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RESEARCH ARTICLE

Field evaluations of biodegradable boric acid hydrogel baits for the control of Argentine ants: Promising results in vineyards and citrus orchards

Boric acid hydrogel baits help control Argentine ants and associated honeydew-producing hemipterans in vineyards and citrus orchards.

by Benning Le, Kathleen Campbell, Hoeun Park, Shu-Ping Tseng, Raju Pandey, Gregory S. Simmons, Ruth Henderson, Carmen Gispert, Michael K. Rust, Chow-Yang Lee, Roghaiyeh Karimzadeh, Yong-Lak Park and Dong-Hwan Choe

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Abstract

Argentine ants are a major pest in California. In this study, a biodegradable calcium alginate hydrogel with an aqueous boric acid bait was tested against Argentine ant populations in a citrus orchard and a vineyard. A new continuous method was developed to produce large quantities of hydrogel bait for the field test. Foraging activity levels of ants were compared between baited and untreated zones. For both study sites, four to five monthly bait applications throughout summer provided a greater than 80% reduction in ant activity. Based on spatial analyses by distance indices, the baited areas were characterized by gaps (areas with lower ant counts) and the untreated control zones were characterized by patches (areas with higher ant counts). This indicated area-wide suppression of Argentine ants. For the citrus orchard, post-baiting panel trap monitoring showed reductions of both ants and Asian citrus psyllid in the baited zone compared to the control. For the vineyard, mid-season soil analyses indicated that the impact of boric acid baiting on soil boron concentration was negligible. In sum, the calcium alginate hydrogel bait with boric acid as an active ingredient may provide a promising solution for Argentine ant baiting.

Outside of their native range, the Argentine ant, *Linepithema humile (Mayr)* (Hymenoptera: Formicidae), is an invasive pest in natural, urban, and agricultural settings (Holway et al. 2002; Global Invasive Species Database 2022; Vega and Rust 2001). In agricultural settings, Argentine ants tend hemipterans in order to consume the hemipterans' "honeydew" (excretions). The ants' activities significantly hinder the effect of natural enemies on these plant pests (Buckley 1987; Tillberg et al. 2007). Therefore, managing Argentine ants is considered an important part of the integrated pest management (IPM) of various honeydew-producing plant pests (Daane et al. 2008; Milosavljević et al. 2021).

In California, Argentine ants and their symbiotic relationship with honeydew-producing plant pests pose a serious problem in two important crop systems:

Applying the experimental calcium alginate hydrogel baits at a vineyard study site. This 2-year field study found that boric acid hydrogel baiting provided area-wide suppression of Argentine ants in vineyards and citrus orchards. *Photo*: Dong-Hwan Choe.

citrus and grape. Huanglongbing, a bacterial disease that has caused billions of dollars in citrus damage, is vectored by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (da Graça et al. 2016). Argentine ants tend nymphal stages of ACP and ward off their natural enemies, increasing the survival of ACP and subsequent risk of pathogen transmission (Milosavljević et al. 2021). In the grape system, mealybugs contaminate the grape bunches, making the crop unmarketable, and also transmit grapevine leafroll virus. Argentine ants tend various types of mealybugs, actively interrupting natural enemies of the mealybugs that could otherwise effectively suppress the mealybug populations (Cooper et al. 2019).

A grape bunch infested with mealybugs. Mealybugs are often hidden in the sheltered locations within the bunches. Heavy trails of tending Argentine ants are typically found to lead to these infested bunches. *Photo*: Dong-Hwan Choe.

Argentine ants feeding the boric acid hydrogel bait applied on the ground. The liquid bait available on the surface of the hydrogel beads will be imbibed by the ants. The foragers will carry the bait in their crop (note distended abdomen of some ants) and share it with other ants within the same population. *Photo*: Dong-Hwan Choe.

Argentine ant infestations in agricultural settings often require chemical control (Klotz et al. 2003; Silverman and Brightwell 2008). As an alternative to broad-spectrum insecticide sprays, liquid baiting has been studied as a possible control approach (Cooper et al. 2008; Daane et al. 2006; Rust et al. 2000). Because the liquid bait can be effectively transferred among colony members of Argentine ants via trophallaxis, the liquid baiting may help achieve a colony-wide impact (Buczkowski et al. 2014; Rust et al. 2004).

However, conventional methods with liquid baiting require the use of bait stations, which is expensive at larger scales (Daane et al. 2008). As a solution, hydrogel compounds have been explored as a carrier of liquid bait for Argentine ant populations (Buczkowski et al. 2014; Tay et al. 2017). Different hydrogel compounds have been tested for ant baiting: initially, synthetic polyacrylamide (Boser et al. 2014; Rust et al. 2015), and, more recently, biodegradable calcium alginate (Tay et al. 2017). Most of these recent investigations on hydrogel baits targeting Argentine ants have used thiamethoxam as an active ingredient, which is a highly effective bait toxicant for Argentine ants (Boser et al. 2014; Buczkowski et al. 2014; McCalla et al. 2020; Rust et al. 2015; Tay et al. 2017). Cooper et al. (2019) recently tested 0.5% boric acid liquid baits delivered in synthetic polyacrylamide hydrogel to suppress Argentine ants in California vineyards. Choe et al. (2021) tested calcium alginate hydrogel with 1% boric acid as one of the treatment methods for an Argentine ant management program in urban residential settings.

