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Evaluating control methods for red imported fire ant and their effects on hibiscus mealybug in citrus

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Abstract

The red imported fire ant (*Solenopsis invicta*) may disrupt biological control of hibiscus mealybug (*Nipaecoccus viridis*) in Florida citrus. Controlling *S. invicta* may therefore be necessary for effective management of hibiscus mealybug. We evaluated four different methods to control *S. invicta* (ground applications of insecticide, two different insecticidal ant baits and spot-treating ant colonies with hot water) to determine how these different treatments affected ants tending to hibiscus mealybug colonies, natural enemy abundance within hibiscus mealybug colonies and hibiscus mealybug colony abundance. While all treatments reduced the number of *S. invicta* colonies in treated areas compared to an untreated control, only ground applications of insecticide and the two ant baits significantly reduced the abundance of *S. invicta* found tending hibiscus mealybug colonies. Additionally, significantly more *Brachymyrmex obscurior* ants were found tending mealybug colonies in plots treated with either of the two ant baits. There was no significant effect of treatment on the abundance of natural enemies in hibiscus mealybug colonies, but significantly fewer natural enemies were found in mealybug colonies tended by *S. invicta*, and significantly more natural enemies were found in mealybug colonies tended by *B. obscurior*. Ground applications of insecticide, the abamectin ant bait Clinch and hot water treatments all reduced the overall number of hibiscus mealybug colonies compared with control areas, while the S-methoprene ant bait Extinguish did not. Our results suggest that reducing the number of *S. invicta* tending hibiscus mealybug colonies can lead to higher natural enemy abundance and lower hibiscus mealybug abundance. However, methods used to control *S. invicta* may have different impacts on ant species like *B. obscurior*, leading to different outcomes with natural enemies.

KEYWORDS
ant-hemiptera interactions, *Brachymyrmex obscurior*, integrated pest management, mutualism, predators, Pseudococcidae

1 | INTRODUCTION

Hibiscus mealybug (*Nippaecoccus viridis* Newstead) is an emerging and serious pest of citrus in Florida capable of damaging citrus flowers, fruit and branches (Cilliers & Bedford, 1978; Diepenbrock & Ahmed, 2020). In other citrus-growing regions of the world, hibiscus mealybug is usually a minor or incidental pest that is normally kept in check by predators and parasitoids (Cilliers & Bedford, 1978; Sharaf & Meyerdirk, 1987). When outbreaks have occurred, it is often because the use of broad-spectrum insecticides reduced populations of natural enemies, which allowed hibiscus mealybug populations to grow (Cilliers & Bedford, 1978; Franco et al., 2004). In Florida, the use of insecticides to curtail damage caused by Asian citrus psyllid (*Diaphorina citri* Kuwayama) and the citrus greening disease it vectors has reduced the abundance of many natural enemies in citrus groves (Monzo et al., 2014; Qureshi & Stansly, 2007). This release from predation and parasitism has likely played a role in allowing hibiscus mealybug to become a serious pest in Florida citrus. Supporting natural enemies and creating the correct conditions for them to survive is therefore critical to develop integrated pest management of hibiscus mealybug in citrus.

Mealybugs are frequently tended to and protected from natural enemies by various ant species. Examples of this include *Crematogaster peringueyi* Emery and *Linepithema humile* Mayr (the Argentine ant) protecting vine mealybug (*Planococcus ficus* Signoret) from parasitoids (Mgocheki & Addison, 2009) and *L. humile* reducing parasitoid abundance in obscure mealybug (*Pseudococcus viburni* Signoret) colonies (Daane et al., 2007). For hibiscus mealybug, the presence of *Technomyrmex albipes* Smith lowered parasitism of 1st instar mealybugs on tamarind from ~100% to ~2% (Nechols & Seibert, 1985). Overall, ants tending honeydew-producing hemipterans such as mealybugs frequently reduce the abundance and species richness of predators (Styrsky & Eubanks, 2007).

