EVALUATION OF COMMERCIAL ANT BAITS AS A COMPONENT OF AN INTEGRATED PEST MANAGEMENT PROGRAM FOR VINE MEALYBUG, *PLANOCOCCUS FICUS*

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ABSTRACT: Vine mealybug (VMB) is a destructive phloem-feeding pest in California vineyards. VMB feeding activity can debilitate vines and contaminate clusters making them unsuitable for harvest. An Integrated Pest Management (IPM) program that relies on several tactics (insecticides, mating disruption and biological control) can provide sustainable control of VMB populations. However, Argentine ants (*Linepithema humile*) that interfere with the activity of biological control agents may disrupt mealybug management programs in commercial vineyards. The objective of this project was to evaluate broadcast applications of commercial and experimental baits targeting *L. humile* populations over 3 growing seasons (2015-2017) in coastal California vineyards. In 2015 and 2016, several commercial granular baits were evaluated. Although the spinosad-laced bait (Seduce; 0.07%) seemed to reduce ant foraging in initial trials, it was subsequently found to have little or no significant effect. Therefore, in 2016 and 2017, trials evaluated the effectiveness of an experimental formulation of thiamethoxam (0.0006%) and boric acid (0.5%) delivered in polyacrylamide crystals. Both polyacrylamide baits rapidly eliminated ants for at least 5-6 weeks following two applications, and provided sustained control of ant populations for up to 6 months. These results suggest that commercial applications of polyacrylamide baits could be highly promising for control of invasive *L. humile* in coastal California vineyards.

LAYPERSON SUMMARY: Vine mealybug is a destructive pest in California vineyards; it contaminates fruit and reduces vine health and productivity. Grape growers may use multiple tactics (IPM) including insecticides, mating disruption and biological control, to achieve control of VMB populations. Argentine ants are invasive insects common in coastal California vineyards. Ants disrupt IPM programs for VMB because they interfere with the activity of a small parasitic wasp that attacks VMB. Ant baits are an effective approach to manage ant populations while minimizing impacts on non-target organisms. We are investigating the potential of commercial and experimental baits to control Argentine ants in vineyards. Both baits reduced ant activity in the treated areas, although the effect was more sustained with the experimental bait, suggesting its potential as a component of sustainable VMB management in coastal California vineyards.

INTRODUCTION: The vine mealybug (VMB), *Planococcus ficus* (Signoret), is a destructive vineyard pest that contaminates fruit, debilitates vines and vectors plant pathogens such as *grapevine leafroll-associated virus-3* (Daane et al. 2012). First reported from vines in the Coachella Valley (Gill 1994), VMB soon spread throughout California, likely on infested nursery stock (Haviland et al. 2005). It is currently found in most California grape-growing regions (Godfrey et al. 2002; Daane et al. 2004a, 2004b) and has the potential to spread throughout the western United States.

Management of VMB populations can prove challenging and often requires the use of multiple tactics, including biological control, mating disruption and insecticides (Daane et al. 2008). Management can be particularly complicated in coastal wine grape growing regions where VMB populations are tended by Argentine ants, *Linepithema humile* [Mayr]. In the presence of tending ants, biological control of mealybugs can be significantly interrupted, resulting in large VMB populations that may be more easily spread to new areas. These populations also contaminate the fruit, causing yield losses and decreased fruit quality. In vineyards where Argentine ant is prevalent, management of ant populations is a critical part of an IPM program for VMB and necessary for containment of insect populations (Nyamukondiwa and Addison 2011; Mgochecki and Addison 2009).