In this study, we tested calcium alginate hydrogel beads to deliver a boric acid liquid bait targeting field populations of Argentine ants in two different crop systems: citrus and grape. For the citrus site, panel traps were also used to monitor the densities of ACP, its parasitoid wasps, and Argentine ants. Because high boron levels are a concern, soil and plant samples in the vineyard site were analyzed to determine whether the baiting had any impact on boron concentration. Genetic analyses were used to confirm that all of the Argentine ants within each of the study sites belong to the same supercolony.

Valencia oranges in Riverside

The experimental site was located within Agricultural Operations, University of California, Riverside (33.970147°N, 117.346431°W). The site consisted of nine blocks of Valencia orange trees (fig. 1) (total 15.6 acres [6.3 hectares], 1.7 acres [0.7 hectares] for each block). The site was divided into three identically sized zones (control, buffer, and baited zones; 5.2 acres [2.1 hectares] for each zone) for the experiment.

For each citrus block, nine trees were systematically selected for monitoring: the third or fourth tree from the road in rows 4, 7, 10, 13, 16, 19, 22, 25, and 28 (i.e., a

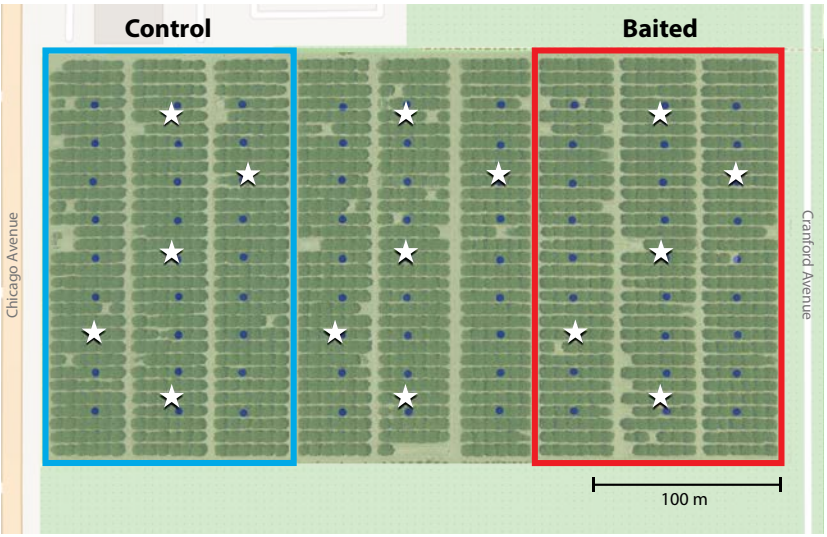

FIG. 1. Field site for the experiment (citrus). Dots indicate trees that were monitored for ant activity. Stars indicate where panel traps for ACP monitoring were placed (see discussion in main text). The equally sized control and baited zones are separated by a buffer zone in the center.

total of 27 monitoring trees per zone) (fig. 1). The foraging activity of Argentine ants on the monitoring trees was quantified using a method adapted from Cooper et al. (2019). Details on the monitoring method are provided in the online supplementary material.

To produce large quantities of hydrogel beads, a continuous production method was developed using a conveyor belt (supplementary fig. 2). Details on the bait production are provided in the online supplementary material.

The hydrogel bait was applied using an automatic spreader (Kubota V5003 spreader, Kubota Corp., Osaka, Japan) connected to a utility vehicle (Kubota RTV, Kubota Corp.) (supplementary fig. 3, supplementary video 1). Based on Cooper et al. (2019), an application rate of 10 gallons (gal)/acre (ac) (93.5 liters/hectare) was chosen for the current study. The baited zone was treated on June 25, July 23, August 20, and September 17, 2021. The field was irrigated with a micro-sprinkler the day before applications but not during the bait application, to allow the ants to forage on the ground and find the hydrogel baits.

On September 17, 2021 (after the last bait application), five yellow-green double-sided panel traps (5.5 inches [14 centimeters (cm)] by 6.9 inches [17.5 cm], ACP-Trap, Alpha Scents, Inc., Canby, Ore.) were set up to monitor insect populations in each of the control, buffer, and baited zones. The traps were hung on randomly selected trees within each zone (fig. 1, stars), about 5 feet (ft) (1.5 meters [m]) above the ground on the south-facing side of a tree. The traps were collected and replaced with new ones every two weeks. Adult ACP, adult *Tamarixia radiata* (a parasitoid wasp of ACP) (Hymenoptera: Eulophidae), and Argentine ants trapped in each trap were counted. Monitoring continued until November 12, 2021, with four two-week trapping intervals.

Argentine ant workers sampled on July 16, July 30, August 13, August 27, September 10, and September 24, 2021 were used for genetic analyses. Details on the genotyping are provided in the online supplementary material.