In central Florida, the red imported fire ant (*Solenopsis invicta* Buren) may play an important role in protecting hibiscus mealybug from natural enemies in citrus groves. Several mutually beneficial associations between honeydew-producing mealybugs and *S. invicta* have been documented (Helms & Vinson, 2003; Zhou et al., 2012), and *S. invicta* has been linked to an increased abundance of other mealybug species (Helms & Vinson, 2002). *Solenopsis invicta* is common in highly disturbed areas (King & Tschinkel, 2008; Steele et al., 2020) such as citrus groves (Deyrup, 2017; Tschinkel, 2006) and has anecdotally been observed to be one of the primary ant species tending and foraging on hibiscus mealybug in Florida citrus (Diepenbrock, personal observation). *Solenopsis invicta* can prevent predation by natural enemies (Zhou et al., 2012), has been linked with decreases in the abundance of spiders, lacewings and *Orius* spp. in cotton (Diaz et al., 2003), and may have a similar impact in a number of other cropping systems including citrus (Tschinkel, 2006). Even in the absence of broad-spectrum insecticides disrupting natural enemy communities, the presence of *S. invicta* may prevent natural enemies from effectively controlling hibiscus mealybug. Properly managing *S. invicta* in citrus groves may be necessary to allow for effective biological control of hibiscus mealybug.

However, the impact that *S. invicta* has on hibiscus mealybug populations or on natural enemies of hibiscus mealybug remains unknown. Additionally, there are numerous methods for managing fire ants in citrus groves, ranging from soil applications of insecticides and commercially available insecticidal baits (Diepenbrock et al., 2020) to spot-treating individual colonies with hot water (Tschinkel & King, 2007). While each has limitations and strengths, it is unclear which method will result in the greatest impact on hibiscus mealybug abundance or on their natural enemies.

To determine how controlling *S. invicta* in citrus impacts hibiscus mealybug and its natural enemies, we asked the following questions:

1. How do ant treatments designed to control *S. invicta* impact ants tending hibiscus mealybug colonies?
2. What effect do ant treatments have on natural enemies preying on hibiscus mealybug colonies?
3. How do ant treatments impact the abundance of hibiscus mealybug colonies?
4. What interactions exist between the presence of ants and the abundance of natural enemies?

We hypothesized that:

1. Ant treatments would reduce the number of all ants tending hibiscus mealybug colonies.
2. Treatments that reduced the number of ants would lead to greater numbers of natural enemies preying on hibiscus mealybug.
3. Treatments that reduced the number of ants would result in fewer hibiscus mealybug colonies.
4. The presence of ants, especially *S. invicta*, would correspond with decreased natural enemy abundance.

2 | MATERIALS AND METHODS

2.1 | Location and experimental design

Experiments were conducted in a 26-acre commercial Valencia orange grove in Lake Wales Florida USA (27°50′34.55″N, 81°34′29.99″W). Plots were laid out in a Randomized Complete Block Design, with five treatments and six blocks. Individual treatments consisted of ~¼ acre plots (~40 m long × ~30 m wide) and were approximately 10 trees long and four rows wide. A gap of two trees (~7 m) was left between treatments in each block, and all blocks were separated by one row of trees. To avoid edge effects that could allow for increased fire ant abundance (proximity to roads, etc.; Forys et al., 2002) all plots were placed at least 7 m away from roads and field margins.

2.2 | Treatments

Five treatments were assessed: (1) An untreated control; (2) Ground applications of insecticide consisting of chlorpyrifos and later, bifenthrin at a rate of 1 qt/acre for chlorpyrifos and ~22.5 oz/acre for Bifenthrin; (3) An abamectin ant bait (Clinch) at a rate of 1 lb/acre; (4) An S-methoprene ant bait (Extinguish) at a rate of 1 lb/acre; and (5) Hot water applications to individual ant mounds. The ground applications of insecticide were representative of a common method of ant control used among Florida growers (Diepenbrock, personal observation), while the ant baits are more targeted methods of ant control and are commercially labelled for use in citrus (Diepenbrock et al., 2020).

Hot water treatments represent a novel method of ant control in citrus. Hot water treatments were carried out using a hot water machine and application method that delivers hot water (~87–95°C) belowground into each fire ant colony within the treatment plots under low pressure at a rate of ~19 L/min (King, 2020). Chlorpyrifos was applied with a boom sprayer (Workhorse Sprayers 25 Gallon Deluxe Spot Sprayer, LG25DSS, Tractor Supply Co.) and consisted of two 30-s passes over treated plots. Due to the impending loss of chlorpyrifos tolerance in citrus, we halted use of chlorpyrifos to ensure the cooperator could sell all harvested fruit and switched to bifenthrin applications in the summer of 2021. Bifenthrin was applied with the same sprayer, in a single 30-s pass. Both ant baits were applied with hand crank fertilizer dispensers (Scotts Whirl Hand-Powered Spreader, The Scotts Company LLC) at approximately 455 g (1 lb) per 0.4 hectare (1 acre) (Barr, 2005). Applications were carried out by walking parallel lines through plots while simultaneously spreading bait, thus ensuring relatively even coverage throughout plots.

