Improving Liquid Bait Programs for Argentine Ant Control: Bait Station Density

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ABSTRACT Argentine ants, *Linepithema humile* (Mayr), have a positive effect on populations of mealybugs (*Pseudococcus* spp.) in California vineyards. Previous studies have shown reductions in both ant activity and mealybug numbers after liquid ant baits were deployed in vineyards at densities of 85–620 bait stations/ha. However, bait station densities may need to be <85 bait stations/ha before bait-based strategies for ant control are economically comparable to spray-based insecticide treatments—a condition that, if met, will encourage the commercial adoption of liquid baits for ant control. This research assessed the effectiveness of baits deployed at lower densities. Two field experiments were conducted in commercial vineyards. In experiment 1, baits were deployed at 54–225 bait stations/ha in 2005 and 2006. In experiment 2, baits were deployed at 34–205 bait stations/ha in 2006 only. In both experiments, ant activity and the density of mealybugs in grape fruit clusters at harvest time declined with increasing bait station density. In 2005 only, European fruit lecanium scale [*Parthenolecanium corni* (Bouché)] were also present in fruit clusters, and scale densities were negatively related to bait station density. The results indicate that the amount of ant and mealybug control achieved by an incremental increase in the number of bait stations per hectare is constant across a broad range of bait station densities. The results are discussed in the context of commercializing liquid ant baits to provide a more sustainable Argentine ant control strategy.

KEY WORDS ant bait, biological control, grapes, mutualism

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), has reached pest status in multiple environments (Human and Gordon 1997, Vega and Rust 2001, Holway et al. 2002). In natural environments, the Argentine ants' exploitative interactions with insects and plants and their competitive interactions with other ant species have threatened native communities at locations in California, Hawaii, South Africa, and Europe (Way et al. 1997, Krushelnycky and Reimer 1998a, 1998b, Christian 2001, Sanders et al. 2001, Wetterer et al. 2001). In urban environments, the presence of Argentine ants as they forage for food, water, and shelter is a major nuisance for people (Klotz et al. 2002). Meanwhile, in agricultural environments, the mutualisms that form between Argentine ants and honeydew-producing hemipterans can result in higher hemipteran densities and greater pest damage and economic loss (Buckley 1987, Styrsky and Eubanks 2007).

Honeydew-producing hemipterans feed on plant sap and are found as pests in most agricultural crops (Blackman and Eastop 2000, Styrsky and Eubanks 2007). Typically, ants that associate with honeydew-producing hemipterans collect the herbivores' sugar-rich excretions as a food source and, in exchange, provide a series of benefits that includes attacking and repelling predators and parasitoids of the hemipterans (Way 1963, Buckley 1987, Styrsky and Eubanks 2007). In citrus groves, Argentine ants collect honeydew from mealybugs, whiteflies, and soft scale and reduce natural enemy abundances, leading to damaging population levels of the hemipteran pests (Moreno et al. 1987). In vineyards in coastal California, Argentine ants form mutualisms with the grape mealybug, *Pseudococcus maritimus* (Ehrhorn), and the obscure mealybug, *P. viburni* (Signoret) (Hemiptera: Pseudococcidae) (Daane et al. 2007). The grape mealybug is predominant in North Coast appellations and the obscure mealybug predominates in Central Coast appellations. These *Pseudococcus* mealybugs have a sporadic history as vineyard pests. The grape mealybug reached primary pest status in vineyards from the 1940s to the 1960s, when insecticide applications may have disrupted biological controls (Flaherty et al. 1992). More recently, these mealybug species were considered secondary pests that rarely rose above economic thresholds. However, the pest status of *Pseudococcus* species began to shift in the late 1980s. The territory of the invasive Argentine ant has expanded in the

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grape-growing regions of coastal California, and the ant's presence in these vineyards has contributed to the reappearance of economically damaging populations of *Pseudococcus* mealybugs (Phillips and Sherk 1991).

Argentine ants actively tend the *Pseudococcus* mealybugs, collecting the carbohydrate-rich excretions of the mealybugs on the grapevines and returning to their nests to share the honeydew with their queens and developing larvae. Ant exclusion experiments showed that mealybugs were 3–82 times more abundant on the grape clusters of ant-tended vines than on the clusters of ant-excluded vines (Daane et al. 2007), exceeding economic damage thresholds. Mealybugs foul the crop directly, through their physical presence in the grape clusters, and indirectly by producing honeydew, which serves as a substrate for mold and rot. Grape growers commonly protect their crop by applying insecticidal sprays targeted at the mealybugs and/or the ants (Addison 2002, Klotz et al. 2003, Daane et al. 2006b). However, growers are interested in alternative methods of achieving mealybug control for at least three reasons. First, the effectiveness of contact insecticides is severely limited by the protected locations of mealybugs and ants: mealybugs feed under the bark and in the crevices of vines, and ants locate their colonies underground. Sprays against ants contact worker ants that are actively foraging but not the workers, queens, eggs, and larvae that reside in the nest. Thus, colonies can recover quickly. Second, sprays must be reapplied after 1–3 mo because their residual effectiveness declines over time. Third, the chemicals commonly applied as a barrier sprays to suppress ant populations are broad-spectrum insecticides that conflict with widely held interests in maintaining a community of beneficial insects and minimizing inputs of pesticides to the environment.

An alternative approach to ant control is to combine a slow-acting insecticide with a feeding attractant to create a toxic bait (Blachly and Forschler 1996, Krushelnycky and Reimer 1998b, Rust et al. 2000, Silverman and Roulston 2001, Klotz et al. 2004, Tollerup et al. 2004, Greenberg et al. 2006). A series of studies using different active ingredients and different bait station designs has adapted the use of liquid baits to the grape agroecosystem (Klotz et al. 2003, Tollerup et al. 2004). In studies by Daane et al. (2006a), bait stations were placed at 5- to 10-vine intervals in plots 0.18 ha or smaller, equivalent to densities of 85–620 baits/ha; the liquid baits resulted in fewer ants, fewer mealybugs, and less damage to grape clusters. Thus, liquid baits are effective when bait stations are deployed at high densities. However, because the costs of materials and maintenance increase with the number of bait stations per hectare, growers will be more likely to use liquid baits for Argentine ant control if the baits are effective at lower densities as well. The goal of this study was to examine the effectiveness of liquid baits across a range of densities. We deployed bait stations at various densities in two California vineyards and assessed the impact of the bait station densities on populations of Argentine ants and grape mealybugs.

