at breast height, for 1 min. All trails on the experimental tree trunk were surveyed.

We ascertained whether the introduction of liquid bait influenced Argentine ant ground foraging and how far the effect extended from the liquid bait treated tree. Ground ant surveys were undertaken in the early afternoon. Ground survey bait cards, 76 by 128 mm, were placed on the mulch adjacent to the experimental trees, a distance of 3–4 m from the experimental trees but with a continuous corridor of pine needle mulch or grass connecting them (softscape) and a distance of >4 m from the experimental trees across tarmac or concrete (hardscape). Ground survey bait cards contained ≈5 g of apple jelly placed in the middle and each card was left in place for 1 h. Bait cards were then carefully placed in a 165- by 149-mm resealable plastic bag (S.C. Johnson & Sons, Inc., Racine, WI) and frozen upon immediate return to the laboratory. Once frozen, captured Argentine ants were identified and counted.

Statistical Analysis. All statistical analyses were performed with JMP version 7 (SAS Institute 2007). Repeated measures multivariate analysis of variance (MANOVA) was performed to compare terrapin scale numbers on horticultural oil, imidacloprid, and dicrotophos treatments to nontreated trees. For each hemipteran control method, the treatment tested was the between subjects variable and time was the within subjects variable. Analysis of change in terrapin scale numbers for both dicrotophos-treated and control trees were analyzed by Wilcoxon signed rank test. Repeated measures MANOVA was performed for Argentine ant nest and 1-min canopy foraging counts with dicrotophos treatment and liquid bait addition as the between-subjects variables and time as the within-subjects variable. Repeated measures MANOVA also was performed for ground forager bait card surveys for both dicrotophos-treated and control trees with bait card location (adjacent, softscape, or hardscape) and liquid bait treatment as between-subjects variable and time again as the within-subjects variable. All data were log transformed to satisfy assumptions of equal variance.

Results and Discussion

Horticultural oil had no effect on terrapin scale numbers ($F_{1,28} = 0.23$; $P = 0.6321$). Horticultural oils have been reported as having some success controlling terrapin scale on peach trees (Simanton 1916, Pless et al. 1995); however, Hubbard and Potter (2006) reported horticultural oils had no effect on calico scale, *Eulecanium cerasorum* (Cockerell). It is unclear whether mineral oil treatment kills coccids through suffocation or contact with exposed membrane. Terrapin scales hibernate through the colder months and possess a tough test that seals tightly to the host plant tissue with waxes (Simanton 1916). It would seem that terrapin scale has some defenses against either mode of action. Soil drenching with imidacloprid was equally ineffective at reducing terrapin scale densities ($F_{1,21} = 0.02$; $P = 0.8663$). Imidacloprid also has proved ineffective against calico scale as both a soil-injected and trunk-injected systemic insecticide (Hubbard and Potter 2006). Imidacloprid typically shows little activity in the phloem (Mota-Sanchez et al. 2009), which may explain the poor results against stem settling phloem feeders.

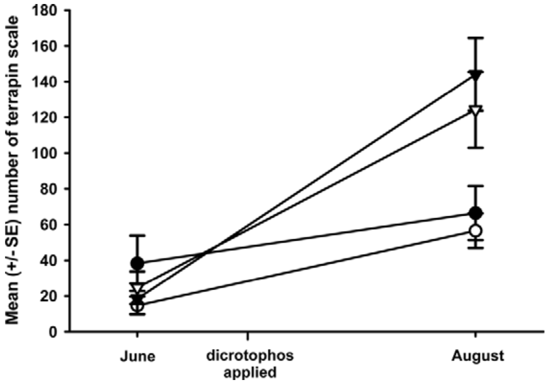

Fig. 1. Mean ± SE number of terrapin scale surveyed on experimental red maples. Terrapin scale was surveyed through branch clippings taken from various strata of the tree canopy. Tree treatments included dicrotophos injected into the trunk of the tree, liquid bait dispenser placed in the mulch at the base of the tree, or both. ●, dicrotophos-treated trees with liquid bait added; ○, dicrotophos-treated trees only; ▼, trees with liquid bait added only; and ▽, control trees.

Dicrotophos produced significant but incomplete control of the honeydew-producing terrapin scale. Terrapin scale numbers on control trees were higher over time than on the systemic treated trees ($F_{1,33}$ = 54.23; $P < 0.0001$; Fig. 1). Our experimental trees were large mature maples with trunk diameter between 210 and 330 mm at chest height. Even though we applied dicrotophos at the label rate, we did not see a decline in terrapin scale numbers. Rather, numbers remained steady on systemic-treated trees during the study ($z = -11.5$, $P = 0.4548$), whereas terrapin scale numbers on control trees increased markedly ($z = 89.5$, $P = 0.0016$). Indirect control of terrapin scale through the exclusion of Argentine ant foragers by Tanglefoot banding saw a collapse of terrapin scale numbers, presumably by allowing natural enemies unhindered access to the coccids (Brightwell and Silverman 2009). Terrapin scale suffers tremendous pressure from numerous parasitoids and predators (Simanton 1916, Williams and Kosztarab 1972, Devorshak 1994, Meyer et al. 2001).