A two-way mixed-design analysis of variance (ANOVA) test was carried out to test whether two zones (baited and control) differed over time in the number of Argentine ants, using R v. 1.4.1717 (R Core Team 2021). Friedman's two-way ANOVA tests were also used to compare ant activity levels within baited or untreated control zones by using Statistix Version 10 (Analytical Software 2017). For this test, three representative sampling dates were chosen: pre-baiting (ten days before the first baiting, June 15), mid-baiting (two weeks after the second baiting, August 6), and post-baiting (two weeks after the fourth and final baiting, October 1). Spatial analysis by distance indices (SADIE) was employed to determine the spatial pattern of Argentine ants (SADIEShell Version 2.0). The clustering maps were created using the inverse distance-weighted interpolation method in ArcGIS Pro (Environmental Systems Research Institute, Redlands, Calif.). Details for these statistical methods are provided in the online supplementary material.

The panel trap data were analyzed using a generalized linear model with a Poisson distribution in JMP 15 (SAS Institute Inc., Cary, N.C.) as a two-factor experiment (treatment and trap setup time), followed by post-hoc pairwise comparisons.

Wine grapes in Temecula

The experimental site was located in Temecula, California (33.55903°N, 117.02856°W). The site included five varieties of grape: pinot gris, sauvignon blanc, chardonnay, syrah, and cabernet sauvignon. The site is naturally divided into two sections by a broad vegetated, seasonal riparian area (minimum width 69 ft [21 m]; maximum width 282 ft [86 m]), which served as a natural buffer zone between the control (northern plot, 7.4 ac [3.0 hectares]) and baited zones (southern plot, 4.2 ac [1.7 hectares]) (fig. 2).

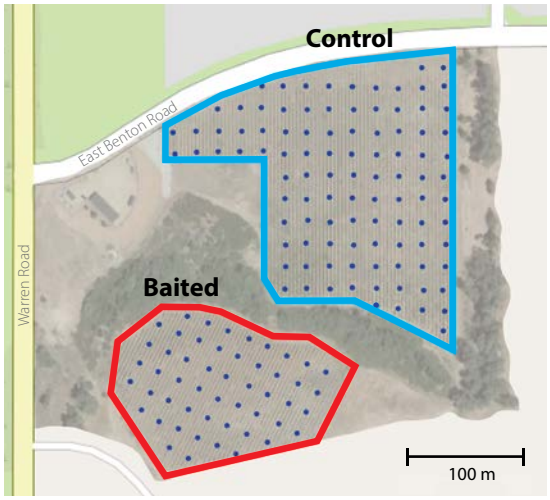

FIG. 2. Field site for the experiment (grape). Dots indicate vines that were monitored for ant activity. The control zone (north) was about 1.7 times larger than the baited zone (south). The control and baited zones were separated by the buffer zone in the middle.

The plots were divided into 57-ft (17.4 m) by 57-ft (17.4 m) grids using an aerial map. The vine located in the center of each grid was selected for monitoring. This arrangement resulted in 101 and 51 monitoring grapevines for the control and treatment zones, respectively (fig. 2). Details on the monitoring method are provided in the online supplementary material.

The hydrogel bait was produced using the method previously described for the citrus system. The bait was applied using hand-held spreaders (Scotts Whirl Hand-Held Spreader, Scotts Company LLC, Marysville, Ohio) at an application rate of 10 gal/ac (93.5 liters/hectare) (supplementary fig. 5). The treatment section received bait applications on April 26, May 24, June 21, July 19, and August 16, 2022. As above, the field was drip irrigated the day before a bait application but not during the application.

Argentine ant workers obtained from the monitoring visits on April 14, May 5, June 2, June 30, July 28, and August 25 were used for genetic analyses, which were carried out using the method previously described for the citrus system (also see the online supplementary material).

Samples of soil (from 0 to 6 inches and 6 to 12 inches in depth), petiole, and leaf were collected from the experimental vineyard on July 5, 2022 (after the third baiting) and sent to a plant diagnostics laboratory for boron analysis (Fruit Growers Laboratory, Inc., Santa Paula, Calif.).

The statistical methods described for the citrus system (also see the online supplementary material) were also used for the vineyard study. For Friedman's two-way ANOVA tests, three representative sampling dates were chosen for comparison: pre-baiting (12 days before the first baiting; April 14), mid-baiting (9 days after the third baiting; June 30), and post-baiting (9 days after the fifth baiting; August 25).