All treatments were initially applied in late February 2021 (Hot water across February 23rd, 24th and 26th; baits on February 24th, and chlorpyrifos on February 26th). Hot water treatments were reapplied to any surviving colonies in late March 2021 (March 23rd, 25th and 26th), and again in late September 2021 (September 22nd and 23rd). Per label instructions, both insecticidal ant baits were reapplied every 3 months (On 10 May 2021, 23 September 2021 and 5 January 2022). Bifenthrin was applied to the same plots as Chlorpyrifos on 17 June 2021.

2.3 | Mealybug sampling

Hibiscus mealybug colonies were collected and destructively sampled to identify natural enemies on and within colonies. Sampling occurred every month when mealybug populations were high enough to reliably find colonies in the field, and took place on 24 May, 21 June, 19 July, 15 October and 9 November 2021. Colonies varied in size and in the number of mealybugs present, ranging from single ovisacs to groups of dozens of ovisacs. Five colonies per treatment per block were collected, for a total of 150 colonies per sampling event. Colonies were removed by excising the fruit, leaves or twigs that mealybug colonies were feeding on, were placed into plastic bags and were brought back to the laboratory to be dissected. Once in the laboratory, hibiscus mealybug colonies were carefully pulled apart with forceps under a dissecting microscope (OM2300S-V3 7X - 45X Zoom Stereo Microscope). The number of hibiscus mealybug ovisacs in the colony was recorded, and predator and parasitoid abundance quantified by counting and identifying all natural enemies present in and on the hibiscus mealybug colonies. For natural enemies that could not be identified from the larvae or pupae, adults were reared out for identification. Larval and pupal predators and parasitoids were placed into Petri dishes sealed with parafilm in a Percival growth chamber at 28°C for up to 3 weeks to rear into adults for identification. Neuropteran predators were identified using keys created by the Florida Department of Agriculture and Consumer Services (https://entnemdept.ufl.edu/creatures/beneficial/green_lacewings.htm and https://entnemdept.ufl.edu/creatures/beneficial/brown_lacewings.htm).

Parasitoids were sent to Dr Michael Gates at the National Museum of Natural History for identification, and lepidopterans were sent to Dr James Hayden at the Florida Department of Agriculture and Consumer Services—Division of Plant Inspection for identification. Dipterans were identified by Dr Andrew Young at the University of Guelph, and Dr Gil Felipe Gonçalves Miranda at the Canadian National Collection of Insects Arachnids and Nematodes.

The abundance of predators, parasitoids and overall natural enemies did not meet assumptions of normality or homogenous variance and were compared between treatments using Kruskal–Wallis tests and Dunn tests with a Benjamini and Hochberg adjustment. To account for differences in mealybug colony size and larger colonies possibly having more natural enemies per colony, the number of natural enemies, predators and parasitoids found in each mealybug colony were divided by the number of ovisacs in the colony. Separate Kruskal–Wallis tests were run to determine whether treatment impacted these ratios of natural enemies per ovisac. All analyses were conducted in R (R Core Team, 2022).

Additionally, the abundance of hibiscus mealybug colonies was quantified. Starting on 17 September 2021 and taking place every 4 weeks for a total of seven times, the number of living and dead hibiscus mealybug colonies were recorded in all different treatments. Hibiscus mealybug abundance was quantified by slowly walking 3 min transects along the row of trees, counting all visible hibiscus mealybug colonies. Colonies were defined as one or more ovisacs physically touching or within ~1 cm of each other. Colonies were considered alive if adult mealybugs were clearly visible, if ovisac wax was a bright white colour, and/or if purple haemolymph (Diepenbrock & Ahmed, 2020) was present when the colony was crushed by technicians. Colonies were considered dead if no adults were visible, ovisacs were broken apart and/or crumbly, and if no haemolymph was present if colonies were crushed. Each technician conducted three transects per treatment in a single block. The total number of transects varied by sampling date based on the number of technicians available (ranging from three to five). The number of living colonies, dead colonies and overall colony abundance were compared between treatments using Kruskal–Wallis tests and Dunn tests with a Benjamini and Hochberg adjustment, and all analyses were conducted in R (R Core Team, 2022).