We chose dicrotophos because of its rapid translocation within the treated tree (Donley 1967). Microinjection of dicrotophos has been successful against several tree-feeding insects (e.g., *Adelges tsugae* Annand, McClure 1992; gall wasp *Callirhytis cornigera* (Osten Sacken), Eliason and Potter 2000; eastern tent caterpillar, *Malacosoma americanum* (F), Potter et al. 2005). Trunk-injected dicrotophos afforded some control over calico scale in sugar maple, *Acer saccharum* Marshall, and sweetgum (*Liquidambar* spp.) (Hubbard and Potter 2006). Koehler and Campbell (1968) found that dicrotophos produced erratic results in Norway maple, *Acer platanoides* L., and silver maple, *Acer saccharinum* L., against aphids *Periphyllus lyropictus* Kessler and *Drepanaphis acerifoliae* Thomas, respectively, and it seems that complete hemipteran control in maples through the use of systemic insecticides may be generally difficult to achieve.

Table 1. Summary of F statistics of the fixed effects of dicrotophos treatment and liquid bait treatment trees vs control trees from repeated measures ANOVA on Argentine ant nest numbers and foraging effort on the host red maple

Effect	*F*	df	Prob
No. Argentine ant nests at the base of the experimental trees			
Dicrotophos treatment	5.2940	1,30	0.0285
Liquid bait treatment	0.7097	1,30	0.4062
Dicrotophos × liquid bait interaction	1.1206	1,30	0.2982
No. Argentine ants trailing up and down the trunk of experimental trees in 1 min			
Dicrotophos treatment	9.3662	1,40	0.0039
Liquid bait treatment	0.6585	1,40	0.4219
Dicrotophos × liquid bait interaction	0.0393	1,40	0.8438

We saw a drop in nest numbers around the base of dicrotophos-treated trees (Table 1; Fig. 2) but not to the same degree when Argentine ant workers were completely excluded from the tree canopy (Brightwell and Silverman 2009). The reduction in nest numbers at the base of dicrotophos-treated trees demonstrated the efficiency of the dispersed central-place foraging strategy, used by the Argentine ant, in distributing available colony resources (Holway and Case 2000). When excluded from the tree canopy, nests numbers drop to virtually zero (Brightwell and Silverman 2009). Canopy foraging by Argentine ants was reduced on trees receiving dicrotophos compared with trees not receiving this systemic insecticide (Table 1; Fig. 3). Argentine ant foraging effort is linearly correlated with increased numbers of hemipterans (Grover et al. 2008) and the reduction in foraging effort, again, reflects the lower terrapin scale numbers on the dicrotophos-treated trees. The reduced numbers of Argentine ants foraging in the canopy after dicrotophos treatment seemed to have still offered the

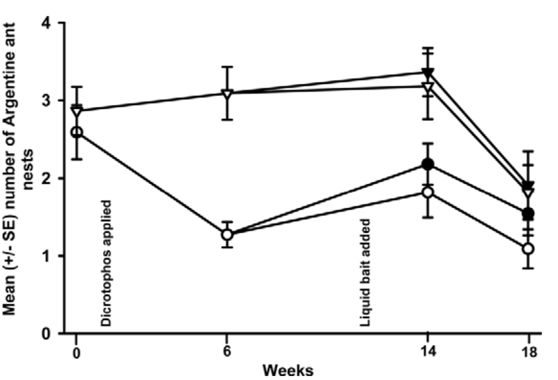

Fig. 2. Mean ± SE number of Argentine ant nests at the base of experimental red maples. Dicrotophos was injected into the treatment tree trunk and liquid bait dispensers were placed in the mulch at the base of the tree. ●, dicrotophos-treated trees with liquid bait added; ○, dicrotophos-treated trees only; ▼, trees with liquid bait added only; and ▽, control trees.

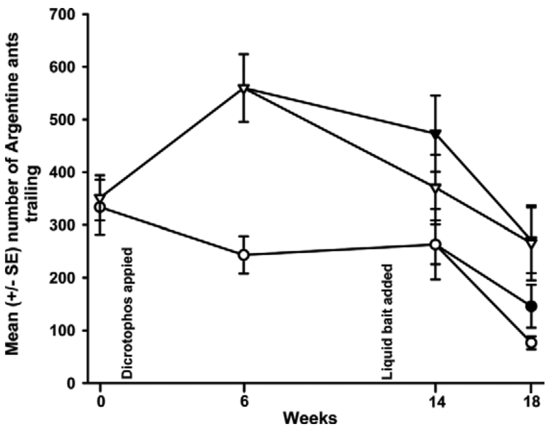

Fig. 3. Mean ± SE number of trailing Argentine ants passing a point on the tree trunk in 1 min. One minute counts included workers moving both up and down trail were counted and all trails on each tree were included. Dicrotophos was injected into the treatment tree trunk and liquid bait dispensers were placed in the mulch at the base of the tree. ●, dicrotophos-treated trees with liquid bait added; ○, dicrotophos-treated trees only; ▼, trees with liquid bait added only; and ▽, control trees.

