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Response of *Wasmannia auropunctata* (Hymenoptera: Formicidae) to water-soaked imported fire ant baits

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Abstract

The little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae), is a stinging invasive ant that can establish extremely large, dominating populations in tropical and subtropical regions. In Florida, it is well established in south and central Florida with a reported northern limit of Marion County, Florida, USA. However, in 2018 to 2019 overwintering populations were discovered farther north in Gainesville, Florida, USA. There is a need to develop effective management options suitable for the site uses of these recently discovered infestations. Most commercial imported fire ant baits are formulated on a corn grit carrier that, when exposed to moisture, is thought to compromise bait effectiveness. Due to the humid and rainy summer weather in this region, the objective of this study was to determine the acceptance and efficacy of water-soaked ant baits on *W. auropunctata*, some of which had purported moisture resistance. Bait acceptance tests conducted in the field with dry baits determined that baits containing the active ingredients spinosad and pyriproxyfen were accepted poorly, while *W. auropunctata* accepted both dry and wet baits containing hydramethylnon, metaflumizone, indoxacarb, and abamectin. Laboratory colonies given access to either dry or wet baits exhibited significant declines in workers, brood, and queens with several colonies being eliminated. The results of this study indicated that water-soaked imported fire ant baits could control *W. auropunctata*, and moisture exposure did not cause baits to become unpalatable. However, these results should be validated under field conditions, where precipitation may reduce the accessibility of baits to foraging ants.

Key Words: electric ant; control; treatment; water resistant; invasive ant

Resumen

La pequeña hormiga de fuego, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae), es una hormiga invasora urticante que puede establecer poblaciones dominantes extremadamente grandes en regiones tropicales y subtropicales. En Florida, está bien establecida en el sur y el centro de la Florida con un límite norte hasta el condado de Marion, Florida, EE. UU. Sin embargo, entre el 2018 y 2019 se descubrieron poblaciones que hibernan más al norte, en Gainesville, Florida. Existe la necesidad de desarrollar opciones de manejo efectivas adecuadas para los usos del sitio de estas infestaciones descubiertas recientemente. La mayoría de los cebos comerciales importados para hormigas de fuego están formulados con grano de maíz como portador que cuando se expone a la humedad, se cree que compromete la eficacia del cebo. Debido al clima húmedo y lluvioso del verano en esta región, el objetivo de este estudio fue determinar la aceptación y eficacia de los cebos para hormigas empapados en agua sobre *W. auropunctata*, algunos de los cuales tenían supuesta resistencia a la humedad. Las pruebas de aceptación de cebos realizadas en el campo con cebos secos determinaron que los cebos que contenían los ingredientes activos spinosad y pyriproxyfen fueron mal aceptados, mientras que *W. auropunctata* aceptó cebos secos y húmedos que contenían hidrametilnon, metaflumizona, indoxacarb y abamectina. Las colonias de laboratorio a las que se les dio acceso a cebos secos o húmedos exhibieron disminuciones significativas de obreras, crías y reinas y con varias eliminadas. Los resultados de este estudio indicaron que los cebos para hormigas de fuego importados empapados en agua podrían controlar *W. auropunctata*, y la exposición a la humedad no hizo que los cebos se volvieran desagradables. Sin embargo, estos resultados deben validarse en condiciones de campo, donde la precipitación puede reducir la accesibilidad de los cebos para las hormigas en busca de alimento.