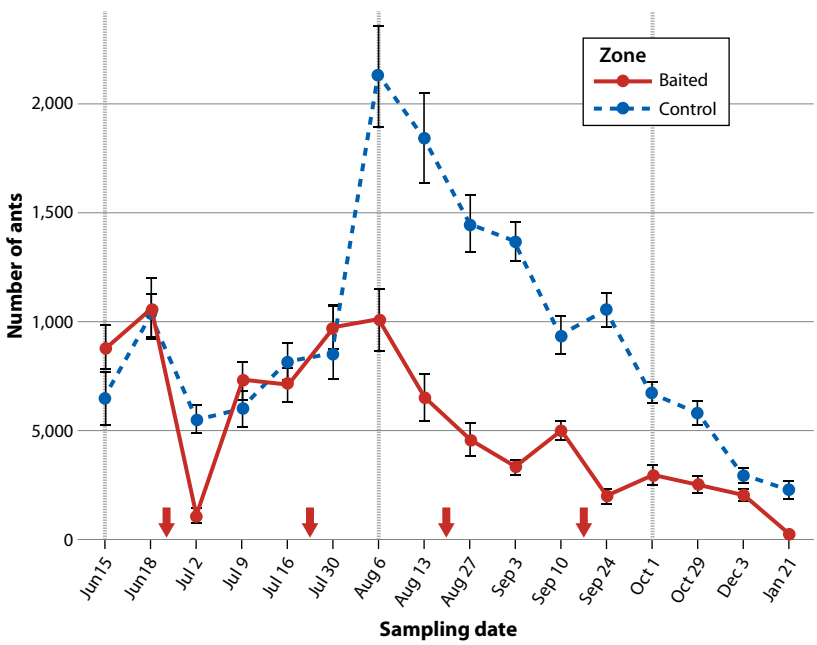

FIG. 3. Average number of ants per monitor for each sampling date in the experimental citrus grove from June 2021 through January 2022. Error bars represent the standard error of means (SEM). Arrows indicate the timing of four bait applications. Dotted vertical lines show three representative time points for comparisons over time within a zone (pre-, mid-, and post-baiting time points from the left). The control zone showed a large increase in ant activity in early August. However, this increase was not observed in the baited zone.

Ants reduced in citrus orchard

The result of two-way mixed-design ANOVA indicated that the number of Argentine ants was significantly different between baited and control zones ($F = 63.1$, df = 1, 52; $P < 0.0001$) (fig. 3). A significant interaction was found between zone and monitoring visits, indicating that control and baited zones were significantly different in their ant abundance throughout the monitoring period ($F = 13.37$, df = 15, 780; $P < 0.0001$).

The results of Friedman's two-way ANOVAs showed differences in ant counts within zones across pre-, mid-, and post-baiting times ($F = 25.51$, $P < 0.0001$ for the baited zone; $F = 15.38$, $P < 0.0001$ for the control zone) (fig. 3). For the baited zone, the ant count from the post-baiting time point was significantly lower than those from pre- and mid-baiting (Dunn's all-pairwise comparisons test: $\alpha = 0.05$). For the control zone, ant counts were similar between pre- and post-baiting time points, with the extremely high ant counts at the mid-baiting time point significantly different from the other two time points (Dunn's all-pairwise comparisons test: $\alpha = 0.05$).

SADIE results indicated that the Argentine ant exhibited frequent spatial aggregations within the experimental site ($I_a > 1$ in 14 out of 16 sampling dates) (table 1). The spatial aggregation was statistically significant for 11 sampling dates ($P_a < 0.05$). Significant clustering into patches (areas with higher ant counts) and gaps (areas with lower ant counts) was detected, especially after the second baiting (both $P\bar{v}_i$ and $P\bar{v}_j < 0.05$). Time series clustering maps (fig. 4) showed that, after the first bait application, the baited zone had a rapid and significant reduction in ant activity (July 2), evidenced

TABLE 1. SADIE parameters for the spatial distribution pattern of Argentine ants in the citrus orchard

Date of sampling	I_a	P_a	$\bar{v}_j$	$\bar{v}_i$	$P\bar{v}_j$	$P\bar{v}_i$
June 15, 2021	1.127	0.2136	−1.072	1.187	0.2869	0.1613
June 18, 2021	0.816	0.8346	−0.783	0.792	0.9026	0.8856
July 2, 2021	3.035	0.0003	−3.006	2.826	0.0000	0.0000
July 9, 2021	0.999	0.3882	−0.977	1.076	0.4323	0.2754
July 16, 2021	1.290	0.1028	−1.235	1.140	0.1272	0.1990
July 30, 2021	1.278	0.1077	−1.246	1.226	0.1172	0.1274
Aug. 6, 2021	2.546	0.0003	−2.503	2.477	0.0003	0.0003
Aug. 13, 2021	3.167	0.0003	−2.888	2.838	0.0000	0.0000
Aug. 27, 2021	2.962	0.0003	−2.682	2.473	0.0000	0.0005
Sept. 3, 2021	3.848	0.0003	−3.482	4.058	0.0000	0.0000
Sept. 10, 2021	2.951	0.0003	−2.804	3.092	0.0000	0.0000
Sept. 24, 2021	4.019	0.0003	−3.956	3.939	0.0000	0.0000
Oct. 1, 2021	2.941	0.0003	−2.902	3.024	0.0000	0.0000
Oct. 29, 2021	2.943	0.0003	−2.652	2.487	0.0000	0.0000
Dec. 3, 2021	1.652	0.0162	−1.502	2.070	0.0292	0.0018
Jan. 21, 2022	2.821	0.0003	−2.768	3.317	0.0000	0.0000

I_a: index of aggregation; P_a: *P*-value of I_a; $\bar{v}_j$ and $\bar{v}_i$: Indices of clustering; $P\bar{v}_j$ and $P\bar{v}_i$: *P*-values of $\bar{v}_j$ and $\bar{v}_i$, respectively.