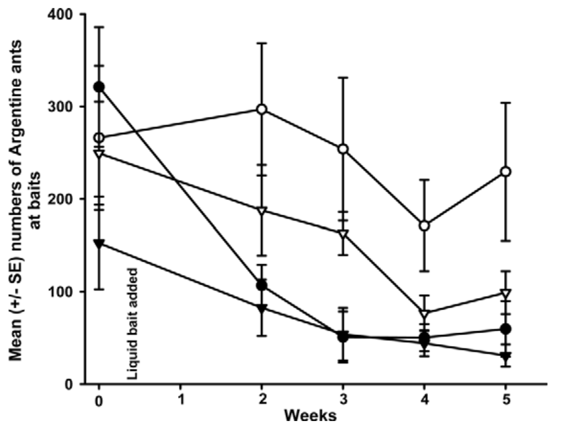

Fig. 4. Mean ± SE number of Argentine ants on bait cards placed at the base of experimental red maples. Surveys were conducted weekly with liquid bait dispensers introduced immediately after the pretreatment count. Liquid bait dispensers were placed in the mulch at the base of the tree. ●, dicrotophos-treated trees with liquid bait added; ○, dicrotophos-treated trees without liquid bait; ▼, trees with liquid bait only; and ▽, neither dicrotophos or liquid bait applied.

surviving terrapin scale adequate protection from their myriad predators.

Our liquid bait treatment had no measurable effect on either Argentine ant nest number or numbers of canopy foraging workers at both dicrotophos-treated and untreated trees (Table 1; Figs. 2 and 3). We had predicted that nest number would initially increase at dicrotophos-treated trees upon the addition of the liquid bait stations. Brightwell and Silverman (2009) also reported no increase in nest numbers upon the addition of liquid bait (0.5% boric acid in 25% sucrose water). Brightwell and Silverman (2009) suggested that the dimensions of their toxicant dispenser were such that simultaneous feeding by many workers was limited; thus, there may not have been adequate incentive for Argentine ant nests to relocate. Our stations contained ≈250 ml of liquid bait, with a surface area ≈100 times larger than that in Brightwell and Silverman (2009) and thus could accommodate large numbers of simultaneously feeding workers. The Argentine ant will readily move nests to new food resources (Newell and Barber 1913, Holway and Case 2000, Silverman and Nsimba 2000) yet did not relocate nests close to the bait stations. We conclude that honeydew availability was still plentiful within the environment, even with our dicrotophos treatment, and was more attractive to Argentine ants than the liquid bait formulation.

Fewer Argentine ant workers were captured at the ground foraging bait cards placed at the base of trees receiving liquid bait for both dicrotophos-treated tress ($F_{1,14} = 5.00$; $P = 0.0421$) and nondicrotophos-treated trees ($F_{1,14} = 5.78$; $P = 0.0305$) compared with trees without liquid bait (Fig. 4). There was no difference in the pre liquid bait count even though there was some variation in initial treatment means ($F_{3,39} = 1.4$, $P = 0.2503$; Fig. 4). The decline in Argentine ant foragers at the bait stations indicated that the toxic bait had a discernible effect on local ant densities. Interestingly, this effect of liquid bait on Argentine ant numbers did not extend beyond the liquid bait-treated tree to nearby trees whether they were separated by softscape ($F_{1,9} = 1.20$; $P = 0.3001$) or hardscape ($F_{1,9} = 0.48$; $P = 0.5019$). This was somewhat surprising as Argentine ant workers are capable of foraging over large distances, up to 60m (Vega and Rust 2003). In addition, trophallaxis among Argentine ant workers and queens should have helped to extend the lethal effect of boric acid across the landscape (Markin 1970). It is possible that the amount of toxicant introduced during this study was insufficient to exert effective control over the entire infestation. The dynamic nature of Argentine ant supercolonies (Newell and Barber 1913, Holway and Case 2000, Heller et al. 2006) may have masked evidence of any spatial effect of the toxicant on the Argentine ant colony.

We selected various insecticide formulations and delivery systems to test our novel approach based on demonstrated effects on Coccidae and Argentine ants from previous reports. Terrapin scale proved difficult to control with the conventional hemipteran control strategies we used. The impact of our combined plant systemic-liquid bait strategy on Argentine ant populations was modest. Although we still consider our strategy sound, other toxicant combinations may prove more effective in reducing local pest ant densities.

Acknowledgments

We thank M. Green, T. Walker, and P. Crump for technical assistance. This study was supported by USDA grant SRIPM

2008-34103-19001 and The Blanton J. Whitmire Endowment at North Carolina State University.

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Received 23 April 2010; accepted 6 July 2010.