2.4 | Ant sampling

To determine the effect of treatment on *S. invicta* and on ants overall, *S. invicta* colony abundance and the identity and abundance of ants tending to hibiscus mealybug colonies were quantified. Starting in February 2021, the number of living *S. invicta* colonies was recorded every 2 weeks through March 2022. *Solenopsis invicta* colony abundance was assessed by slowly walking through rows and counting all visible *S. invicta* colonies. Colonies were confirmed to be alive and active by slightly digging into the colony with a shoe or stick, thereby eliciting a worker response and exposing brood close to the surface. Separate mounds that were within ~2 m of each other were classified as the same colony. We opted to quantify *S. invicta* abundance by counting colonies as opposed to using cookie baits to capture foragers because counting colonies gave us a direct measure of the effect of treatments on colony survival. The effect of treatment on colony abundance was assessed with Kruskal–Wallis tests and Dunn tests to determine means separation.

The abundance and species identity of ants on mealybug colonies was also recorded. When mealybug colonies were destructively sampled for predators, any ants found tending or foraging on the colonies were collected. Ants on mealybug colonies were either aspirated off into a vial or were picked off the collected mealybug colony and surrounding plant matter with forceps back in the lab. Ants on mealybug colonies were counted to obtain abundance data and were identified to species level. The effect of treatment on the presence and abundance of tending/foraging ants was assessed with Kruskal–Wallis tests and Dunn tests to determine means separation. Additionally, Kruskal–Wallis tests were used to test the impact of the presence of any ant species, of just *S. invicta*, and of just *Brachymyrmex obscurior* Forel on predator abundance, parasitoid abundance, and overall natural enemy abundance within mealybug colonies.

3 | RESULTS

3.1 | *Solenopsis invicta* Colony abundance

There was a significant effect of treatment on the number of living *S. invicta* colonies ($X^2 = 301.93$, df = 4, $p < 0.001$). All four ant treatments (Hot water treatments, Chlorpyrifos/Bifenthrin treatments, Clinch and Extinguish) led to significantly fewer colonies compared with the control. Additionally, both Clinch and Extinguish resulted in significantly fewer colonies compared to hot water treatments and Chlorpyrifos/Bifenthrin treatments, and hot water treatments led to significantly fewer colonies than Chlorpyrifos/Bifenthrin treatments (Table 1).

3.2 | Ants on hibiscus mealybug colonies

Seven species of ants were found tending hibiscus mealybug colonies. Pooled across all sampling dates and treatments, a total of 443 *Solenopsis invicta*, 501 *Brachymyrmex obscurior*, three *Pseudomyrmex gracilis* Fabricius, five *Cardiocondyla emeryi* Forel, seven *Dorymyrmex bureni* Trager, two *Crematogaster ashmeadi* Mayr and two *Camponotus floridanus* Buckley were collected.

There was no significant overall effect of treatment on the total abundance of ants tending hibiscus mealybug colonies ($X^2 = 8.9661$, df = 4, $p = 0.06195$; Figure 1a) or on whether or not ants were present on mealybug colonies ($X^2 = 7.2374$, df = 4, $p = 0.1239$). However, there was a significant effect of treatment on *S. invicta* abundance ($X^2 = 17.805$, df = 4, $p = 0.001347$) and the presence of *S. invicta* ($X^2 = 17.023$, df = 4, $p = 0.001913$). Significantly more *S. invicta* were found on mealybug colonies in control plots than in Clinch, Extinguish and Chlorpyrifos/Bifenthrin plots (Figure 1b). There was also a significant effect of treatment on the abundance of *Brachymyrmex obscurior* ($X^2 = 16.865$, df = 4, $p = 0.002053$) and the presence of *B. obscurior* ($X^2 = 17.643$, df = 4, $p = 0.001449$). There were significantly more *B. obscurior* tending mealybugs in Clinch and Extinguish plots compared with control and Chlorpyrifos/Bifenthrin plots (Figure 1c).

3.3 | Hibiscus mealybug natural enemies

Several natural enemies were identified from hibiscus mealybug colonies. These consisted of *Fragosa* sp., *Anatrachyntis badia* Hodges, *Ceraeochrysa* sp., *Cryptolaemus montrouzieri* Mulsant and *Anagyrus dactylopii* Howard. (Table 2). *Fragosa* sp., *Anatrachyntis badia*, *Ceraeochrysa* sp. and *Cryptolaemus montrouzieri* are all predatory species, while *Anagyrus dactylopii* is a parasitoid. In many cases, most or all of the ovisacs comprising mealybug colonies that were collected had been consumed by predators or parasitoids or were in the process of being consumed. Additionally, we frequently found pupal casings of *Fragosa* sp., *Anatrachyntis badia* and *Anagyrus dactylopii* in mealybug colonies even when living natural enemies were not present. Overall, 24.5% of all collected mealybug colonies contained at least one predator, 20.9% contained parasitoids, and 36.9% contained natural enemies of some kind.