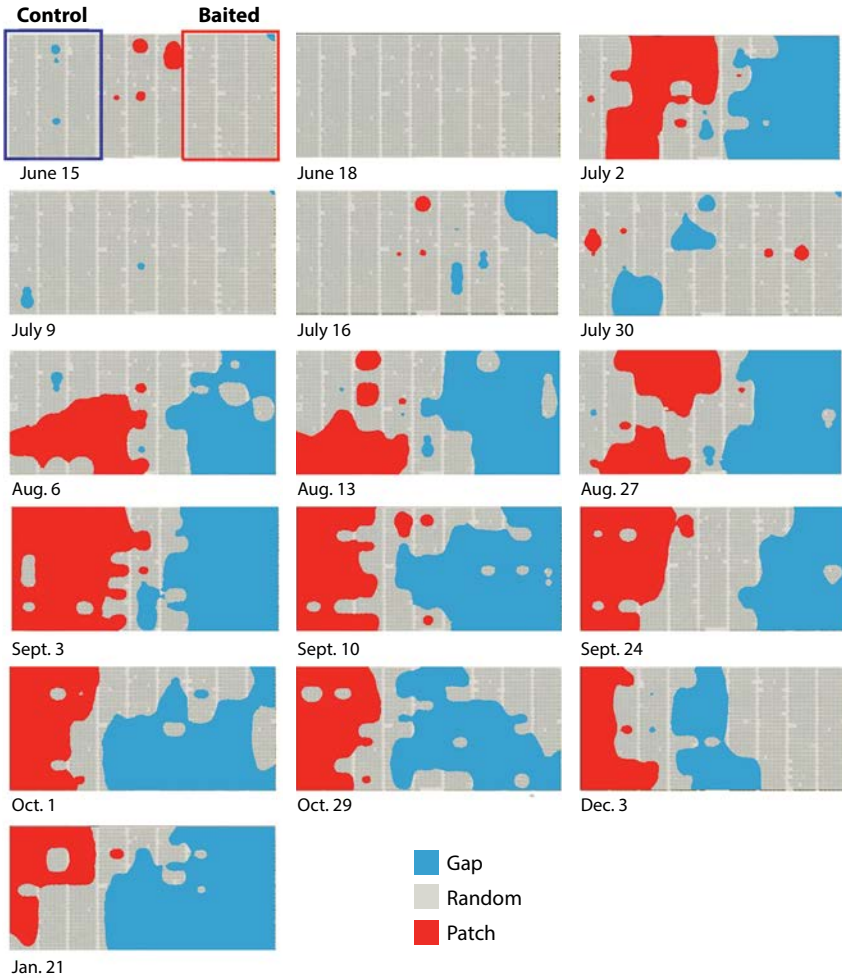

FIG. 4. Clustering maps including patches (red) and gaps (blue) of the Argentine ant in the baited, control, and buffer zones in the citrus orchard from June 2021 through January 2022. Patches and gaps indicate areas with significantly higher and lower ant counts, respectively. From early August onward, the control zone is characterized by the presence of patches, while the baited zone is characterized by the presence of gaps.

by patches in the control zone and gaps in the baited zone. However, the spatial pattern of the ants became random on July 9, possibly caused by the rebounded ant activity in the baited zone. The control zone experienced a large increase in ant activity around late July and early August, while the ant activity in the baited zone remained low (August 6 and onward), creating gaps in the baited zone.

The number of ACP caught per trap (mean ± SEM) in the baited (0.85 ± 0.20) and control zones (2.75 ± 0.57) differed significantly ($\chi^2 = 21.11$, $P < 0.001$) (table 2). The numbers of *T. radiata* trapped did not differ between the baited and control zones ($\chi^2 = 2.91$, $P = 0.088$). Argentine ant workers were also caught in the panel traps. Significantly fewer ants were trapped in the baited zone than in the untreated control zone ($\chi^2 = 75.85$, $P < 0.001$) (table 2). The number of ants trapped in the control and buffer zones did not differ significantly ($\chi^2 = 1.88$, $P = 0.170$).

TABLE 2. Mean (± SEM) numbers of ACP, *T. radiata*, and Argentine ants captured on panel traps set up in four two-week intervals

Zone	No. of ACP	No. of *T. radiata*	No. of Argentine ants
Baited	0.85 ± 0.20 a	0.05 ± 0.05 a	1.25 ± 0.62 a
Buffer	1.60 ± 0.45 b	0.40 ± 0.21 b	5.35 ± 2.20 b
Control	2.75 ± 0.57 c	0.25 ± 0.16 ab	6.40 ± 2.53 b

Numbers not followed by the same letter differed significantly ($P < 0.05$).