Liquid ant baits adapted from the urban environment (Klotz et al. 2002) for use in vineyards (Cooper et al. 2008), significantly reduce mealybug populations in vineyards by contributing to increases in biological control (Daane et al. 2007). The costs associated with the manufacture, deployment and maintenance of bait stations have been prohibitive to widespread adoption of Argentine ant management in vineyards, despite the benefits that could result from such programs (Nelson and Daane 2007). There is continued interest among coastal grape growers in the development of a simpler and more economical bait program that could be widely implemented. Baits formulated as granular products or polyacrylamide gels that can be broadcast with a fertilizer spreader could be distributed more quickly and frequently over a large area, and would not require the manufacture and maintenance of bait stations. The sustained use of the granular or polyacrylamide baits could lead to longer-term containment and control of Argentine ant populations (Boser et al. 2014; Krushelnycky et al. 2004). We are evaluating granular and polyacrylamide ant baits that can be broadcast to reduce populations of Argentine ant. Ant control would in turn contribute to the sustainable control of VMB populations. In the absence of an economical bait program, ant suppression must be achieved with the broad-spectrum insecticide—chlorpyrifos—that can affect water quality, disrupt populations of beneficial insects and pose vertebrate health risks.

OBJECTIVE: The broad goal of this research is to increase the efficacy and adoption of integrated pest management programs for vine mealybug, a destructive pest of grapevines in California. Our specific objective is to: Evaluate the efficacy of two bait formulations to reduce Argentine ant populations as part of an integrated pest management program for vine mealybug.

RESULTS AND DISCUSSION:

2015 FIELD SEASON

GRANULAR BAIT TRIAL: In 2015, our experiment was established in two vineyard blocks in Napa, California (Carneros American Viticultural Area (AVA)). Both blocks were planted in 1999 and are a mix of Chardonnay clones (17-Robert Young and 6) on SO4 rootstock (*Vitis berlandieri* x. *V. riparia*). We used a randomized complete block design, and established six, 6-row replicates of each treatment. The treatments were 3 commercial granular bait products (Table 1).

Table 1. Ant bait products applied in trial blocks in a Napa County vineyard.

Treatment	Active Ingredient (concentration)	Rate per acre	Bait applications (2015)
Altrevin	metaflumizone (0.063%)	1.5 lb.	March 14 & 15; April 15 & 16; June 15 & 16
Altrevin & powdered sugar	metaflumizone (0.063%)	1.5 lb.	
Extinguish	hydramethylnon (0.365%) & methoprene (0.25%)	1.5 lb.	
Seduce	Spinosad (0.07%)	20 lb.	
Untreated	none	none	none

In March, April and June 2015, the cooperating vineyard manager applied the bait in the vine row with a modified broadcast spreader mounted on an all-terrain vehicle (ATV). Because Altrevin and Extinguish are formulated with a protein attractant specifically for control of red imported fire ant (*Solenopsis invicta*), we included one Altrevin treatment in which the bait was coated with powdered sugar before application to make it more attractive to Argentine ants. The spinosad bait, Seduce, is formulated with a carbohydrate attractant (sugar) specifically to target the Argentine ant (Figure 1B). Additionally, Seduce has been approved for use in organic vineyards. Since there are a limited number of insecticides approved for VMB management in organic vineyards, ant bait can be an essential component of an IPM program in these vineyards.

Ant densities were determined indirectly as a measure of feeding activity, assessed as the amount of nontoxic sucrose water removed from 50-milliliter (ml) polypropylene centrifuge tubes (Corning Inc., Corning, NY) tied to the vine trunk (Klotz et al. 2002, Daane et al. 2008a) in the center two rows of each plot. The 50-ml tubes are henceforth referred to as monitoring tubes. A 2-centimeter (cm) hole was drilled in the cap, and a square of permeable plastic mesh (Weedblock, Easy Gardener Inc., Waco, TX), was placed between the cap and the filled tube, covering the hole. The mesh is fine enough to retain the liquid when the tube is inverted, but coarse enough to allow ants to remove the liquid on contact. A second lid was fixed to the original lid, and covered with a permanent mesh to discourage feeding by honeybees and wasps. Before the tubes were deployed in the vineyard, each tube was filled to 45 ml with 25% sucrose water and the weight of each tube was recorded. Tubes were inverted on a vine trunk for 4 to 7 days (depending on ant activity), at a density of 12 tubes per plot, or a total of 72 tubes per treatment. At the end of the monitoring period, the tubes were brought back to the laboratory and the new weights were recorded. One additional monitoring tube per plot was attached to an ant-excluded bamboo stake to measure the amount of water lost to evaporation; this amount was averaged across all plots and used to adjust the final weight.

