Research Article

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Assessing colony elimination in multicolonial ants: Estimating field efficacy of insecticidal baits against the invasive dark rover ant (*Brachymyrmex patagonicus*)

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Abstract

BACKGROUND: A frequent goal of pest management strategies targeting social insects is total colony elimination. Insecticidal baits are highly effective at controlling social insect pests, although their ability to provide total colony elimination has only been well studied in a few species. Genetically testing colony elimination in many urban pest ants can be challenging due to indistinct colony boundaries observed in unicolonial, invasive species; however, some pest ants, such as the dark rover ant (*Brachymyrmex patagonicus*), maintain strict colony borders through aggression towards non-nestmates. Each of these distinct colonies can be identified using molecular markers, allowing for the tracking of individual colonies pre- and post-treatment to measure colony density. While counting the number of foraging workers to assess treatment efficacy may suffice in some cases, it offers little insight into the colony-level impacts of a treatment.

RESULTS: Using microsatellite markers, distinct rover ant colonies were identified and tracked around residential structures before and after the application of an imidacloprid bait. The number of foraging ants at the treated structures was reduced by an average of 83.0% over a 28-day observation period. Baiting also significantly reduced the total number of colonies present. At the treatment structures, only ~25% of the original colonies remained at the end of the study. Colonies with foraging trails <1.5 m from a bait station had a higher chance of being eliminated.

CONCLUSION: Using insecticidal baits against *B. patagonicus* can be highly effective at colony elimination; however, with such small foraging ranges and high colony densities, proper placement is required to ensure enough bait is properly positioned to treat all colonies affecting a structure.
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Keywords: microsatellite; Formicidae; urban pest management; imidacloprid

1 INTRODUCTION

Social insects, such as ants and termites, are among the most significant structural pests worldwide.[1] These species are characterized by a reproductive division of labor which ensures that the reproductive caste is confined to the safety of the nest,[2–4] while the worker caste engages in more dangerous activities like foraging and defense.[5,6] This strategy has allowed social insects to become ecologically successful,[7,8] and it makes the control of these some species quite challenging.[9] Because new workers can easily be produced by the reproductives, effective control strategies must target the entire colony (i.e. total colony elimination). Many social insects form cryptic nests in the soil, so applying a liquid insecticide such that it reaches the reproductive caste of these soil-dwelling species can be difficult. Baits and some nonrepellent sprays are often used against social insects as they take advantage of the behaviors that make these species so dominant.[10] Foraging workers bring the active ingredient back to the nest, where it is shared *Via* trophallaxis or contact between nestmates, including queens, and disseminated throughout the colony.[11,12] One advantage of using baits over a broad-spectrum insecticide is that they are formulated to exclude many nontarget species.[13] Additionally, advances in bait formulations have lowered the costs associated with bait application and monitoring, allowing for increased versatility with this approach to pest management.[14–18]

Ants, and in particular invasive ants, are some of the most economically important insect pests in both urban and agricultural

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settings.[19,20] Invasive ant species can substantially alter ecosystems by outcompeting native species or by facilitating the growth of other pest or invasive populations.[21–23] Consequently, a substantial amount of effort and resources is spent annually on the management and monitoring of pest ants.[24–26] Population density estimates, using food lures or counting foragers, are often carried out to assess treatment efficacy against ants[27]; however, these strategies provide no information regarding colony densities. Molecular markers, such as microsatellites, can be used to determine the colony identity of individual workers collected from the field. Microsatellite markers are short regions of repetitive DNA with a high level of genetic diversity well suited for kinship analyses. Workers belonging to the same colony are usually more related to each other than to workers from a different colony, thus the shared alleles among nestmates make them easily distinguishable from non-nestmates.[28–30] Regarding pest management, a sample of individuals collected during an initial infestation can be genetically analyzed to determine if subsequent infestations are due to a new or preexisting colony. Using microsatellites to monitor pest populations and assess the efficacy of management efforts has been well studied in subterranean termites[31–36]; however, this method has not been widely applied in ant pests.[37]