Materials and Methods

The effect of bait density on ant and mealybug suppression was tested in two experiments at commercial vineyards in the Carneros appellation of Napa and Sonoma Counties, CA, an area with historically high Argentine ant densities. Vineyards were 3–16 yr old, managed for wine production, and irrigated by drip lines. The row middles were cultivated by discing alternate rows and mowing the resident vegetation in the untilled rows; the berms beneath the vines received applications of a glyphosate herbicide. Sterol inhibitors and/or sulfur was applied preventively against powdery mildew (*Uncinula necator* Burrill). All sites had active populations of Argentine ants and grape mealybugs.

Experiment 1: 2005 Season. Twenty-four plots were established in the spring, arranged as six plots in each of four experimental blocks. Experimental blocks differed in location, planting date, and grape varietal (one block each of Pinot Meunier and Chardonnay and two of Pinot Noir). Within each block, plots were randomly assigned to one of six bait station density treatments, 0, 5, 10, 14, 18, and 21 stations per plot, which correspond to 0, 54, 107, 150, 193, and 225 stations/ha. Plots were seven rows of 30 vines each, planted with 1.8 m between vines and 2.4 m between rows, covering 0.094 ha. Bait stations were evenly distributed within plots: for example, a plot with 14 bait stations had two bait stations in each of its seven rows. Plots were separated from each other by eight rows (24.4 m). No insecticides were applied during the 2005 growing season.

Bait stations consisted of 250-ml polypropylene centrifuge tubes with a porous plastic membrane (Weedblock, Easy Gardener, Waco, TX) covering their openings, as described in Daane et al. (2006a). The bait stations were positioned upside down and tied to vines with flagging tape. The membrane held the liquid in the tubes but allowed ants to feed through the pores. The membrane was held in place by the threaded cap, which had a hole drilled in it. The liquid bait was a solution of 25% sugar, 0.50% boric acid, and 0.15% citric acid in tap water (all percentages are wt:vol). The sugar was the attractant, the boric acid was the toxin (Klotz et al. 2000, Daane et al. 2006a, Greenberg et al. 2006), and the citric acid lowered the pH of the solution to retard mold growth. Bait stations were initially placed on 1 June 2005 and replaced every 2–4 wk thereafter, as needed to provide a nearly continuous supply of liquid bait. When bait stations were replaced, the remaining amount of bait was recorded. Similar amounts of bait were removed from all bait stations, indicating that more bait was dispensed in plots with more bait stations, as intended. In some instances bait stations were empty, indicating that there were some interruptions in bait availability.

The effects of the baits on the Argentine ant and mealybug populations were evaluated by measuring ant activity and mealybug abundance at intervals throughout the experiment. Ant activity was measured using "monitoring tubes," which operated on a design similar to that of the bait stations (Daane et al. 2006a, Greenberg et al. 2006). Monitoring tubes consisted of 50-ml polypropylene centrifuge tubes that were filled with 45 ml nontoxic 25% sugar water, capped with a plastic membrane, and weighed. Six monitoring tubes were evenly distributed in each plot. The monitoring tubes were inverted and tied to vines in the plots, allowing ants to feed on them. After 2–7 d, the tubes were returned to the laboratory and reweighed. The difference in weight reflected the amount of sugar water removed by ants plus the amount evaporated. To measure the amount evaporated, 12 monitoring tubes per block (two per plot) were attached to bamboo garden stakes from which ants were excluded by a coating of sticky resin (Stikem Special, Seabright, Emeryville, CA). The average weight loss of the 12 evaporation tubes was subtracted from each of the monitoring tubes in the block to calculate the amount removed by ants for each tube. The values for the six monitoring tubes in each plot were divided by the number of days they were in the field and averaged, yielding the mean rate of sugar water removal by ants for each plot. Because each milliliter of sugar water removed represents ≈3,300 ant visits (Greenberg et al. 2006), sugar water removal rates are related to ant activity. However, ant activity varies with weather conditions and other environmental factors in addition to ant abundance and therefore does not provide an absolute measure of ant density, but a relative measure that is appropriate for comparing ant abundance at locations measured in the same region at the same time. Bait stations were removed from plots during some, but not all, ant activity sampling periods. Ants readily located and fed from monitoring tubes, whether bait stations were present or not.

Ant activity was measured before bait station placement, on 31 May 2005, and after grapes were harvested, on 19 September 2005. Ant activity data were analyzed by MANOVA with sample date, block, and bait density as the independent variables and removal rates on the different sample dates as the dependent variables (von Ende 2001, Hopkins 2003b). When the sample date × bait density interaction term was significant, the MANOVA was followed by univariate linear regression models for individual sample dates with bait density as the sole effect in the model.

Mealybug densities were measured on entire grape vines during the growing season and on fruit clusters near harvest time, based on sampling programs developed by Geiger and Daane (2001). Twelve vines per plot were visually searched for a fixed time period (1.5 min/vine), and all mealybugs were counted. Visual counts were performed twice: once when the baits were placed and again in the middle of the season, on 27 July 2005. The 12 sampled vines (6% of all vines in each plot) were selected in a stratified random sampling scheme, using random numbers generated by Research Randomizer at www.randomizer.org. Selected vines were excluded from the second sample date. Mealybugs move among vine microhabitats (e.g., trunk, leaves) during the growing season (Geiger and Daane 2001). Therefore, each sampling session was initiated by examining several nonexperimental vines to learn the mealybugs' primary location on that date. Observers scanned all parts of the selected vines and concentrated their searches on the plant parts expected to harbor mealybugs. Selected vines were searched for 1.5 min each by turning leaves and peeling bark and recording the number of juveniles (instars II-IV), adult females, and ovisacs. Crawlers and settled first instars are too small to count reliably given the time constraints of this field sampling program and were therefore not included in the counts. To test for the effect of bait station density on mealybug abundance over the course of the growing season, mealybug count data were analyzed by MANOVA with sample date, block, and bait density as the independent variables and total mealybug counts on the different sample dates as the dependent variables.