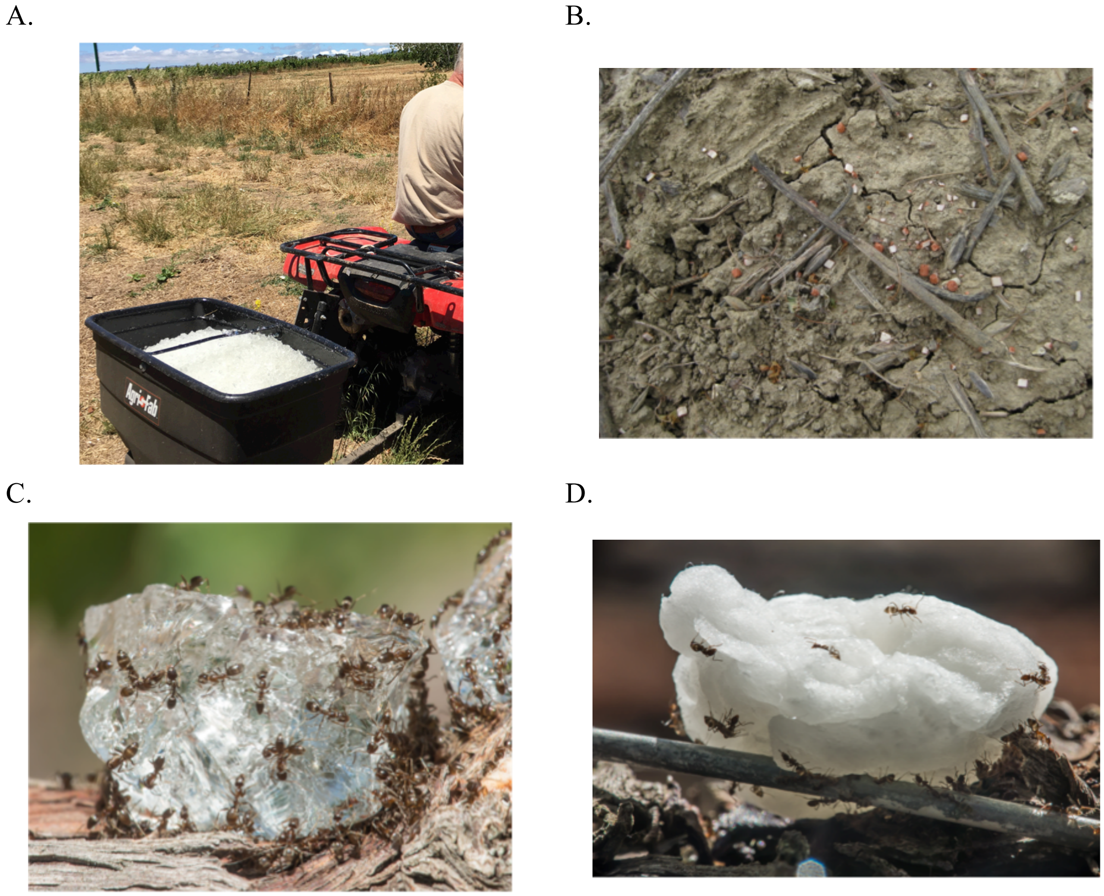

Figure 1. (A) broadcast spreader with polyacrylamide bait mounted on ATV; (B) Seduce bait (reddish pellets) under the vine row; (C) Argentine ants feeding on polyacrylamide bait; (D) Argentine ants feeding on cotton ball used for monitoring ant activity.
Photo credits: (A) C. Bianucci; (B) M. Cooper, UC Cooperative Extension (UCCE); (C) & (D): M. Hobbs, UCCE