There was no effect of treatment on the overall abundance of natural enemies ($X^2 = 4.9865$, df = 4, $p = 0.2887$; Figure 2), nor was there an effect of treatment on the abundance of predators ($X^2 = 5.0862$, df = 4, $p = 0.2786$) or parasitoids ($X^2 = 3.8325$, df = 4, $p = 0.4291$). When accounting for the number of hibiscus mealybug ovisacs contained in each colony, there was still no effect of treatment on natural enemy abundance ($X^2 = 6.808$, df = 4, $p = 0.1464$), predator abundance ($X^2 = 5.562$, df = 4, $p = 0.2343$) or parasitoid abundance ($X^2 = 4.5403$, df = 4, $p = 0.3378$).

TABLE 1 Mean number of *Solenopsis invicta* colonies (±1 standard error) found in plots with different treatments.

Control	Chlorpyrifos/bifenthrin	Clinch	Extinguish	Hot water
26.9 ± 0.89 a[†]	19.98 ± 0.72 b	9.57 ± 0.43 d	10.07 ± 0.42 d	15.05 ± 0.64 c

[†]Different letters denote statistically significant differences between treatments (Dunn tests, $p < 0.05$).

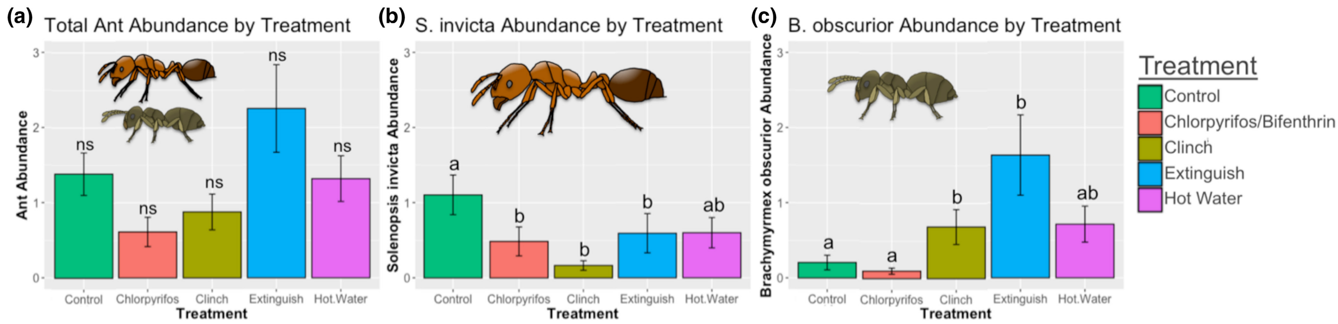

FIGURE 1 Average abundance (±1 SE) of (a) all ants; (b) *Solenopsis invicta*; and (c) *Brachymyrmex obscurior* found tending hibiscus mealybug colonies in plots with different ant treatments. Different letters denote statistically significant differences between treatments (Dunn tests $p<0.05$). 'ns' indicates no significant differences treatments.

TABLE 2 Abundance and identity of natural enemies found in field-collected hibiscus mealybug colonies, summed across all sampling dates and treatments.

Natural enemy	Lifestage	Abundance
Fragosa sp.	Larvae	93
	Pupae	55
	Pupal Casings	50
	Total	198
Anatrachyntis badia	Larvae	177
	Pupae	12
	Pupal Casings	13
	Total	202
Ceraeochrysa sp.	Larvae	16
Cryptolaemus montrouzieri	Larvae	2
Anagyrus dactylopii	Pupae	81
	Pupal Casings	98
	Adults	219
	Total	398
Total		816

3.4 | Hibiscus mealybug abundance

There was no significant effect of treatment on the number of living hibiscus mealybug colonies found among transects ($X^2 = 5.6705$, df = 4, $p = 0.2251$). However, there was a significant effect of treatment on the number of dead mealybug colonies ($X^2 = 17.854$, df = 4, $p = 0.001318$), and on the overall number of both living and dead colonies ($X^2 = 15.27$, df = 4, $p = 0.004173$). Plots treated with Clinch had significantly fewer dead colonies compared with the control plots and to plots treated with Extinguish. Plots treated with Clinch also had significantly fewer overall colonies compared to control plots, as did Chlorpyrifos/Bifenthrin and hot water plots (Table 3).