The dark rover ant (*Brachymyrmex patagonicus* Mayr) is an invasive species from South America that has spread across most of the southern United States (US)[38,39] and is continuing to invade new areas.[40–42] This species can utilize a wide range of environments,[43] especially disturbed habitats, and has become a relatively common nuisance pest inside urban structures.[39] Colonies feed on a variety of food sources,[44] although they are most commonly associated with sugary food sources such as extrafloral nectar and honeydew.[39,45] Most colonies of *B. patagonicus* occupy a single nest and are predominantly headed by a single queen.[38] Colonies maintain strong nest-mate recognition and strict colony boundaries, with as many as 24 colonies coexisting at a single structure.[46] Pest management professionals commonly report reinfestations when treating structures for dark rover ants.[47] The high density of colonies of *B. patagonicus*, together with their multicolonial structure, calls into question how treatments impact colony densities around residential structures. By assessing management efforts at the colony level, it is possible to delve into the factors hampering proper control of this pest species.

Here, we measured both foraging worker activity and colony density of *B. patagonicus* around residential structures before and after the use of bait stations. For each residential structure, ant activity was assessed weekly using counts of foraging ants. Colony density was examined through the genetic assignment of foraging workers to individual colonies using microsatellite markers. With this method, we were able to illustrate the amount of pest pressure faced by each structure due to the high number of colonies present. Subsequently, by tracking the fate of each colony over the length of the study, we were able to measure the effects of baiting on colony density. Additionally, we investigated the correlation between colony elimination and the proximity of foraging trails to the bait stations.

2 METHODS

2.1 Ant activity assessment and sample collection

Assessments of overall *B. patagonicus* activity were performed on 12 residential structures in the Bryan/College Station area, Texas, USA by counting the number of foraging workers.[48,49] These assessments were made weekly, starting 1–3 days pre-treatment and then 7-, 14-, 21-, and 28-days post-treatment. To measure population densities, each structure was divided into four quadrants (Fig. S1). A single foraging trail of *B. patagonicus* was located in each quadrant and the total number of ants passing over a fixed point along the trail was recorded for 1 min. Only trails directly on the structure or in areas up to 1.5 m out (i.e. decks or flowerbeds) were counted. If multiple trails were identified in a quadrant, the most active trail was selected for data collection. If after 5 min of searching no trails were found, this quadrant was determined to have no ants present. Each selected trail in each quadrant was counted three times per assessment (with at least 5 min between readings) and the average of these counts was recorded as the trail density for this quadrant (Fig. S1). For each structure, the sum of the trail density counts for each structure was recorded as ant activity.

To measure the effect of baiting on colony densities, workers from all active foraging trails were sampled from each structure during the pre-treatment assessments as well as 28 days post-treatment. All walls and neighboring surfaces were exhaustively searched for foraging trails up to 3.0 m away from the structure. Collection locations were mapped for each structure to estimate the distance between foraging trails as well as their proximity to the closest bait station. For each collection, individuals were directly stored in 95% ethanol for subsequent genetic analyses to identify the number of unique colonies on each property. The presence of observable foraging trails was used as an indicator of the presence/absence of a colony. The comparison of colonies present pre- and post-treatment was used to estimate colony elimination and colony stability over time with and without baiting.

2.2 Bait application

The pre-treatment ant activity assessments were used to randomly assign half of the houses to the control group (A–F) and half of the houses to the treatment group (G–L). Unfortunately, control structure E had to be discarded as an outside pest control company treated the structure during the trial. In the treatment group, four to six plastic Maxforce bait stations were installed around the structures with a minimum of 10–12 m between stations. When possible, stations were placed in the shade and in areas with a high number of foraging ants. Approximately 1.0 g of MaxForce Quantum Ant Gel Bait (active ingredient 0.03% imidacloprid; Bayer Environmental Science, Cary, NC, USA) was added to each station. All chemical applications were made following the manufacturer's instruction by a licensed Noncommercial Applicator (Texas Department of Agriculture License Number 0756973).