Ant feeding activity is reported as grams (g) of sugar water removed from monitoring tubes per day (Figure 2). During the February and March monitoring periods (pre-treatment and 10 days after the first treatment, respectively) ant feeding activity was not significantly different across all treatments. This is not surprising since we blocked for consistent ant populations prior to treatment; also, and most importantly, baits have delayed toxicity and would not be expected to control populations so quickly (10 days) after application. During the April 24 to 28, 2015 monitoring period, feeding activity was significantly reduced in the Seduce bait treatment (Tukey's pairwise comparison, $p=0.0099$); this is roughly 6 weeks after the first bait application and 1 week after the second. From May 26 to June 3, 2015, feeding activity in the Seduce treatment (-0.007 +/-0.12 g per day) was reduced compared to other treatments (0.52 to 0.92 +/- 0.35 to 0.55 g per day), although the difference was not statistically significant due to the high variability in ant feeding—particularly in the Altrevin and untreated blocks. During the July and August monitoring periods, ant feeding was low to none in all treatments. In other ant bait trials, we have detected similar feeding lulls at our monitoring tubes during the summer (Daane et al. 2006, 2008a). We did not see any differences in population suppression between the powdered sugar-coated bait and those protein-based baits without powdered sugar. Since the sugar is not an inert ingredient of the bait, it may not adhere well to the bait. It could have been removed during the application process or not durable in the field. At this point, there does not appear to be a measurable improvement in bait performance through the addition of the powdered sugar under these conditions. And adverse effects were noticed as the sugar heated (and melted) in the spreader, thereby clogging the mechanisms of the spreader that impacted application efficiency and necessitated additional disassembly/cleaning time. Overall, the Seduce bait was the easiest to apply; we attributed this to weight and consistency of the bait as well as application rates (higher rates made the applied bait more visible, and therefore easier to calibrate the spreader and adjust drive speeds).