3.5 | Relationships between ants and natural enemies

There was no effect of the overall presence of ants on the abundance of natural enemies in mealybug colonies ($X^2 = 0.7195$, df = 1, $p = 0.3963$; Figure 3a) or the abundance of predators ($X^2 = 1.1902$, df = 1, $p = 0.2753$) or parasitoids ($X^2 = 0.82129$, df = 1, $p = 0.3648$). However, there was an effect of the presence of *S. invicta* on overall natural enemy abundance ($X^2 = 6.1673$, df = 1, $p = 0.01301$) and on the abundance of predators ($X^2 = 6.5456$, df = 1, $p = 0.01051$) with *S. invicta* resulting in fewer overall natural enemies and predators (Figure 3b). There was no effect of the presence of *S. invicta* on parasitoid abundance ($X^2 = 0.23246$, df = 1, $p = 0.6297$). When *B. obscurior* were present, mealybug colonies had significantly more overall natural enemies ($X^2 = 9.5683$, df = 1, $p = 0.00198$; Figure 3c), more predators ($X^2 = 10.664$, df = 1, $p = 0.001092$) and more parasitoids ($X^2 = 4.7397$, df = 1, $p = 0.02947$).

4 | DISCUSSION

4.1 | Effect of treatment on ants

While all of the treatments we tested resulted in significantly fewer *S. invicta* colonies compared with control plots, only Chlorpyrifos/Bifenthrin, Clinch and Extinguish also resulted in fewer *S. invicta* tending hibiscus mealybugs. Considering both Clinch and Extinguish led to significantly fewer *S. invicta* colonies compared to Chlorpyrifos/Bifenthrin and hot water treatments, it follows that both led to low *S. invicta* forager abundance as well. It is possible that *S. invicta* colony numbers must be heavily reduced before there will be a corresponding effect on workers tending mealybugs. While the exact foraging distance of *S. invicta* remains unclear (Dhami, 2008), they can reach food sources far from the central colony. This may have enabled *S. invicta* workers to still find and tend mealybugs in plots treated with hot water despite significant reductions in colony numbers. Additionally, hot water treatments may not have resulted in lower *S. invicta* forager abundance on mealybug colonies because the treatments specifically target brood and queens and leave numerous workers who are out foraging still alive. Similarly, Chlorpyrifos/Bifenthrin treatments may have reduced *S. invicta* forager abundance despite not reducing *S. invicta* colony abundance as much as the other treatments because applications of insecticides such as Bifenthrin are highly lethal to workers that they come into contact with (Wiltz et al., 2010) but may not have the same impact on colonies.

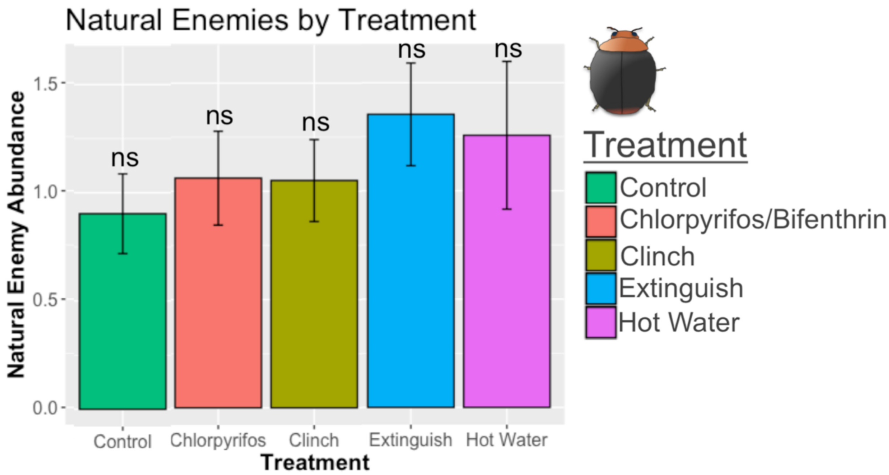

FIGURE 2 Mean number of natural enemies (±1 SE) found in field-collected hibiscus mealybug colonies from plots with different ant treatments. Different letters represent statistically significant differences between treatments (Dunn tests, $p < 0.05$). 'ns' indicates no significant differences treatments.

TABLE 3 Mean number of living, dead and both living and dead (overall) hibiscus mealybug ovisacs (±1 standard error) found during transects in plots with different ant treatments.