Fruit was harvested for wine production from three experimental blocks on 8–10 September 2005, and the fourth block was harvested on 8 October 2005. As a measure of crop damage, mealybugs in the fruit clusters were counted 22–23 August 2005. Stratified random sampling was used to select 35 vines in each plot (17% of all vines), and one cluster was removed from each selected vine. Clusters in contact with woody tissue were preferentially sampled because they were more likely to harbor mealybugs (Geiger and Daane 2001). For each plot, the rachis and laterals of the selected clusters were cut into pieces with pruning shears, the berries were inspected, all mealybugs were counted, and the number of mealybugs per cluster was calculated. In addition, individuals of another insect pest of grapes, the European fruit lecanium scale, *Parthenolecanium corni* (Bouché) (Hemiptera: Coccidae), were counted and recorded. Because initial regression analysis yielded residuals that were not uniformly distributed, the mealybug and scale count data were rank transformed. Logarithmic transformations were inappropriate because the data included values between 0 and 1 (Hopkins 2003a). The figures present the untransformed cluster count data. Separate linear regressions were applied to the rank-transformed mealybug and scale data, with block and bait density as effects in both models. Because bait density was expected to reduce mealybug and scale abundance, these were one-tailed tests: for each herbivore, the null hypothesis that the slope of the regression was equal to or greater than zero was tested against the alternative that the slope was less than zero.

To illustrate the relationship between ants and honeydew-producing hemipterans, we tested across all treatments and blocks for correlations between the numbers of mealybugs and scale observed in grape clusters at harvest time and ant activity measured at the sample date immediately after harvest. Because the count data for mealybugs and scale were not normally distributed, their relationship was evaluated with Spearman's rank correlation test.

Experiment 1: 2006 Season. Experiment 1 was originally designed to continue through the 2006 season to gauge the effects of our bait density treatments across multiple seasons. However, infestations of vine mealybug, *Planococcus ficus* (Signoret), were detected in neighboring vineyard blocks during 2005, and after the conclusion of the growing season, the broad-spectrum insecticide chlorpyrifos was applied to all experimental plots as part of a ranch-wide eradication program. The chlorpyrifos application reduced densities of all arthropods, including the Argentine ants. The drastic change in the arthropod community hampered our ability to measure the across-season effects of our treatments, but it also provided us with a new opportunity. Whereas our previous studies were conducted at locations with initially high ant densities, our experiment 1 plots were positioned to study the effect of liquid baits in an area of low ant densities. Thus, the 2006 season tested for the ability of liquid ant baits to suppress the growth of an initially low ant population across a range of bait station densities.

In addition to the experimentwide application of chlorpyrifos, one of the four blocks received two applications of methomyl and was excluded from our analysis for 2006. The other three experimental blocks were not treated with insecticides during the 2006 growing season. The exclusion of the methomyl-treated block did not qualitatively affect the results.

Ant activity was measured on 18 May 2006, and bait stations were placed on 25 May 2006. Subsequently, ant activity was measured on 14 July and 6 October 2006. Liquid bait was depleted more slowly than in the 2005 season; thus, baits were changed less frequently. Bait station changes occurred on 14 August and 11 September 2006. Because no mealybugs were observed in preliminary inspections, we did not conduct visual searches of whole vines. Grape clusters were collected on 11–12 September 2006, 2 wk before harvest, and taken to the laboratory where they were cut apart and inspected for mealybugs. The response variables of the 2006 season—ant activity and mealybugs per cluster—were calculated and analyzed in the same manner as the 2005 season.

Experiment 2: 2006 Season. Because experiment 1 was altered by a pesticide application, we established a second experiment in new locations. Experiment 2 repeated experiment 1, aside from the following methodological differences. In experiment 2, the plots were smaller: each plot was four rows of 35 vines, and plots were separated by six rows. In three blocks, the vines (Pinot Noir) were planted on a 1.8 by 2.4-m spacing, yielding plots that were 0.062 ha. In the fourth block, the vines (Chardonnay) were planted with 1.5 m between vines and 2.7 m between rows, yielding plots that were 6.3% smaller at 0.058 ha. Bait stations were placed on 28 April 2006. The six treatments were 0, 2, 4, 6, 8, and 12 bait stations per plot, corresponding to 0, 34, 68, 102, 137, and 205 baits/ha; in the smaller plots, the bait densities were 6.3% higher. Three of the four experimental blocks were not treated with insecticides. One block was treated with an insecticidal spray of imidacloprid on 21 July 2006, but *t*-tests showed that ant and mealybug populations were not reduced by the imidacloprid, and therefore, all four blocks were included in the analyses. The analyses yielded qualitatively similar results whether the imidacloprid-treated block was included or not.

Monitoring tubes were used to measure ant activity on five dates: a pretreatment count on 27 April, in-season counts on 27 June and 25 July, and postharvest counts on 11 September and 13 October 2006. The monitoring tubes were exposed to ant activity in the field for 1–6 d. Mealybug densities on vines were assessed on two dates, 15 June and 1 September 2006, by visually inspecting eight vines per plot (6% of all vines) for 2.5 min each. End-of-season counts of mealybugs in grape clusters were performed in three of the four experimental blocks; the omitted block was harvested earlier than expected. On 7–8 September 2006, mealybugs were counted on the fruit clusters of 20 vines per plot (14% of all vines); European fruit lecanium scale were negligible on the fruit clusters in 2006 and were not counted. Grapes were harvested on 6–7 September in blocks 1–3 and on 19–20 October in block 4. The calculations and analyses of the response variables in experiment 2—ant activity, mealybug counts on whole vines, and mealybugs per cluster—were similar to experiment 1.

Results

Experiment 1: 2005 Season. Ant activity data showed a significant interaction between bait density and sample date, indicating that bait density caused ant activity to change between May and September (Table 1). The ant activity measurements taken in May showed there was no pretreatment relationship between bait density and ant activity, but by September 2005, ant activity was lower in plots with more baits (Table 2; Fig. 1). Thus, over the course of a growing season, higher densities of bait stations yielded greater ant control.