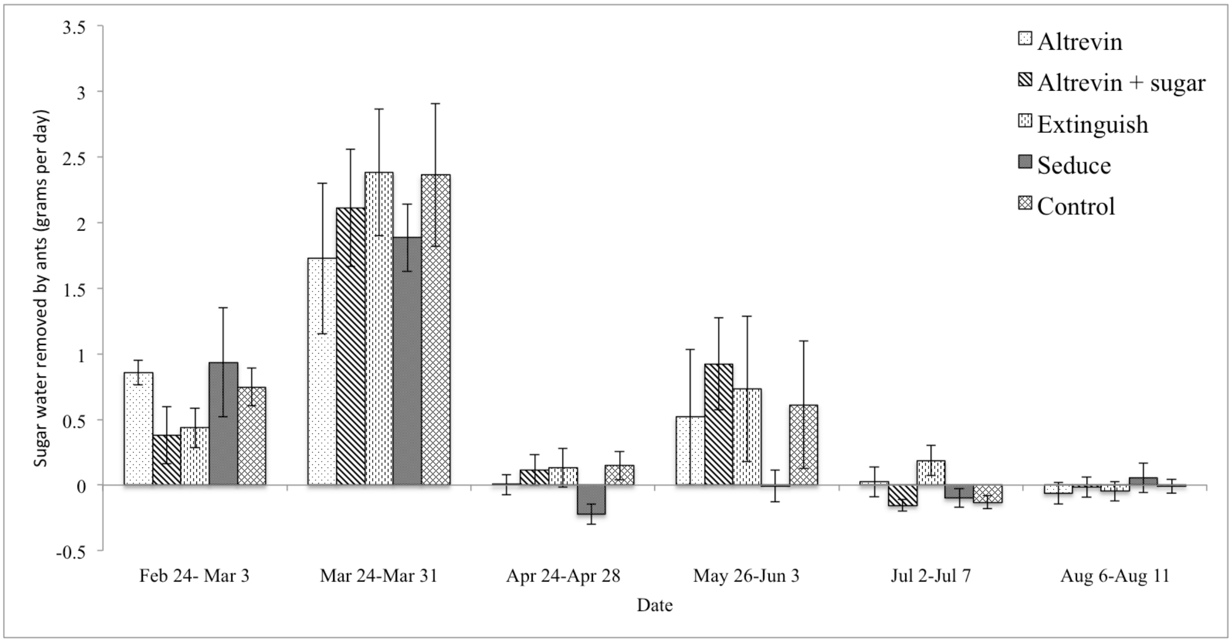

Figure 2. Average sucrose water removed (grams per day) from monitoring tubes by Argentine ants, during six monitoring periods in a Chardonnay vineyard (Carneros AVA) in 2015. Results are reported for each bait treatment and the untreated control. During the April 24 to 28, 2015 monitoring period, feeding activity was significantly reduced in the Seduce bait treatment (Tukey's pairwise comparison, p=0.0099). On all other dates, there were no significant differences among treatments.

2016 FIELD SEASON

GRANULAR BAIT TRIAL: Based on results of our 2015 trials, we eliminated both Altrevin and Extinguish ant baits from our 2016 trials, focusing solely on Seduce (0.07% spinosad)—the product that was most efficacious in preliminary trials. We selected 5 experimental blocks (Oakville and Rutherford appellations of Napa Valley AVA), and established split-plot design (bait and untreated) in all blocks. In two of those blocks (designated I1 and I2), Seduce ant bait was applied at a rate of 20 lbs per ac on April 15 and 16, 2016. In the remaining three blocks (designated T1, T2, F1), Seduce ant bait was applied at a rate of 28 lbs per ac (slightly higher than the target rate due to challenges with calibration and the spreader equipment) on May 19 and 20, 2016; a second application at the rate of 20 lbs per ac was applied in blocks T1, T2 and F1 on June 25 and 27, 2016. The spreader equipment was the same as that used in the 2015 trial. The cooperating vineyard managers made all the bait applications.

We monitored ant activity pre- and post-application using cotton balls (Fisher Scientific) soaked in 25% sucrose solution (Figure 1C). Ant activity was measured once every 2 weeks. Forty-five or fifty vines per treatment per block were selected as monitoring vines. One saturated cotton ball was deployed on each monitoring vine, either on the ground (early season) or on the vine (after fruit set), depending on where the ants were predicted to be most active. After 2.5 to 3 hours, cotton balls were retrieved from each monitoring vine, and ant activity on the cotton ball was assessed using a 0 to 3 scale where '0' equals no ants, '1' equals the presence of 1 to 10 ants, a value of '2' is assigned to cotton balls with 11 to 50 ants, and a rating of '3' assigned for the presence of greater than 50 ants.

Due to some challenges with site selection, the first bait applications in blocks T1, T2 and F1 occurred later (May 19 and May 20, 2016) than would be desired to optimize results. In blocks I1 and I2, bait applications were initiated early in the growing season (April 15 and 16, 2016), and within 14 days of when ants were detected and temperatures were adequate for foraging to occur. We tested for significant differences between baits and control at each sampling date using Mann-Whitney U tests (Table 2). Our analyses suggest that (1) the dry bait treatment at sites I1, I2 was only significantly different from control on one date after treatment; given that ant levels were near zero pre-treatment, it seems unlikely this was due to the treatment. (2) Dry bait in block F1 was no different from control until after the 2nd treatment. After the 2nd treatment, ant levels were significantly lower than the control but were not statistically different on the last sampling date on 7th October. (3) Dry bait in blocks T1, T2 was significantly lower than control at every sampling date. As ants were significantly lower than control pre-treatment and actually increased after the first treatment, there is no convincing evidence that the bait had an effect. In conclusion, this study did not generate convincing evidence that dry bait (Seduce) reduced ant levels in two vineyards (I and T). At vineyard F, the dry bait treatment was lower than control after the 2nd treatment but ant levels were not actually reduced until October when they also had decreased in the control. At best there was as very limited effect of dry bait in only one vineyard in this study. These results are not encouraging with regards to the efficacy of Seduce ant bait for controlling Argentine ants in commercial vineyards. Although future studies should evaluate a higher product rate, and/or more applications to determine whether improved control can be achieved. More applications were not explored during the current study, as the cooperating vineyard managers did not find this to be an economically attractive strategy.