Mealybug colonies	Control	Chlorpyrifos/bifenthrin	Clinch	Extinguish	Hot water
Living	1.49 ±0.22 a[†]	1.00 ±0.15 a	0.77 ±0.13 a	1.26 ±0.24 a	0.91 ±0.17 a
Dead	1.30 ±0.17 a	0.86 ±0.14 ab	0.55 ±0.12 b	1.17 ±0.16 a	0.93 ±0.15 ab
Overall	2.80 ±0.28 a	1.86 ±0.23 b	1.32 ±0.17 b	2.43 ±0.31 ab	1.84 ±0.25 b

[†]Different letters denote statistically significant differences between treatments in the same row (Dunn tests, $p < 0.05$).

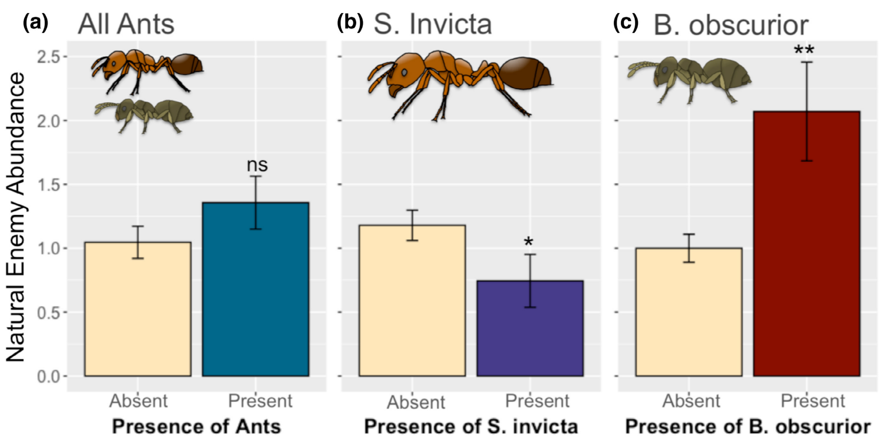

FIGURE 3 Average abundance (±1 SE) of natural enemies found in hibiscus mealybug colonies with and without ants present with (a) all ant species; (b) *Solenopsis invicta*; and (c) *Brachymyrmex obscurior*. Asterisks signify statistically significant differences between treatments (Kruskal–Wallis Tests, $*p < 0.05$, $**p < 0.01$). 'ns' indicates no significant difference treatments.

Contrary to our hypothesis, we did not see a reduction in the overall number of ants tending mealybugs in any of our treatments. Although we observed a significant decrease in *S. invicta* in Clinch, Extinguish and Chlorpyrifos/Bifenthrin plots, we saw a significant increase in *B. obscurior* worker abundance on mealybug colonies in Clinch and Extinguish plots compared with control plots. This opposite effect of treatment was unexpected and resulted in an overall unchanged number of ants across all treatments. Our results suggest *B. obscurior* is not attracted to either Clinch or Extinguish, as both had a strong negative impact on *S. invicta* colonies and workers. It is also possible *B. obscurior* experienced competitive release when *S. invicta* numbers were reduced, thereby leading to an increase in the number of *B. obscurior* foragers. More direct study of *B. obscurior*, their preference for ant baits, and how different treatments affect the number of *B. obscurior* colonies could help better explain our data.

4.2 | Ants and natural enemies

Beyond an opposing impact of treatment on *S. invicta* and *B. obscurior* abundance, there was also an opposing impact of the two ant species on the number of natural enemies in hibiscus mealybug colonies. Natural enemy abundance was negatively correlated with the presence of *S. invicta*, while it was positively correlated with the presence of *B. obscurior*.

We expected to find a lower abundance of natural enemies when *S. invicta* was present. *Solenopsis invicta* has been documented actively protecting and gathering significant honeydew resources from colonies of invasive *Antonia graminis* Maskell mealybugs in Texas (Helms & Vinson, 2002), and it follows *S. invicta* may similarly protect and utilize other invasive mealybugs such as hibiscus mealybug. In our study, *S. invicta* reduced predator abundance, while not affecting parasitoid abundance. *Solenopsis invicta* has previously been linked to lower abundance of some predators in cotton including lacewings (Diaz et al., 2003) which were predators we found in our study, and controlling ants is a recognized method for promoting biological control of mealybugs in citrus (Franco et al., 2004). However, *S. invicta* was previously also found to decrease parasitism in brown citrus aphid (*Toxoptera citricida* Kirkaldy) in Florida citrus by removing parasitized individuals (Persad & Hoy, 2004), unlike our results. While there is relatively little literature on *S. invicta* interactions with mealybugs, what does exist indicates that *S. invicta* reduces natural enemy abundance, especially of certain predators.