Palabras Claves: hormiga eléctrica; control; tratamiento; resistente al agua; hormiga invasora

The little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae), is a stinging, invasive ant that can establish extremely large, dominating populations especially in agricultural and forestry lands in regions outside its native range of South America. Established non-native infestations are found in tropical and subtropical regions (Wetterer & Porter 2003; Wetterer 2013). In the US, *W. auropunctata* is well established on the island of Hawaii (Conant & Hirayama 2000), the US territory of Guam (Raymundo & Miller 2012), and in central and south Florida. In Florida, *W. auropunctata* initially was observed in south Florida (i.e., Miami) in 1924 (Smith 1929; Spencer 1941). At that time, their establishment in north Florida was considered to be unlikely due to the cold winters (unless protected by buildings), with their northern presence reported as far north as Marion County, Florida, USA (Deyrup et al. 2000; Deyrup 2016). In 2018 to 2019, we observed 3 overwintering populations of *W. auropunctata* north of Marion County, in Gainesville, Alachua County,, Florida, that currently persist. At 1 of the infested sites, an urban farm and garden, numerous incidences of stings from these ants were reported (DML personal observation). Spencer (1941) has reported more severe problems with stinging *W. auropunctata* curtailing citrus fruit harvesting in Florida.

Certain commercial red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), baits can suppress or even eradicate

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W. auropunctata populations or activity (Causton et al. 2005; Vanderwoude et al. 2010; Lach & Barker 2013). These baits consist of a toxicant, a phagostimulant (typically soybean oil) which also serves as the solvent for the toxicant, and a defatted corn-grit oil-absorbent carrier (Williams et al. 2001). However, control of *W. auropunctata* is thought to be impeded in tropical climates where frequent precipitation can degrade standard corn grit and other bait carriers (Souza et al. 2008; Hara et al. 2014). Evaluations of ant baits for control of *W. auropunctata* under high moisture conditions have been conducted in small laboratory colonies, where imported fire ant bait containing metaflumizone that was exposed to rainfall and intermittent sunshine for 7 and 14 d, had > 90% worker, and 80% and 63% queen mortality for each time period, respectively (Hara et al. 2011). Other imported fire ant bait formulations exposed to sunlight and irrigation had significantly less *W. auropunctata* foraging activity on the baits than on fresh bait (Hara et al. 2014).

With the discovery, to our knowledge, of the most northern established populations of *W. auropunctata* in Florida, coupled with the typically humid and rainy summer weather in this region, there is a need to further examine commercial ant baits exposed to moisture for their effectiveness to control *W. auropunctata*. The objective of this study was to determine the acceptance and the efficacy of ant baits, primarily targeted at imported fire ants, to *W. auropunctata*. These baits contained various active ingredients and carriers, some with purported moisture resistance.

Materials and Methods

BAIT ACCEPTANCE TESTS

Wasmannia auropunctata acceptance of various commercially available ant baits was determined in the field at a site located on a small urban farm and garden (29.6447101°N, 82.3625787°W) on the University of Florida campus in Gainesville, Florida, USA. The tested baits listed imported fire ants or other ants on their product labels. Granular bait formulations were selected because they relatively are easy to apply because they do not require premixing before broadcasting over infested landscapes or can be scattered directly around nesting sites. Some baits contained active ingredients that have been used in *W. auropunctata* eradication projects (Causton et al. 2005; Vanderwoude et al. 2010), or the baits had published or purported claims of resisting degradation from moisture such as dew or precipitation (Kafle et al. 2010) (Table 1).

Two tests initially assessed dry baits to determine acceptance by *W. auropunctata* without the potential confounding effect of moisture degradation. Each bait (0.5 g) was placed on individual polystyrene weigh dishes (25 mm base diam), which were cut in half to allow 1 side to be level to the ground, thus permitting easier ant access to the bait. Baits were spaced a minimum of 30 cm apart to minimize bait competition and were positioned in areas where *W. auropunctata* were seen foraging or congregated under harborage such as rocks. The number of *W. auropunctata* on the baits were counted at 15 min intervals for 1 h. If ants were difficult to count due to high numbers, digital photos were taken, and counts were obtained from a magnified image. The number of ants on each bait was summed over the 4 time intervals. If there was a minimal number of ants on a bait after 1 h, peanut butter, a highly accepted lure of *W. auropunctata* (Williams & Whelan 1992), was added to the weighing dish to confirm ants were foraging in the area. The following baits (Table 1) were used in test 1: Amdro®, Maxforce® Complete, Esteem®, and Siesta™. In test 2, Advance®, Advion®, Erasant-Hydro, Payback, and Seduce were evaluated. There were 4 replicates of each bait with 1 to 3 replicates tested per d in Aug 2018. A separate assessment of Clinch® Ant Bait acceptance was conducted in Sep 2018 using the methods stated above.