Table 2. Results of Mann-U tests comparing dry bait vs. control for each sampling date.

Sampling Trial	Blocks I1/I2			Block F1			Blocks T1, T2	
	Trial Date	*p* value		Trial Date	*p* value		Trial Date	*p* value
1	8-Mar	.55	1	6-May	.19	1	6-May	**.01***
2	23-Mar	.56	TREATMENT 20-MAY			TREATMENT 19-MAY		
3	6-Apr	.45	2	27-May	.70	2	27-May	**<.01***
TREATMENT 15, 16-APR			3	10-Jun	.86	3	10-Jun	**<.01***
3	19-Apr	1.0	TREATMENT 20-JUN			TREATMENT 25-JUN		
4	6-May	**<.01***	4	24-Jun	**<.01***	4	24-Jun	**<.01***
5	3-Jun	.53	5	8-Jul	**<.01***	5	8-Jul	**<.01***
6	10-Jun	.23	6	22-Jul	**<.01***	6	22-Jul	**<.01***
7	24-Jun	.02	7	7-Oct	.82	7	7-Oct	**<.01***
8	8-Jul	.97						
9	22-Jul	.68						
10	11-Oct	.04						

POLYACRYLAMIDE GEL BAIT TRIAL: Based on a pilot study that eliminated >99% of ants from treated plots in the California Channel Islands (Boser et al. 2014), and a preliminary vineyard study conducted by the Principal Investigators in 2015 (unpublished data), we are evaluating the efficacy of a polyacrylamide gel bait formulation in vineyards. We established three experimental blocks (split-plot design: treated and untreated treatments); two of these blocks (designated C1 and C2) are located in the Carneros appellation (Napa Valley AVA) and one (designated M1) is located in the St. Helena appellation. Blocks C1 and C2 are populated with the invasive vine mealybug; block M1 is populated with the native grape mealybug (*Pseudococcus maritimus*). In addition to the economic damage sustained by VMB populations, the spread of grapevine leafroll-associated virus 3 (GLRaV-3) is a major concern in all of these blocks.

The bait solution consists of 0.0006% thiamethoxam (Platinum insecticide, Syngenta US) in 25% sucrose solution, deployed at a rate of 10 gal per ac in polyacrylamide Water Storing Crystals (MiracleGro®) (figure 1C). These crystals absorb water and water-soluble chemicals, and when hydrated present a thin layer of liquid bait solution on the surface for 24 to 72 hours following application. To allow sufficient time for the crystals to absorb the bait solution, they were added to the mixture 24 hours prior to the application. The hydrated crystals were deployed using an 85 lb tow spreader (Agri-Fab, model #45-0315) pulled with an all-terrain vehicle (ATV) (Figure 1A). Bait applications were initiated once foraging ants were detected at sugar-soaked cotton balls. The cooperating vineyard manager made the bait applications on March 16 and April 14, 2016 in blocks C1 and C2, and on April 15 and May 26, 2016 in block M1. Because block M1 is in a more northerly location within Napa County, ants did not become active until later in the season (ant foraging is reduced below 60 F (15 C)). Ant monitoring pre- and post-application followed the method described previously, using cotton ball soaked with a 25% sucrose solution (figure 1D).