Unexpectedly, we observed an increase in natural enemy abundance when *B. obscurior* was present. It is possible *B. obscurior* actively supported hibiscus mealybug colonies, thereby making them more appealing to predators and parasitoids. In a previous study, *Iridomyrmex rufoniger* Lowne ants aided black scale (*Saissetia oleae* Olivier) populations on citrus by removing honeydew that would otherwise have smothered them, without impacting natural enemies (Dao et al., 2014). A similar mechanism, whereby hibiscus mealybug is supported and possibly made more attractive to natural enemies, may have occurred with *B. obscurior*. Future work could help determine why *B. obscurior* appears to promote both predators and parasitoids in hibiscus mealybug. Whatever the reason, there are important implications for ant management. Managing specific ants such as *S. invicta* is more important than excluding all ant species and having some ant species such as *B. obscurior* present may actually be beneficial for biological control of hibiscus mealybug.

4.3 | Impact of ants and natural enemies on hibiscus mealybug abundance

Unfortunately, we were unable to directly correlate mealybug abundance with natural enemies or ants in our data. By the time we started counting mealybug colonies, hibiscus mealybug abundance had dropped low enough where it was not feasible to gather colonies to check for natural enemies or ants. So while we can directly say what impact different treatments had on ant abundance and presence, natural enemy abundance and mealybugs colony abundance, we have to infer from our data the impact of ants and natural enemies on mealybugs. However, our results are highly suggestive that there are important interactions between ant presence and hibiscus mealybug abundance.

Significantly fewer overall hibiscus mealybug colonies were found in plots treated with Clinch, Chlorpyrifos/Bifenthrin and hot water compared to control plots, with Clinch plots containing the fewest colonies. These results support our hypothesis that ant treatments that reduced *S. invicta* numbers would lead to fewer mealybugs, especially in the case of Clinch. However, Extinguish plots did not have fewer mealybugs, which we would have expected considering both *S. invicta* colony abundance and worker abundance on mealybug colonies were significantly reduced in Extinguish plots. As previously stated, a possible explanation is that *B. obscurior*, which was most abundant in Extinguish plots, positively benefits hibiscus mealybug while also benefiting natural enemies. However, this is currently speculative and primarily comes from post hoc reasoning.

4.4 | Future directions and implications for pest management

Our hypotheses followed a particular chain of logic: ant treatments would reduce *S. invicta* abundance and activity, which would in turn increase natural enemy abundance and activity, thereby decreasing hibiscus mealybug numbers. Our results indicate these hypotheses appear to have been mostly supported. Ant treatments did reduce *S. invicta* colony abundance, and three of the treatments (Clinch, Extinguish, and Chlorpyrifos/Bifenthrin) also reduced *S. invicta* worker abundance on hibiscus mealybug colonies. While there was no effect of treatment on natural enemy abundance, natural enemy abundance was significantly greater if *S. invicta* was absent. Finally, most of our ant treatments reduced the number of hibiscus mealybug colonies present. However, our results from Extinguish plots do not fit our hypotheses, and the effect appears to be driven largely by the presence of *B. obscurior*. Further research more directly assessing the links between *S. invicta*, *B. obscurior*, natural enemies and hibiscus mealybug could help explain some of our unexpected results and could inform best management practices for hibiscus mealybug.

As a practical matter, indirect management of hibiscus mealybug by directly managing ants may require growers to commit to one or more ant management strategies. Our results suggest that broadcast bait applications for area-wide (grove-scale) management will likely be the most effective, however, any ant management programme will entail costs that may be limiting. It is also important to note that the ant management strategies employed in this study are not mutually exclusive and combined treatment approaches (e.g. hot water and baiting) may provide sufficient controls while limiting inputs of pesticide needed for ant control. Future work aimed at understanding the cost/benefit ratio of individual and combined ant management methods in citrus and the full effect that these treatments have on *S. invicta* and other ant species over time is an important next step.

AUTHOR CONTRIBUTIONS

Eric G. Middleton: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Joshua R. King:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – review and editing. **Abigail Johnson:** Data curation; investigation; resources; writing – review and editing. **Lauren M. Diepenbrock:**

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CONFLICT OF INTEREST STATEMENT

The author Joshua R. King holds a patent on the device used to apply hot water treatments in this study.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the current study are available via Dryad at https://datadryad.org/stash/share/bDLjBeA0QCENhUzQmUHXWjgryE2RychFh9dfX2IM7IQ.

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