2.3 Genetic procedures and analyses

To assign the ants collected during pre- and post-treatment to their colony of origin, genomic DNA was extracted from four to eight workers per trail. Extractions were performed using a modified Gentra Puregene method (Gentra Systems, Inc. Minneapolis, MN, USA). Each worker was genotyped at seven microsatellite markers using the primer sets and thermocycle profiles listed in Eyer *et al*. (2020) (Bpa7, Bpa8, Bpa9, Bpa13, Bpa14, Bpa16, and Bpa23).[38] PCR reactions were carried out on a Bio-Rad thermocycler T100 (Bio-Rad, Pleasanton, CA, USA) and the PCR products for each individual were combined into a single well for fragment analysis. These samples were compared against the LIZ500 standard ladder and fragment analysis was performed on an ABI 3500 capillary sequencer (Applied

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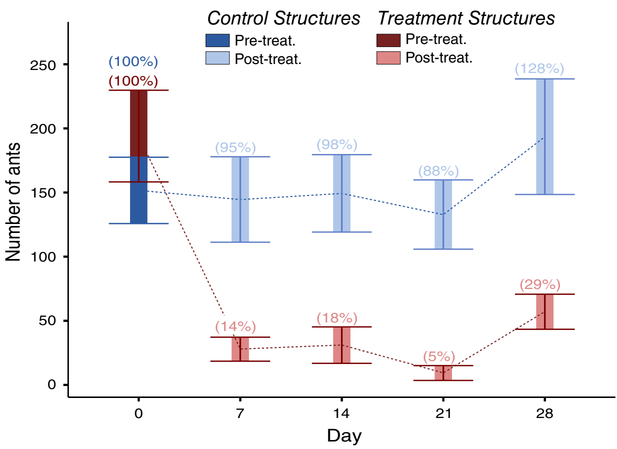

Figure 1. Ant activity over time (mean ± SE). The percentage of the original density counts is shown in parentheses.

Table 1. Ant densities over time

	Days post-treatment				
	0	7	14	21	28
Control	151.8 a (100%)	144.6 a (95.3%)	149.4 a (98.4%)	132.8 a (87.5%)	193.8 a (127.7%)
Treatment	194.3 a (100%)	27.5 b (14.2%)	30.7 b (17.5%)	8.8 b (4.5%)	56.8 b (29.2%)

The percentage of the original pre-treatment ant activity (0-day assessment) is shown in parentheses. Numbers followed by different letters were found to be significantly different (Student's *t*-test, $P \leq 0.05$).

Biosystems, Foster City, CA, USA). The alleles for each sample were identified on Geneious v.9.1.[50]

Genotypic differentiation was tested between each pair of trails collected from the same structure. To determine colony identity, log-likelihood G-tests were implemented in GENEPOP v.4.7.[51] A standard Bonferroni correction was performed on the Fisher's probability test to account for multiple comparisons. The assignment of trails to colonies was visualized using the Bayesian clustering method implemented in STRUCTURE v.2.3.4.[52] For each structure, simulations were run with K ranging from one to the number of trails sampled (max = 30), with 20 replications for each number of K. Each run comprised a first step of a 50 000 burn-in period and 100 000 iterations of the Markov chain Monte Carlo (MCMC). The most likely number of genetic clusters was determined using the method of Puechmaille[53] implemented in StructureSelector.[54]

2.4 Statistical analyses

The mean ant activity level was analyzed using a repeated-measures ANOVA with a Student's *t*-test to compare the control and treatment groups at each assessment interval. The mean number of colonies was analyzed using an ANOVA with Student's *t*-test. These tests were used to compare the number of colonies between the treatment and control groups during the pre-treatment assessments, as well as to compare the pre- and post-treatment colony numbers for each group. Both ant activity and colony density are reported as the mean ± standard error. A linear regression was used to compare the correlation between colony elimination and a colony's proximity to the bait stations. A chi-squared test was used to compare the proportion of colonies eliminated between the distances of <1.5, 1.5–3.0, 3.1–4.5, and >4.5 m. All analyses were performed with JMP Pro version 14.