We tested for significant differences between baits and control at each sampling date using Mann-Whitney U tests (Table 3). In summary, pre-treatment ant ratings were no different between the bait and control vines at either vineyard. After the first treatment, the bait treatment had significantly fewer ants (near zero) than the control in vineyard M1; this continued throughout the season—until the final sampling date on October 7, 2016. In the C1 and C2 blocks, there were significantly fewer ants on the first sampling date following the first treatment. From one month after the 2nd bait application until the end of the season (October 11, 2016), ant populations in the baited blocks in vineyards C1 and C2 remained significantly lower than in the untreated control. In summary, our trials indicate that the polyacrylamide bait laced with thiamethoxam nearly eliminated ants for 1.5 months, and provided sustained control of ants for up to 6 months after the 2nd bait treatment.

Table 3. Results of Mann-U tests comparing polyacrylamide bait vs. control for each sampling date

Sampling Trial	Blocks C1, C2			Block M1	
	Trial Date	*p* value		Trial Date	*p* value
1	26-Feb	.29	1	8-Mar	1.0
2	8-Mar	.16	2	23-Mar	.06
TREATMENT 16th MAR			3	6-Apr	.49
3	23-Mar	**<.01***	TREATMENT 15th APR		
4	15-Apr	1.0	4	19-Apr	**<.01***
TREATMENT 15th APR			5	6-May	**<.01***
5	28-Apr	1.0	TREATMENT 25th MAY		
6	11-May	**<.01***	6	3-Jun	**<.01***
7	30-May	**<.01***	7	10-Jun	**<.01***
8	14-Jun	**<.01***	8	27-Jun	**<.01***
9	30-Jun	**<.01***	9	8-Jul	**<.01***
10	11-Jul	**<.01***	10	27-Jul	**<.01***
11	25-Jul	**<.01***	11	7-Oct	.026
12	11-Oct	**<.01***			

2017 FIELD SEASON

POLYACRYLAMIDE BAIT TRIAL: These trials are a continuation of our 2016 trials with polyacrylamide crystals laced with 6 ppm thiamethoxam (Platinum insecticide, Syngenta US). We are also evaluating bait laced with boric acid (0.5%), as an option for organic growers. The bait was mixed and applied as described previously (figure 1C). Because ants re-invaded the treated areas in 2016 (split-plot design), in 2017 we designated entire blocks as either treated or untreated, and paired the treated and untreated blocks. We have 2 pairs of blocks in the Carneros region and 2 pairs in the Oakville region. We also continued our trial in one split-plot block in St Helena. The Carneros blocks were treated with thiamethoxam-laced bait on May 3 and June 9, 2017. The Oakville blocks were treated with boric acid-laced bait on May 4 and May 26-31, 2017. And the St Helena block was treated with thiamethoxam-laced bait on May 5 and June 10, 2017. Ant monitoring pre- and post-application followed the method described previously, using cotton ball soaked with a 25% sucrose solution (figure 1D).

The polyacrylamide gel bait reduced ants at all sites; however, the extremely variable ant populations in the untreated blocks made it more challenging to attribute an explanation to the effects than in previous years. In the treated blocks in Oakville, ant numbers decreased significantly only after the 2nd treatment (within 2 weeks), where they remained close to (or at) zero for up to 2 months. This appeared to show an effect of the bait treatment. However, the control did not show much variation in ant numbers, remaining just above zero for the study period and ending at the same level as the bait blocks (despite some significant fluctuation). In the first Carneros site, ant numbers in the bait block were significantly reduced after the 1st treatment and remained at zero until the end of the study period, 2 months after the 2nd treatment. Ants in the control block were low generally with little increase (if any) from March to August. However, they were almost always significantly higher than the control block after the 1st treatment. In the second Carneros site, ant numbers in the control block were low but there was an overall small increase from March to August. In comparison, ant numbers were initially higher in the bait block, but crashed to zero immediately after the 1st treatment, only rising to match the control 2 months after the 2nd treatment. This provides evidence that the thiamethoxam-laced polyacrylamide bait reduced ants. At the St. Helena site, ant numbers in the control block started at zero in March and increased over the season until August (Fig 3). In comparison, ant populations in the bait block were low/zero pre-treatment and continued at this low level for the entire study period, becoming significantly lower than the control within 2 weeks of the first bait application. This is evidence that the wet bait suppressed ant numbers from first treatment (although it should be noted that a decline in ant number due to the bait was not measured because of the lack of ants pre-treatment.)