There was no treatment effect on mealybug abundance on whole vines between the pretreatment and midseason samples taken on 1 June and 27 July 2005 (Table 1; Fig. 2). However, higher densities of bait stations resulted in fewer mealybugs present in grape clusters at the time of harvest (linear regression model: $r^2 = 0.54$; $F = 5.5$; df = 4,19; $P = 0.004$; effect of baits/ha: $F = 6.2$, $P = 0.042$ [one-tailed]; Fig. 3). The number of scale per cluster also declined with increasing bait station density (linear regression model: $r^2 = 0.74$; $F = 13.7$; df = 4,19; $P < 0.001$; effect of baits/ha: $F = 16.0$, $P = 0.008$ [one-tailed]; Fig. 3).

The addition of a nonlinear term [$(\text{baits/ha})^2$] to the models resulted in poorer fits to the data, indicating the relationships between bait station density and ant and mealybug abundances were best fit by linear models. Correlations between ant activity and mealybug and scale densities in grape clusters showed positive associations with Argentine ants (mealybugs: $r_s = 0.66$, $P < 0.001$; scale: $r_s = 0.88$, $P < 0.001$).

Experiment 1: 2006 Season. Ant activity was low throughout the experiment 1 plots in 2006, both in absolute terms and relative to the 2005 season. MANOVA showed no main effect of bait density (between subjects test, bait density: $F = 3.7$; df = 1,14; $P =$

Table 1. Within-subject significance tests for MANOVAs of ant activity and mealybug counts in bait density experiments 1 (2005 and 2006) and 2 (2006 only)

Experiment and response variable	Source of variation	df	Test statistic and value	*P*
Experiment 1, 2005 season: ant activity	Sample date	1,19	$F = 4.3$	0.053
	Sample date × block	3,19	$F = 38.1$	<0.001
	Sample date × bait density	1,19	$F = 9.1$	0.007
Experiment 1, 2005 season: mealybug counts	Sample date	1,19	$F = 6.5$	0.019
	Sample date × block	3,19	$F = 1.1$	0.388
	Sample date × bait density	1,19	$F = 2.2$	0.153
Experiment 1, 2006 season: ant activity	Sample date	2,13	$F = 6.4$	0.011
	Sample date × block	4,28	Pillai's trace = 0.94	0.001
	Sample date × bait density	2,13	$F = 0.31$	0.736
Experiment 2, 2006 season: ant activity	Sample date	4,16	$F = 45.2$	<0.001
	Sample date × block	12,54	Pillai's trace = 1.4	<0.001
	Sample date × bait density	4,16	$F = 6.2$	0.003
Experiment 2, 2006 season: mealybug counts	Sample date	1,19	$F = 0.12$	0.726
	Sample date × block	3,19	$F = 1.5$	0.250
	Sample date × bait density	1,19	$F = 2.4$	0.135

A significant effect of sample date indicates that the response variable changed within plots through time. A significant interaction between sample date and block indicates that the effect of block was different on different dates. Likewise, a significant interaction between sample date and bait density indicates that the effect of bait density was different on different dates.

0.075). The effect of bait density on ant activity did not change during the 2006 season (Table 1). Univariate regressions showed no significant relationships between bait density and ant activity in May, July, or October (Table 2; Fig. 4). In 2006, there was no relationship between bait density and the rank-transformed number of mealybugs in grape clusters (whole model: $r^2 = 0.48$; $F = 4.3$; df = 3,14; $P = 0.025$; effect of baits/ha: $F = 0.34$, $P = 0.283$ [one-tailed]; Fig. 5). Cluster counts of mealybugs were not correlated with October ant activity ($r_s = 0.35$, $P = 0.151$).

Experiment 2: 2006 Season. The effect of bait density on ant activity varied from date to date, as indicated by the significant interaction between bait density and sample date (Table 1). On the pretreatment sample date in April 2006, there was no relationship between bait density and ant activity. In June, ant activity was lower in plots with more bait stations. However, no effect of bait density was observed in July, September, or October (Table 2; Fig. 6).

Bait density had no effect on the abundance of mealybugs on grape vines over the course of the growing season (Table 1; Fig. 7). However, bait station density had a suppressive effect on the number of mealybugs per cluster at harvest time (linear regression model: $r^2 = 0.51$, $F_{3,14} = 4.8$, $P = 0.017$; effect of baits/ha: $F = 0.018$, $P = 0.032$ [one-tailed]; Fig. 8).

Ant activity and cluster count data were better explained by linear models than nonlinear models, suggesting that incremental increases in bait density lead to constant decreases in ant activity and mealybug abundance. There was a positive association between the number of mealybugs per cluster and ant activity at harvest time ($r_s = 0.65$, $P = 0.003$).

Discussion

The two experiments reported here were conducted in fields with initially high ant densities. Within one growing season, each experiment showed suppressive effects of bait density on ant activity and mealybug numbers. Across the range of bait station densities tested, incremental increases in bait station density had a constantly suppressive effect on ant activity and mealybug abundance in clusters. However, the data did not indicate a particular bait density that maximized ant or mealybug suppression. Rather, the results suggest that all densities of baits, from 54 to 225 bait stations/ha, will provide some reduction in ant activity and mealybug clusters. Higher bait station densities will provide greater reductions of ant populations and mealybug damage (Daane et al. 2006a); this study implies that lower bait station densities can

Table 2. Univariate regressions of ant activity (g sugar water removed per day) on bait density (baits per ha) in bait density experiments 1 and 2

Experiment	Month	r^2	Slope	SE	Model fit
Experiment 1, 2005 season	May	<0.001	0.0010	0.0204	$F < 0.01$, $P = 0.960$
	Sept	0.210	−0.0237	0.0010	$F = 6.0$, $P = 0.023$
Experiment 1, 2006 season	May	0.210	−0.0033	0.0016	$F = 4.2$, $P = 0.058$
	July	0.140	−0.0036	0.0023	$F = 2.6$, $P = 0.126$
	Oct.	0.020	−0.0022	0.0038	$F = 0.34$, $P = 0.570$
Experiment 2, 2006 season	April	0.008	0.0076	0.0174	$F = 0.2$, $P = 0.664$
	June	0.400	−0.0620	0.0162	$F = 14.6$, $P < 0.001$
	July	0.070	−0.0282	0.0217	$F = 1.7$, $P = 0.206$
	Sept	0.002	0.0021	0.0097	$F = 0.05$, $P = 0.828$
	Oct.	0.004	0.0037	0.0126	$F = 0.09$, $P = 0.769$