Table 2. Number of rover ant colonies collected during the pre- and post-treatment collection as well as the number of original colonies recovered

Group	Structure ID	Collection	Total number of colonies	Number of original colonies recovered
Control	A	Pre-treat	17	
		Post-treat	15	12
Control	B	Pre-treat	12	
		Post-treat	14	8
Control	C	Pre-treat	18	
		Post-treat	21	14
Control	D	Pre-treat	6	
		Post-treat	4	4
Control	F	Pre-treat	8	
		Post-treat	6	5
Treatment	G	Pre-treat	4	
		Post-treat	0	0
Treatment	H	Pre-treat	7	
		Post-treat	2	1
Treatment	I	Pre-treat	9	
		Post-treat	6	4
Treatment	J	Pre-treat	6	
		Post-treat	3	2
Treatment	K	Pre-treat	9	
		Post-treat	4	3
Treatment	L	Pre-treat	7	
		Post-treat	1	1

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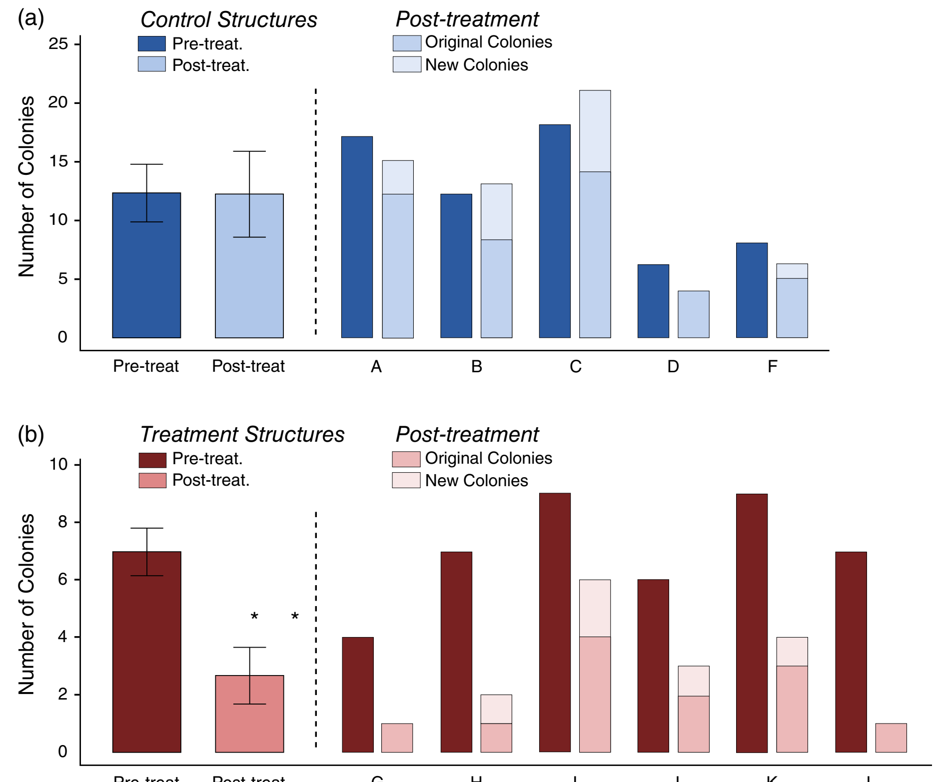

Figure 2. Change in colony number 28 days after bait treatment in the control (a) and treatment (b) groups. The total number of original and new colonies found at each structure (A–L). Note that the *y* axes of (a) and (b) are different scales.