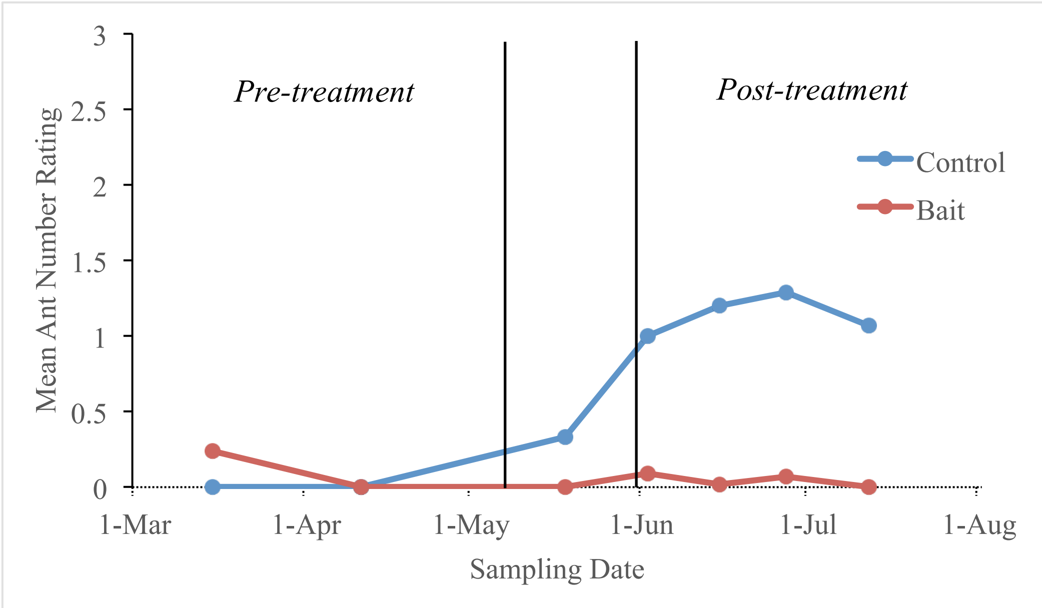

Figure 3. Mean ant level rating for St Helena vineyard block. Ants were rated on a 0 to 3 scale, where a value of '0' was assigned to cotton balls with no ants, a value of '1' assigned for the presence of 1 to 10 ants, a value of '2' is assigned for 11 to 50 ants, and a rating of '3' assigned for greater than 50 ants. Vertical lines represent dates of two bait applications.

CONCLUSIONS: We evaluated two baits (one commercial and one experimental product) to reduce Argentine ant populations in a coastal California vineyard. Because Argentine ants disrupt biological control of VMB by interfering with the activity of predators and parasitoids, control of Argentine ants can be an essential component of IPM programs for VMB. Handling and distribution of baits that can be broadcast is simpler and more efficient than liquid baits that must be contained within bait stations. Additionally, Argentine ant nests are typically multiple and widely dispersed throughout agricultural ecosystems in the spring, summer and fall (Markin 1970) so multiple point-sources make bait more accessible to all nests within an infested area (Boser et al. 2014). Our results suggest that an experimental bait treatment (0.0006% thiamethoxam in polyacrylamide crystals) has the potential to reduce populations of Argentine ant, whereas a commercially available spinosad-laced bait (Seduce) was less effective in our trials. Future studies may explore the use of multiple applications or higher rates of Seduce to obtain adequate control of Argentine ant populations. Two applications of a thiamethoxam-laced polyacrylamide bait reduced ant populations for 2 to 6 months after treatment. Because ants may reinvade from untreated areas, large-scale, regional treatments (such as those conducted in the California Channel Islands (Boser et al. 2014)) could be more successful and future studies should concentrate in this area.

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