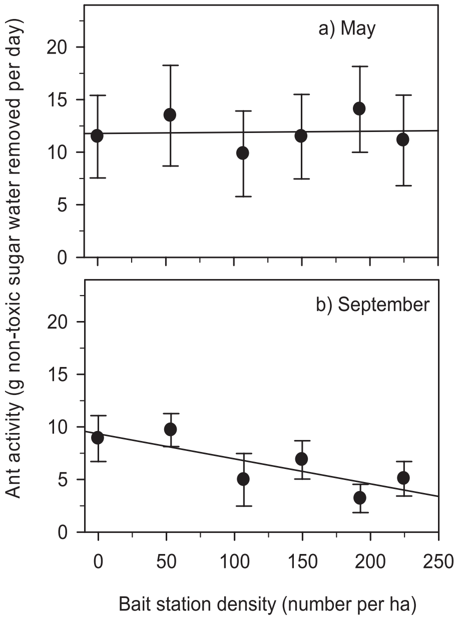

Fig. 1. Linear regressions of ant activity on bait station density, on the (a) May and (b) September sample dates in experiment 1 during the 2005 season, when ant densities were relatively high. The June sample date preceded bait placement; the September sample date followed grape harvest.

provide some benefits, even at high ant densities. The optimal bait station density may depend on the size of the local Argentine ant population.

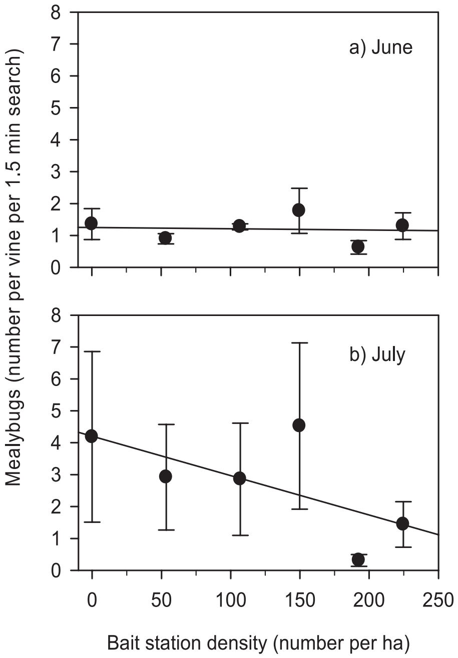

Fig. 2. Linear regressions of the number of mealybugs per vine on bait station density in (a) June and (b) July of experiment 1, 2005 season.

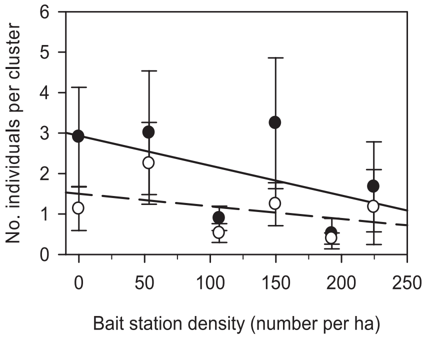

Fig. 3. The number of mealybugs (filled circles, solid line) and the number of scale (open circles, dashed line) per grape cluster at harvest time in experiment 1, 2005 season, as a function of bait station density.

Previous studies of ant baits in vineyards have shown that both ant activity levels and mealybug damage levels decline more strongly in the second year of

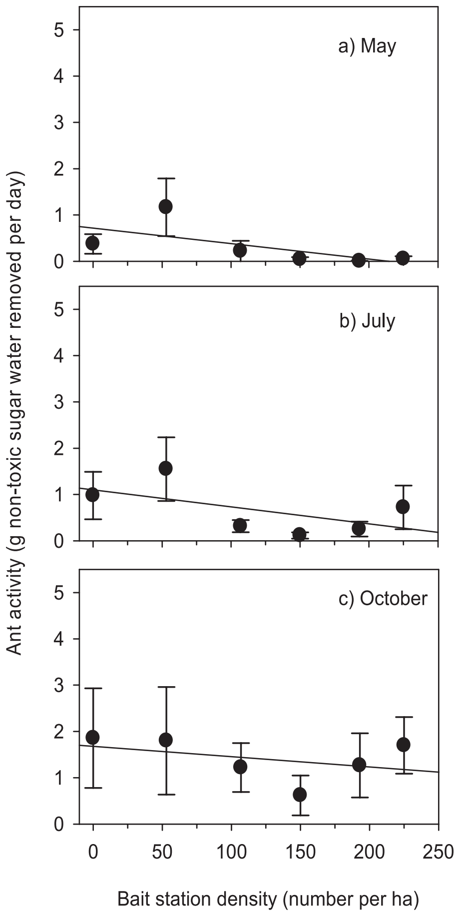

Fig. 4. Linear regressions of ant activity on bait station density in (a) May, (b) July, and (c) October during the 2006 season of experiment 1. The May sample date preceded bait deployment, and the October sample was taken after harvest. Overall ant activity was low compared with the 2005 season.

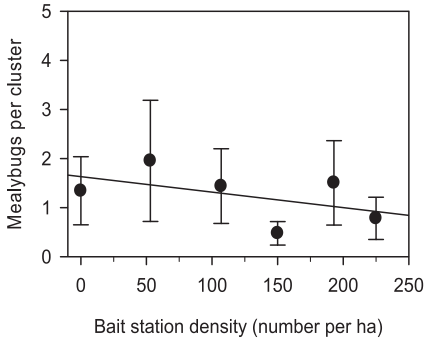

Fig. 5. The number of mealybugs per grape cluster at harvest time in experiment 1, 2006 season, as a function of bait station density.

a bait program, especially when ant populations are initially high (Daane et al. 2006a). Therefore, we expect that continued use of liquid baits would result in continued declines in ant activity and mealybug numbers. Higher bait station densities may be needed to achieve measurable ant control within one or two seasons, particularly in vineyards with higher ant densities. In subsequent seasons, as the ant population declines, continued suppression may be achieved with fewer bait stations per acre.