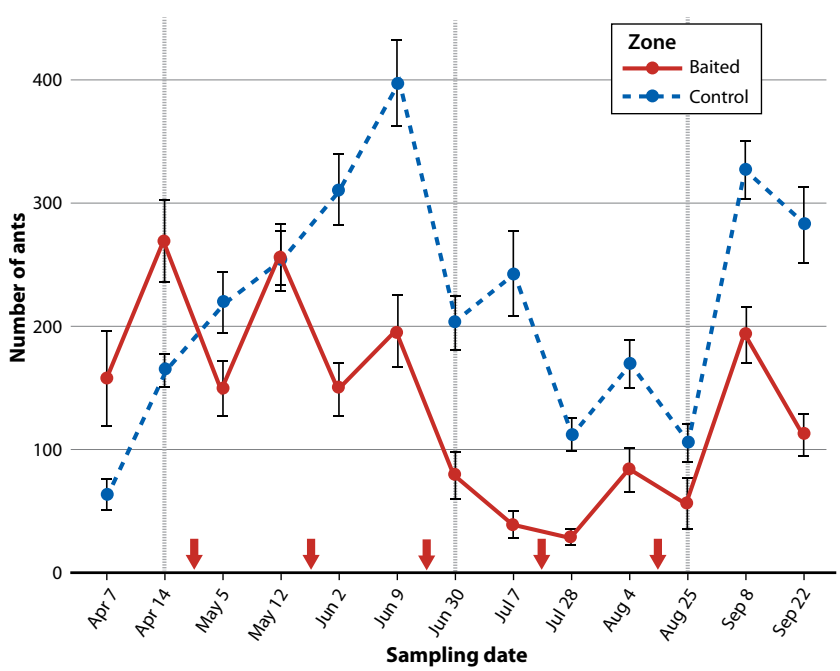

FIG. 5. Average number of ants per monitor for each sampling date in the experimental vineyard from April through September 2022. Error bars represent the standard error of means (SEM). Arrows indicate the timing of five bait applications. Dotted vertical lines indicate three representative time points for comparisons over time within a zone (pre-, mid-, and post-baiting time points from the left). The control zone showed a large increase in ant activity in early June. However, this increase was not observed in the baited zone.

The genetic differentiation between all pairs of ant samples was low (F_{ST} range from −0.028 to 0.075) and not significant (supplementary table 1). Principal coordinate analysis (PCoA) demonstrated no clustering among individuals from the same area or sampling dates (supplementary fig. 6). This finding suggests that the Argentine ants in the experimental orchard belong to one large supercolony.

Control of ants in vineyard

The results of two-way mixed-design ANOVA indicated that the ant counts were significantly different between baited and control zones ($F = 12.2$, df = 1, 150; $P = 0.0006$) (fig. 5). A significant interaction was found between zone and monitoring visits, indicating that control and baited zones were significantly different in their ant abundance throughout the monitoring period (two-way mixed ANOVA: $F = 8.52$, df = 12, 1,800; $P < 0.0001$).

The results of Friedman's two-way ANOVAs showed differences in ant counts within zones across pre-, mid-, and post-baiting time points ($F = 18.33$, $P < 0.0001$ for the baited zone; $F = 12.39$, $P < 0.0001$ for the control zone) (fig. 5). For the baited zone, the ant counts from the mid- and post-baiting time points were significantly lower than those from pre-baiting (Dunn's all-pairwise comparisons test: $\alpha = 0.05$). For the control zone, the ant counts stayed similar between pre- and mid-baiting time points. However, the ant count at the post-baiting time point was significantly lower than those from pre- and mid-baiting time points (Dunn's all-pairwise comparisons test: $\alpha = 0.05$).

SADIE results showed that the Argentine ant exhibited a strong spatial aggregation in the vineyard throughout the monitoring period (table 3). Statistically significant spatial clustering (both P and $P < 0.05$) also indicated significant spatial clustering into patches and gaps. Time series clustering maps (fig. 6) revealed that, after the first two sampling dates (when patches were located within both baited and control zones), the baited zone had a rapid reduction in ant activity as measured on May 5. This created gaps in the baited zone and patches in the control zone.

Boron analysis data are shown in supplementary table 2. Boron concentrations in the soil sample were comparable between the control zone (2.04–2.26 pounds [lbs]/acre foot [AF]) and baited zone (1.40–3.84 lbs/AF) after the third bait application. Boron concentrations in the petiole or leaf samples appear to be slightly elevated for the baited zone compared to the control zone. However, the boron concentrations were within optimal ranges for all samples except the leaf sample from the baited zone (e.g., 107 ppm, while the optimal range is 30 to 100 ppm).

The genetic analysis result indicated that the genetic differentiation between all pairs of ant samples was low (F_{ST} range from −0.0148 to 0.0287) and not significant (supplementary table 3). PCoA demonstrated that there

TABLE 3. SADIE parameters for the spatial distribution pattern of Argentine ants in the vineyard