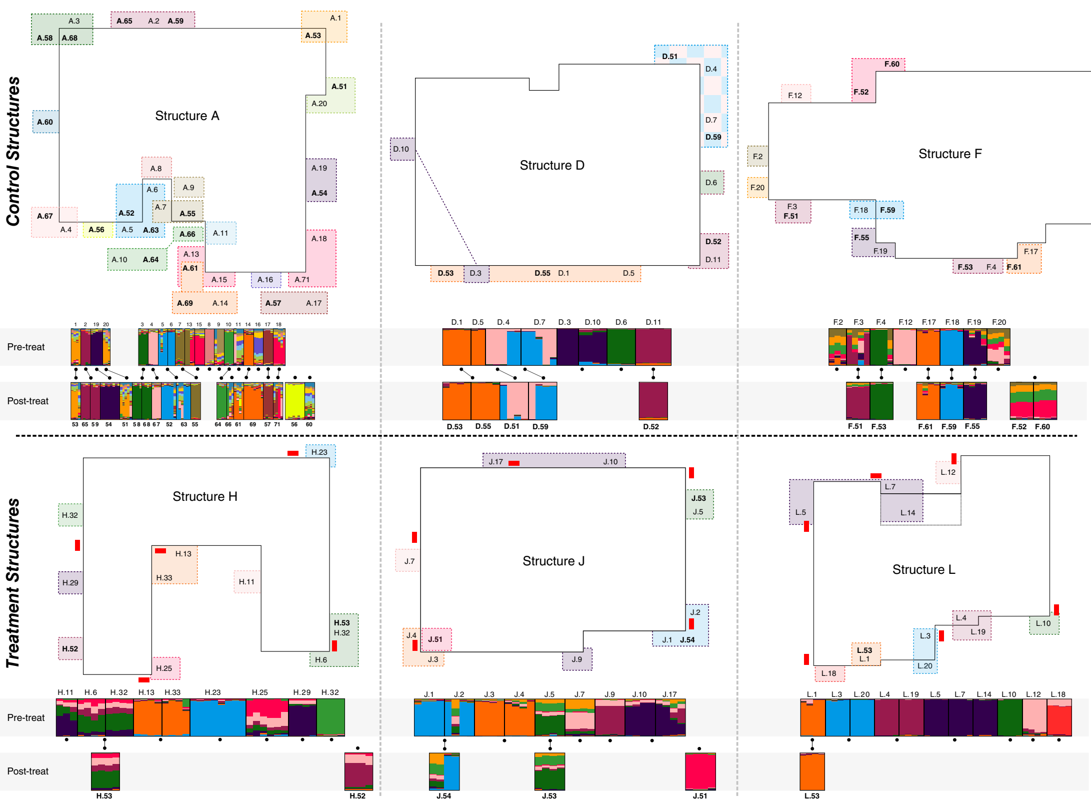

Figure 3. Some of the structures used in this study. Sample collections (denoted with letters followed by a number) mapped to the location around the structure. The post-treatment collections are denoted in bold. For the treatment structures, the location of the bait stations is represented by a red rectangle. Sample collections that were found to be the same colony are grouped together and denoted with a shaded area. Below each diagram are the structure plots assigning colony designations for the pre- (top) and post-treatment collections. Colonies present at both the initial and ending collection are connected with a solid line.

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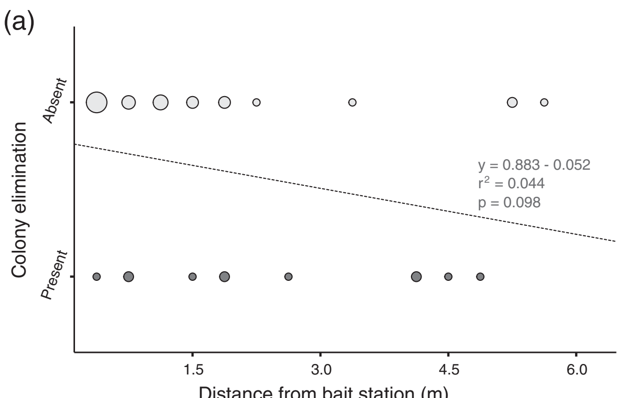
3 RESULTS

During the pre-treatment assessments, the level of ant activity observed at structures subsequently used as control (151.8 $\pm$ 25.9) was not significantly different from the activity observed at structures later assigned to the treatment (194.3 $\pm$ 35.9, P = 0.615; Table 1). Seven days post-treatment, there was an 85% reduction in the number of foraging ants at the treatment structures (27.5 $\pm$ 9.4), and this reduction reached 95% at 21 days post-treatment (8.8 $\pm$ 5.8; Fig. 1). Overall, the bait treatment significantly reduced the ant activity of the treated structures for all assessment periods after treatment (all $P < 0.05$). In contrast, the number of foraging ants found on the control structures during each assessment period was comparable throughout the trial (Fig. 1). Interestingly, a slight increase in activity was observed in both the treatment and control groups 28 days after treatment, potentially due to seasonality or a rain event.