The effectiveness of bait stations may also depend on the date at which they are deployed. The foraging activity of Argentine ants varies seasonally, in conjunction with the reproductive phenology of the colonies (Markin 1970, Rust et al. 2000; K.M.D., unpublished data). We suspect that spring may be a particularly efficient time to deploy baits for two reasons. First, because colonies are developing their brood at this time, spring represents an opportunity to transfer toxic bait to ant larvae and disrupt colony growth. Second, alternative sugar sources such as honeydew and grape juice that are present at other times of year are not available in the spring, causing ants to concentrate their foraging effort on baits.

Experiment 1 was continued into a second growing season, after an insecticide application disrupted the arthropod community across the entire experiment. The insecticide reduced the ant population: in the 2005 season, the median amount of sugar water removed from monitoring tubes was 8.8 g/d; in the 2006 season, it was 0.2 g/d (Figs. 1 and 4). In the 2006 season we measured no effect of bait station density on ants or mealybugs (Figs. 4 and 5). We conclude either that baits have no effect at low ant density or that the effect is too small to detect and is not biologically meaningful. In experiment 2, the effect of bait station density on ant activity changed unexpectedly over the course of the growing season (Fig. 6). In June, ant activity decreased with increasing bait density; but rather than persisting as the season progressed, this pattern dissipated. Why did the negative effect of bait density on ant activity become more moderate during the growing season? We see three possible explanations. First, periods of low ant activity often occur in late summer and fall (Daane et al. 2006a), and our sampling may have occurred at times when ant activity was generally low, making it difficult to detect relative differences. Second, because Argentine ants are extremely vagile

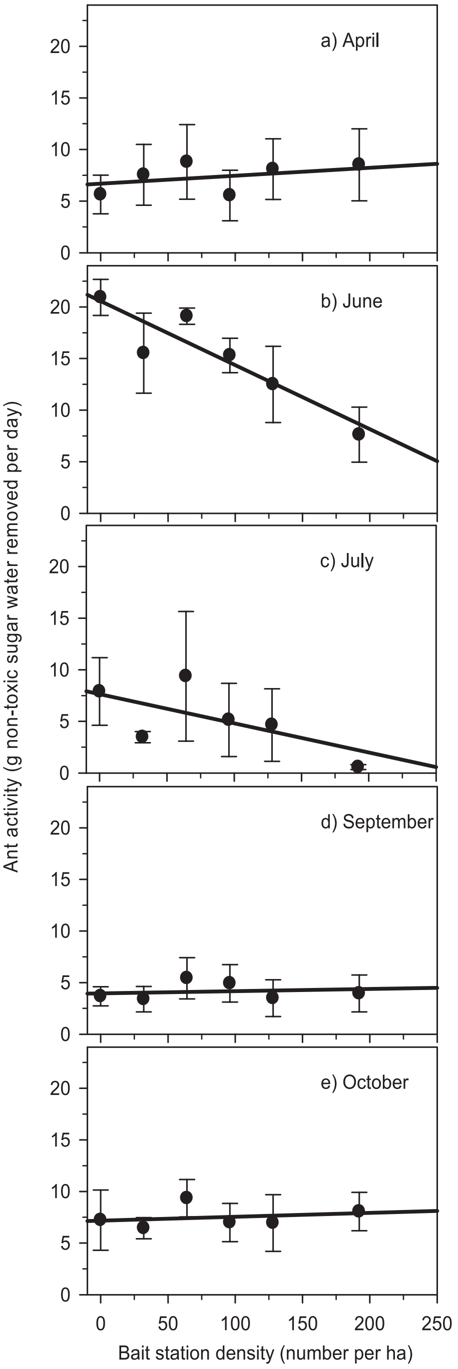

Fig. 6. Linear regressions of ant activity on bait station density, on five sample dates in experiment 2, 2006 season: (a) April, (b) June, (c) July, (d) September, and (e) October. The April sample date preceded bait placement, and the September and October sample dates followed grape harvest.

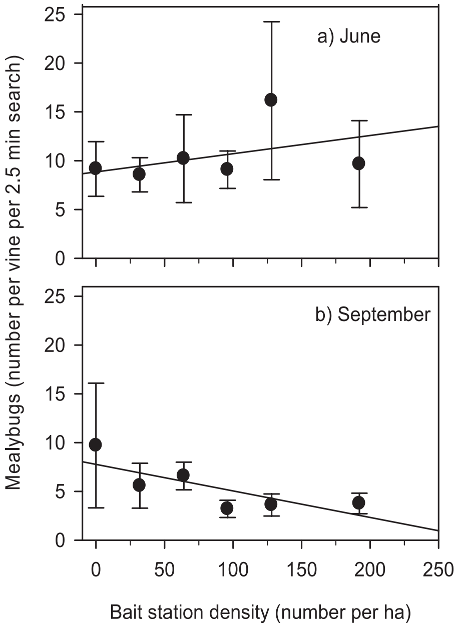

Fig. 7. Linear regressions of the number of mealybugs per vine on bait station density in (a) June and (b) September of experiment 2, 2006 season.

and because there is extensive resource sharing among the polydomous colonies of Argentine ants in their introduced range (Markin 1968, Holway and Case 2000, Tsutsui and Case 2000, Vega and Rust 2003), ants living outside our experimental plots may have migrated into the plots where baits were reducing the local populations. Third, the long-distance foraging and sharing of boric acid baits could have spread the suppressive effect of bait in high bait station density plots across plot boundaries.

These latter two explanations, related to the movement of Argentine ants, have broader implications for our study. We accounted for ant mobility in our experimental design by establishing large plots and separating them with buffers wider than the plots themselves. Even so, in any of our three experiments, ants may have repopulated plots where they were being killed, or they may have transported bait into low bait density plots. These movements would have caused our study to observe less ant suppression than was actually occurring in high bait density plots and more ant suppression than was actually occurring in low bait density plots, particularly as the season progressed. Thus, ant movement may have reduced the accuracy of our results, but if so, it also made our conclusions conservative: the effects of bait density we measured may be smaller than the actual effects that occurred in the field.