Date of sampling	I_a	P_a	$\bar{v}_j$	$\bar{v}_i$	$P\bar{v}_j$	$P\bar{v}_i$
Apr. 7, 2022	2.176	0.0003	−2.329	1.728	0.0000	0.0051
Apr. 14, 2022	2.656	0.0003	−2.656	2.060	0.0000	0.0000
May 5, 2022	1.765	0.0046	−1.795	1.426	0.0044	0.0395
May 12, 2022	1.600	0.0141	−1.633	1.329	0.0087	0.0667
June 2, 2022	1.861	0.0021	−1.943	1.524	0.0018	0.0190
June 9, 2022	2.160	0.0003	−2.213	1.920	0.0000	0.0013
June 30, 2022	1.783	0.0046	−1.904	1.423	0.0015	0.0405
July 7, 2022	1.884	0.0023	−1.843	1.286	0.0028	0.0838
July 28, 2022	2.476	0.0003	−2.576	1.946	0.0000	0.0015
Aug. 4, 2022	2.201	0.0003	−2.325	1.798	0.0000	0.0026
Aug. 25, 2022	1.881	0.0010	−2.000	1.746	0.0021	0.0077
Sept. 8, 2022	2.443	0.0003	−2.501	2.028	0.0000	0.0010
Sept. 22, 2022	2.255	0.0003	−2.316	1.822	0.0000	0.0031

I_a: index of aggregation; P_a: P-value of I_a; $\bar{v}_j$ and $\bar{v}_i$: Indices of clustering; $P\bar{v}_j$ and $P\bar{v}_i$: P-values of $\bar{v}_j$ and $\bar{v}_i$, respectively.

was no clustering among individuals from the same plot or sampling dates (supplementary fig. 7). This finding suggested that the Argentine ants in the experimental vineyard belong to one large supercolony.

Control achieved in orchard

Repeated application of 1% boric acid hydrogel bait effectively suppressed the field population of Argentine ants over three months (August–October). The monitoring data indicated that the ant population in the baited zone was substantially suppressed compared to the control zone (e.g., 47% to 81% reduction between August and October). The difference in Argentine ant counts between the baited and control zones was also shown in the panel trap data, which were obtained later in the season (September–November). The results of SADIE showed that Argentine ants were randomly distributed in all three zones (baited, control, and buffer) before baiting. However, after baiting, the ants became spatially aggregated in the control zone, while the gaps (areas with significantly lower ant counts) were created in the baited zone.

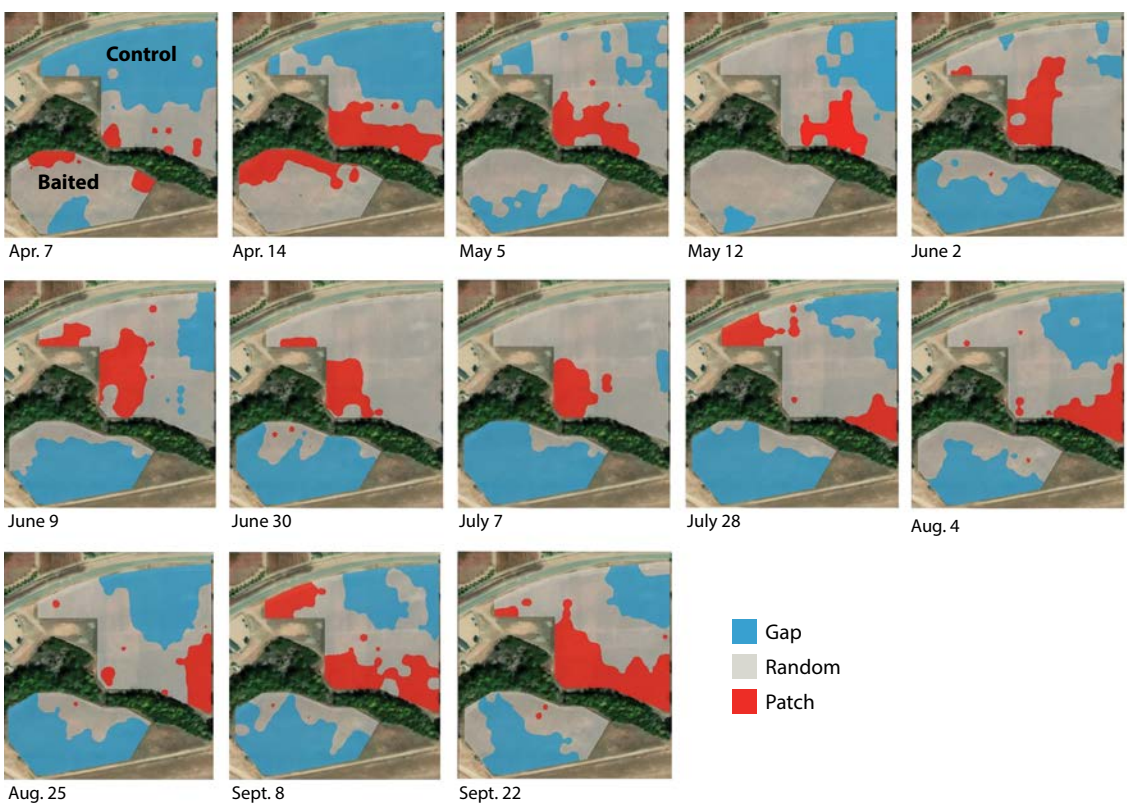

FIG. 6. Clustering maps including patches (red) and gaps (blue) of the Argentine ant in the baited, control, and buffer zones in the vineyard from April through September 2022. Patches and gaps indicate areas with significantly higher and lower ant counts, respectively. From early May onward, the control zone is characterized by the presence of patches while the baited zone is characterized by the presence of gaps.