In total, 1001 workers were genotyped at seven polymorphic loci. From the pre-treatment collections, a total of 103 *B. patagonicus* colonies were identified: 61 from the structures in the control group and 42 from the structures in the treatment group (Table 2). This corresponds to an average of nine colonies per structure (9.36 $\pm$ 1.36), with a range of 4 to 18. In the post-treatment collections, 60 colonies were identified from the control structures and 16 from the treatment structures. A lower number of colonies was found in the treatment group (7.0 $\pm$ 0.78) during the pre-treatment assessment compared to the control group (12.2 $\pm$ 2.38, P = 0.051; Fig. 2). The number of colonies found at the control structures pre-treatment was not significantly different from the number observed after treatment (12.0; P = 0.961). Conversely, there was a significant reduction in the mean number of colonies per structure in the treatment group 28 days post-treatment (2.67 $\pm$ 0.88) compared to the number of colonies found pre-treatment (P = 0.004; Fig. 2).

The number of colonies at the control structures remained relatively constant throughout the trial (Fig. 3). Of the colonies identified from the pre-treatment assessment, ~71% of the original colonies were recovered after 28 days (Table 2). In contrast, only about 26% of the original colonies were recollected from the treated structures (Fig. 3, see also Figs S2 and S3). There was a slight correlation between whether a colony was eliminated and the proximity of their foraging trails to a bait station, although this was not highly significant (P = 0.098; Fig. 4(a)). Most of the colonies (20 of 23) with foraging trails located less than 1.5 m from a bait station were eliminated; a significantly higher proportion than was observed in colonies located further away (11 of 19) (P = 0.005; Fig. 4b). Many of the colonies located between 1.5 and 3.0 m away from the bait stations were also eliminated (seven of 11). Colony elimination was more inconsistent when colonies with foraging trails were more than 3 m from a bait station. Half of these colonies (four of eight) persisted until the end of the trial (Fig. 4(b)).



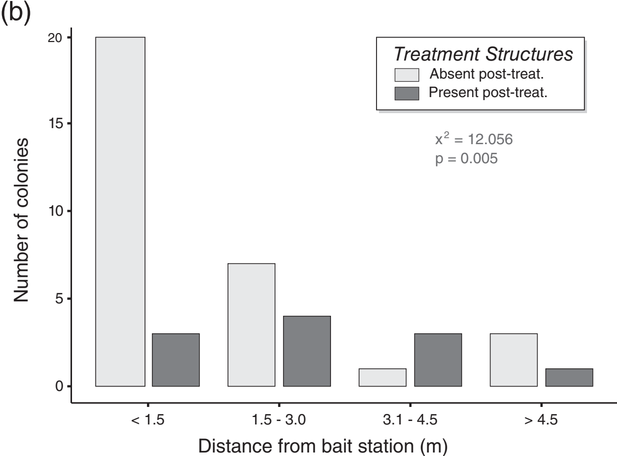


Figure 4. (a) Effect of proximity of colonies to bait stations on colony elimination (linear regression). The size of the circles represents the number of colonies found at that distance from a bait station. (b) The total number of original colonies that were present or absent at day 28, and their distance from the closest bait station (chi-squared).

4 DISCUSSION

Counting the number of foraging workers can be informative but offers limited insight into the colony-level effects of a treatment application. By incorporating both methods into our study, we gained a better understanding of the overall rover ant population at each structure and were therefore able to better assess the success of this treatment strategy. We found that the use of imidacloprid in bait stations significantly reduced both the level of ant activity and colony density of *B. patagonicus* around residential structures. Of the original colonies identified pre-treatment, only 25% remained at the treated structures by the end of the study; far fewer than the number persisting at the control structures (~70%) (Table 2, Figs S2 and S3). Keefer[43] tested five different active ingredients as liquid perimeter treatments against *B. patagonicus* and found variability in efficacy across these products. The mean percentage reduction in the number of foraging ants across the top-performing insecticides was between 61.6% and 84.3% at 30 days. Here, the use of bait stations alone showed a similar reduction in the number of foraging ants (70.8%) after 28 days. While highly effective, none of these products were able to provide complete control of dark rover ant infestations, and thus we propose and discuss some of the biological reasons that could be contributing to the difficulty in controlling this species.