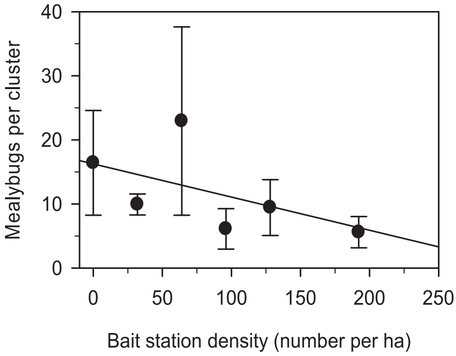

Fig. 8. The number of mealybugs per grape cluster at harvest time in experiment 2, 2006 season, as a function of bait station density.

We estimated mealybug abundance in our research plots using two methods: timed searches of whole vines and counts of mealybugs infesting ripe grape clusters. Whereas the counts of mealybugs on grape clusters supported the conclusion that mealybug populations were more strongly suppressed at higher bait densities, the counts of mealybugs on whole vines did not. The precision of our measurements may partly account for this difference. The cluster counts may provide more precise measurements of the true mean within each plot, for two reasons: first, we sampled 14–17% of vines in each plot for the cluster counts but only 6% of vines for the whole vine searches, potentially reducing our sampling error, and second, the migration of the mealybugs onto the ripening clusters concentrates their populations on a smaller plant structure, potentially reducing our measurement error. We surmise that the more precise within-plot measurements of the cluster counts resulted in relatively less unexplained between-plot variation and more power to detect a treatment effect.

Like mealybugs, European fruit lecanium scale are attended by ants and cause damage to grapes. Argentine ant attendance has been shown to benefit various hemipterans on citrus and other plants (Bartlett 1961, Buckley and Gullan 1991, Barzman and Daane 2001, Martinez-Ferrer et al. 2003). Our 2005 experiment shows that ant and scale abundances can be positively correlated in vineyards and that ant suppression can reduce scale densities. Thus, the pest control benefits of ant baits may extend beyond the *Pseudococcus* mealybugs to other vineyard pest insects. Ant baits may also aid in controlling new hemipteran pests. A recent addition to the mealybug pest complex in California vineyards is the invasive *P. ficus* (Godfrey et al. 2003, Daane et al. 2006b). This invasive pest is more damaging than the *Pseudococcus* mealybugs, and the effect of ant attendance on vine mealybug populations is a topic of current research.

Insecticidal baits applied through bait stations have both disadvantages and advantages relative to insecticidal sprays. The disadvantages of using insecticidal baits include the cost of new materials and equipment, the labor and attention needed for their use and maintenance, and potential for conflicts between bait deployment and other farm operations. The advantages of applying insecticides through bait stations result from keeping the toxin contained rather than broadcasting it and from feeding the toxin directly to the ants. In comparison to sprayed insecticides, insecticidal baits do not contact the crop and they result in less exposure of nontarget organisms, less input to the environment, and longer-term effectiveness. Bait stations can be deployed once per season, whereas sprays have short residuals and require multiple reapplications. In California vineyards, liquid baits containing thiamethoxam, imidacloprid, and boric acid have been used to reduce ant densities (Daane et al. 2006a). Liquid baits have also been used to reduce ant activity on citrus trees (Klotz et al. 2003, Klotz et al. 2004, Tollerup et al. 2004, Greenberg et al. 2006). The advantages of insecticidal baits make them an attractive tool for Argentine ant control in urban and natural environments as well as in agricultural systems. Indeed, safety concerns regarding contact with people and nontarget animals generally restrict the use of sprayed insecticides in urban and natural habitats. Liquid bait has been shown to reduce ant activity around homes and other buildings (Klotz et al. 2002), and granular baits containing hydramethylnon were tested for their ability to suppress Argentine ants in Haleakala National Park in Hawaii (Krushelnycky and Reimer 1998a). In each of these settings, the degree to which liquid baits are ultimately adopted and used will depend on the balance between the costs and benefits of baits and the constraints of the specific application. However, the threats posed by Argentine ants, and the challenges faced by alternative control methods in agricultural, urban, and natural habitats recommend the full development of this promising method for Argentine ant control.

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References Cited

Addison, P. 2002. Chemical stem barriers for the control of ants (Hymenoptera: Formicidae). South Afr. J. Enol. Viticult. 23: 1–8.

Bartlett, B. 1961. The influence of ants upon parasites, predators, and scale insects. Ann. Entomol. Soc. Am. 54: 543–551.

Barzman, M. S., and K. M. Daane. 2001. Host-handling behaviours in parasitoids of the black scale: a case for ant-mediated evolution. J. Anim. Ecol. 70: 237–247.

Blachly, J. S., and B. T. Forschler. 1996. Suppression of late-season Argentine ant (Hymenoptera: Formicidae) field population using a perimeter treatment with containerized baits. J. Econ. Entomol. 89: 1497–1500.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the world's crops: an identification and information guide. Wiley, Chichester, UK.

Buckley, R., and P. Gullan. 1991. More aggressive ant species (Hymenoptera: Formicidae) provide better protection for soft scales and mealybugs (Homoptera: Coccidae, Pseudococcidae). Biotropica 23: 5.

Buckley, R. C. 1987. Interactions involving plants, Homoptera, and ants. Annu. Rev. Ecol. Syst. 18: 111–135.

Christian, C. 2001. Consequences of a biological invasion reveal the importance of mutualism for plant communities. Nature (Lond.) 413: 635–639.

Daane, K. M., K. R. Sime, J. Fallon, and M. L. Cooper. 2007. Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. Ecol. Entomol. (in press).

Daane, K. M., K. R. Sime, B. N. Hogg, M. L. Bianchi, M. L. Cooper, M. K. Rust, and J. H. Klotz. 2006a. Effects of liquid insecticide baits on Argentine ants in California's coastal vineyards. Crop. Prot. 25: 592–603.

Daane, K. M., W. J. Bentley, V. M. Walton, R. Malakar-Kuenen, J. G. Millar, C. A. Ingels, E. A. Weber, and C. Gispert. 2006b. New controls investigated for vine mealybug. Calif. Agric. 60: 31–38.

Flaherty, D., L. Christiensen, and W. Lanini. 1992. Mealybugs, pp. 159–165. *In* W. Lanini, J. Marois, P. Phillips, and L. Wilson (eds.), Grape pest management. University of California Division of Agriculture and Natural Resources. Oakland, CA.