Boric acid bait delivered in calcium alginate hydrogel may provide an environmentally sustainable method to manage Argentine ants in agricultural systems.

At the beginning of August (between July 30 and August 6), the control zone had a dramatic increase in Argentine ant activity (fig. 3). This increased ant activity was not detected for the baited zone during the same period. In Southern California, August to September has been a typical time when Argentine ant activity levels reach their peak in citrus groves (Markin 1970; Rust et al. 2000). The current study demonstrated that the significant increase in Argentine ant activity in mid-summer could be eliminated or suppressed by repeated application (e.g., twice in June and July) of the boric acid hydrogel bait. After August 6, ant activity levels gradually decreased across the field, which is a seasonal trend of Argentine ant activity previously reported in Southern California (Markin 1970; Rust et al. 2000).

Ant control in vineyard

Repeated application of 1% boric acid hydrogel bait effectively suppressed the field population of Argentine ants over four months (June–September). The monitoring data indicated that the ant population in the baited zone was substantially suppressed compared to the control zone (e.g., 41% to 84% reduction between June and September). After the first bait application, the ant activity level quickly decreased. This quick reduction of ant activity also was observed immediately after the second bait application. These sudden reductions in ant count were not observed in the control plot during the same period.

After the initial reduction, the ant activity level in the baited zone bounced back within seven days (fig. 5; see May 12 and June 9). This pattern observed in the early part of baiting (i.e., quick reduction and bounce-back in ant counts) may be due to several factors, including immediate impact on the existing populations and repopulation and recovery by new ants from the same or adjacent locations. In contrast, ant activity in the control zone showed a consistent increase from the beginning of the experimental period until it reached its peak in early June. In particular, between May and June, the control zone had a dramatic increase in Argentine ant activity (fig. 5). This increase in ant activity was not detected for the baited zone during the same period. In California, June is a typical time of year when Argentine ant activity level peaks in the vineyards (Cooper et al. 2008; Cooper et al. 2019). The current study demonstrated that the significant increase in Argentine ant activity in early summer could be eliminated or suppressed by repeated application (e.g., three times from April to June) of the boric acid hydrogel bait.

After June 9, there was a general reduction in ant activity levels across the field, likely reflecting a seasonal trend of Argentine ant activity seen in previous reports. However, in September, the ant activity level was substantially increased in both baited and control plots. This second peak of Argentine ant activity in the later part of the season (September and October), which was smaller than the first peak in early or mid-summer, has been observed in former field experiments conducted in California vineyards (Cooper et al. 2008; Daane et al. 2008).

Promising biodegradable approach

One critical question is whether the level of Argentine ant suppression achieved in the current study would be sufficient to increase the susceptibility of honeydew-producing hemipteran pests to their natural enemies. Even though the present study did not directly address that question, the panel trap data from the citrus study might provide some insights. Based on the panel trap surveys conducted at the end of the season (September–November), the number of ACPs caught in the traps was consistently lower in the baited zone than in the control zone. This finding might indicate that the boric acid hydrogel baiting suppressed Argentine ant foraging to the point that ACP populations in the baited zone were more effectively managed by their natural enemies. In the current study, the mealybug population at the experimental vineyard was not large enough for any formal assessment and monitoring.

Boric acid bait delivered in calcium alginate hydrogel may provide an environmentally sustainable method to manage Argentine ants in agricultural systems. There are several useful attributes of boric acid as a bait toxicant in this particular application. Boric acid used at insecticidal levels is considered non-toxic to organisms that are not being targeted, such as non-insect invertebrates and vertebrates (US EPA 1993). Boric acid baits delivered in bait stations can be used in groves and vineyards while maintaining organic status (Greenberg et al. 2006). In addition, calcium alginate is readily biodegradable after application on the soil surface, without leaving any toxic degradants behind (Kim et al. 2021; Tay et al. 2020).

Because multiple applications might be necessary for this boric acid hydrogel bait to achieve the desired level of ant control, repeated introduction of boron in the soil may be of concern. However, boric acid can be removed from soils by leaching and plant uptake (Harper et al. 2012). Based on the boron analysis, we can conclude that the amount of boron introduced into the soil by the current baiting program is negligible. Additionally, boron is a vital micronutrient for the vegetative and reproductive growth of plants, and boron deficiency is present in various crops worldwide (Kohli et al. 2022; Shorrocks 1997). However, it is recommended to periodically take plant or soil samples for laboratory analysis to monitor boron levels and prevent toxicity issues from overapplication.

Current research demonstrates that boric acid hydrogel baiting is a promising method to control Argentine ant populations in citrus orchard and vineyard systems. Future testing in different crop and ornamental systems would expand the scope of the boric

acid hydrogel baiting. Tests with other organically proven active ingredients (e.g., spinosad) might be of some value, as the other active ingredients also might be effective in achieving a sufficient level of control of Argentine ants when incorporated in calcium alginate hydrogels as a bait (Milosavljević et al. 2024). CA

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