Providing sufficient evidence for colony elimination in field experiments is challenging as there is no consensus on what constitutes an appropriately rigorous test of elimination. We have chosen to use the presence/absence of a colony relative to the pre-treatment assessments as an indicator of colony elimination, although other possibilities exist (i.e. relocation) that could explain the reduction in the number of colonies observed. More detailed studies involving more thorough inspections over prolonged periods of time would be needed to determine if the reduction in the number of colonies at the treatment structures was truly due to total colony elimination or merely elimination

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from the structure. In our study, we carefully sampled ants from all trails within 3 m of the structure, so if colonies relocated they would have had to move at least 3 m away for us to miss them. Regardless, the presence of the bait stations clearly provides a level of control for these structures against *B. patagonicus*. While there was no reduction in the colony density at the control structures, many new colonies were identified after 28 days (Fig. 2(a)). These were either missed during the initial collection or outcompeted and replaced an existing colony. This replacement was also observed at the treatment structures, although not enough to counteract the effect of the treatment (Fig. 2(b)). Determining the rate at which these colonies can be replenished once eliminated will be important in understanding the overall effectiveness of using baits against this species. Additionally, the high rate of colony turnover could be contributing to the difficulty in controlling dark rover ant infestations.

Laboratory evidence suggests that several common active ingredients have reduced efficacy against dark rover ants.[44,55] However, baits containing imidacloprid are highly effective against this species,[55] thus the colonies that persisted around the treatment structures may not have acquired the bait. Many of the rover ant colonies were found in the same general area after 28 days (Fig. 3), and this would indicate that their foraging range is quite small and very consistent. Some colonies' reluctance to venture far from their established foraging area could have contributed to their persistence through the treatment. In our study, only three colonies with foraging trails <1.5 m from the bait station were not eliminated (located 0.5, 1.0, and 1.0 m from the bait). The high consistency of trail foraging over time and short range suggests that baits should be applied directly on the trail to ensure feeding by the targeted colony. Additionally, the only way for numerous colonies to cohabitate in such a small area is if there are abundant resources to sustain them. These alternative food sources could have also reduced the effectiveness of these bait stations.

To date, much of invasive ant control has been focused on highly dominant and destructive ant species, which mainly exhibit reduced intraspecific aggression and supercolonial populations.[29,56,57] In these species, aggression is lost toward nonnestmates and individual nests can contain numerous queens.[56,58,59] This leads to the collapse of colony boundaries and populations consist of a network of interconnected nests exchanging brood, workers, and resources. This network of genetically similar nests is called a supercolony and may cover up to thousands of kilometers.[23] In these instances, a reduction in the overall number of workers is preferable as the total elimination of the whole supercolony would be nearly impossible. When baiting for supercolonial species, the number of bait stations required to achieve significant control can be directly proportional to the density of foraging ants,[14] although this can vary depending on foraging strategy and nest density.[60,61] The introduced population of *B. patagonicus* contrasts with these commonly treated ant species,[56,58,62] as it maintains colony boundaries.[46] Consequently, a high number of distinct colonies can infest a single structure. In this case, it appears that the number of bait stations required for higher rates of control is proportional to the colony density rather than overall forager activity.

Due to the unicolonial structure observed in most treated species, using microsatellite markers to test for colony elimination has not been readily applied in ants as it would not provide useful information regarding the fate of colonies. Yet, like *B. patagonicus*, several pest ant species are not supercolonial, and exhibit multicolonial populations.[63–66] For these species, the assessment of colony density could therefore be useful in determining the overall success of a treatment.[67] In *B. patagonicus*, most colonies forage using a single trail[46]; however, other multicolonial ant species are likely to exhibit different foraging strategies and different colony densities. These factors would affect the overall efficacy of a treatment. Investigating how different ants compete across the landscape as well as the extent of the foraging range of each species will surely enhance specific baiting strategies for the various pest species.

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CONFLICT OF INTEREST

Bayer Environmental Sciences had no role in the collection, analyses, or interpretation of data; in the writing of the original manuscript; or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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