Geiger, C., and K. Daane. 2001. Seasonal movement and distribution of the grape mealybug (Homoptera: Pseudococcidae): developing a sampling program for San Joaquin Valley vineyards. J. Econ. Entomol. 94: 291–301.

Godfrey, K., J. Ball, D. Gonzalez, and E. Reeves. 2003. Biology of the vine mealybug in vineyards in the Coachella Valley, California. Southwest. Entomol. 28: 183–196.

Greenberg, L., J. H. Klotz, and M. K. Rust. 2006. Liquid borate bait for control of the Argentine ant, *Linepithema humile,* in organic citrus (Hymenoptera: Formicidae). Fla. Entomol. 89: 469–474.

Holway, D., and T. Case. 2000. Mechanisms of dispersed central-place foraging in polydomous colonies of the Argentine ant. Anim. Behav. 59: 433–441.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. Annu. Rev. Ecol. Syst. 33: 181–233.

Hopkins, W. G. 2003a. Rank transformation: non-parametric models. A new view of statistics (http://www.sportsci.org/resource/stats/threetrials.html).

Hopkins, W. G. 2003b. Repeated-measures ANOVA with three or more trials and no between-subjects effect. A new view of statistics (http://www.sportsci.org/resource/stats/threetrials.html).

Human, K., and D. Gordon. 1997. Effects of Argentine ants on invertebrate biodiversity in northern California. Conserv. Biol. 9: 1242–1248.

Klotz, J., M. Rust, and P. Phillips. 2004. Liquid bait delivery systems for controlling Argentine ant in citrus groves (Hymenoptera: Formicidae). Sociobiology 43: 419–427.

Klotz, J., L. Greenberg, C. Amrhein, and M. Rust. 2000. Toxicity and repellency of borate-sucrose water baits to Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 93: 1256–1258.

Klotz, J. H., M. Rust, H. Costa, D. Reierson, and K. Kido. 2002. Strategies for controlling Argentine ants (Hymenoptera: Formicidae) with sprays and baits. J. Agric. Urban Entomol. 19: 85–94.

Klotz, J. H., M. K. Rust, D. Gonzalez, L. Greenberg, H. Costa, P. Phillips, C. Gispert, D. A. Reierson, and K. Kido. 2003. Directed sprays and liquid baits to manage ants in vineyards and citrus groves. J. Agric. Urban Entomol. 20: 31–40.

Krushelnycky, P. D., and N. J. Reimer. 1998a. Efficacy of Maxforce bait for control of the Argentine ant (Hymenoptera: Formicidae) in Haleakala National Park, Maui, Hawaii. Environ. Entomol. 27: 1473–1481.

Krushelnycky, P. D., and N. J. Reimer. 1998b. Bait preference by the Argentine ant (Hymenoptera: Formicidae) in Haleakala National Park, Hawaii. Environ. Entomol. 27: 1482–1487.

Markin, G. 1968. Nest relationship of the Argentine ant. J. Kans. Entomol. Soc. 41: 511–516.

Markin, G. 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in Southern California. Ann. Entomol. Soc. Am. 63: 1238–1242.

Martinez-Ferrer, M., E. Grafton-Cardwell, and H. Shorey. 2003. Disruption of parasitism of the California red scale (Homoptera: Diaspididae) by three ant species (Hymenoptera: Formicidae). Biol. Control 26: 279–286.

Moreno, D., P. Haney, and R. Luck. 1987. Chlorpyrifos and diazinon as barriers to Argentine ant (Hymenoptera: Formicicae) foraging on citrus trees. J. Econ. Entomol. 80: 208–214.

Phillips, P. A., and C. J. Sherk. 1991. To control mealybugs, stop honeydew seeking ants. Calif. Agric. 45: 26–28.

Rust, M., D. Reierson, E. Paine, and L. Blum. 2000. Seasonal activity and bait preferences of the Argentine ant (Hymenoptera: Formicidae). J. Agric. Urban Entomol. 17: 201–212.

Sanders, N., K. Barton, and D. Gordon. 2001. Long-term dynamics of the distribution of the invasive Argentine ant, *Linepithema humile,* and native ant taxa in northern California. Oecologia (Berl.) 127: 123–130.

Silverman, J., and T. Roulston. 2001. Acceptance and intake of gel and liquid sucrose compositions by the Argentine ant (Hymenoptera: Formicidae). J. Econ. Entomol. 94: 511–515.

Styrsky, J. D., and M. D. Eubanks. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proc. R. Soc. Biol. Sci. Ser. B. 274: 151–164.

Tollerup, K. E., M. K. Rust, K. W. Dorschner, P. A. Philips, and J. H. Klotz. 2004. Low toxicity baits control ants in citrus orchards and grape vineyards. Calif. Agric. 58: 213–217.

Tsutsui, N., and T. Case. 2000. Population genetics and colony stucture of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. Evolution 55: 976–985.

Vega, S., and M. Rust. 2001. The Argentine ant—a significant invasive species in agricultural, urban and natural environments. Sociobiology 37: 3–25.

Vega, S., and M. Rust. 2003. Determining the foraging range and origin of resurgence after treatment of Argentine ant (Hymenoptera: Formicidae) in urban areas. J. Econ. Entomol. 96: 844–849.

von Ende, C. N. 2001. Repeated-measures analysis: growth and other time-dependent measures, pp. 134–157. *In* S. M. Scheiner and J. Gurevitch (eds.), Design and analysis of ecological experiments, 2nd ed. Oxford University Press, New York.

Way, M. 1963. Mutualism between ants and honeydew-producing Homoptera. Annu. Rev. Entomol. 8: 307–344.

Way, M., M. Cammell, M. Paiva, and C. Collingwood. 1997. Distribution and dynamics of the argentine ant *Linepithema (Iridomyrmex) humile* (Mayr) in relation to vegetation, soil conditions, topograpy and native competitor ants in Portugal. Insectes Soc. 44: 415–433.

Wetterer, J., A. Wetterer, and E. Hebard. 2001. Impact of the argentine ant, *Linepithema humile*, on the native ants of Santa Cruz Island, California. Sociobiology 38: 709–721.

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