The acceptance of wet baits by *W. auropunctata* was determined by following the methods described for dry baits with several modifications. Baits were wetted by soaking 0.5 g of each bait in water (15 mL) for 30 min within 50 mL polypropylene centrifuge tubes, then allowing the water to drain (Kafle et al. 2010). Within 30 min of draining the soaked baits, each wet bait was scraped onto individual weigh dishes that were placed on the ground at approximately 4.6 m intervals in heavily infested areas within the test site. The wide spacing between baits was to prevent competition among the baits. Based on the dry bait acceptance, product labeling that listed imported fire ants, *W. auropunctata*, or use sites that listed food crops, the following were baits were selected for the wet bait evaluation: Advion, Amdro Pro, Clinch, Siesta, and a control (20% [w/w] once-refined soybean oil on a carrier of pregel defatted corn grit) (Balsley & Marei 1982). Other baits that were tested included Amdro, despite not being registered for food crops, because of its common retail availability, and Erasant-Hydro because it was specifically developed for water resistance. Lastly, dry Amdro Pro was included as a standard because its label includes *W. auropunctata*. There were 4 replicates of each bait with a single replicate tested on separate d in Apr 2019.

The summed ant counts per bait were compared by analysis of variance and Tukey's HSD test (PROC ANOVA, SAS version 9.4, SAS Institute, Cary, North Carolina, USA). The logarithmic transformation

Table 1. Ant baits used in the bait acceptance or colony efficacy tests. Information from product labels dated from 2013 to 2018 or accessed online in 2020.

Bait product (manufacturer)	Active ingredient	Pertinent ants on product label	Moisture guidance on label
Advance® granular carpenter ant bait (BASF)	0.011% abamectin	Fire ants	No guidance
Advion® fire ant bait (Syngenta)	0.045% indoxacarb	Imported fire ants, *Solenopsis* spp.	Avoid moisture
Amdro® fire ant bait (Ambrands)	0.73% hydramethylnon	Imported fire ants	Avoid moisture
Amdro® pro fire ant bait (BASF)	0.73% hydramethylnon	Imported fire ants, little fire ants	Avoid rain
Clinch® ant bait (Syngenta)	0.011% abamectin	Fire ants	Avoid moisture
Maxforce® complete granular insect bait (Bayer)	1.0% hydramethylnon	Imported fire ants	No guidance
Erasant-Hydro hydrophobic fire ant bait (Chung Hsi Chemical))	0.9% hydramethylnon[a]	Fire ant[a]	Moisture resistant[a]
Esteem® ant bait (Valent)	0.05% pyriproxyfen	Imported fire ant, red imported fire ant	Avoid rain
Payback fire ant bait (Southern Agricultural)	0.015% spinosad	Fire ants	Avoid moisture
Seduce insect bait (Certis)	0.07% spinosad	Ants (excluding fire ants)	Moisture resistant[b]
Siesta™ insecticide fire ant bait (BASF)	0.063% metaflumizone	Imported fire ants	Avoid moisture

[a]Information from safety data sheet; product not registered in the USA.
[b]Advertised as "The weatherproof pellets resist degradation by rain and UV" (2013).

($\log_{10}$ [x + 1]) was applied to the ant counts to obtain homogeneous variances. Analyses were conducted separately on each of the dry and wet bait tests.

BAIT EFFICACY ON COLONIES

Dry Bait Colony Test

Dry baits that were foraged upon by significant numbers of *W. auropunctata* in the acceptance tests, labeled for imported fire ants or fire ants, or had use sites with various food crops, were examined further to determine their efficacy for control of *W. auropunctata* colonies under laboratory conditions. Amdro also was evaluated as a standard; however, it is not labeled for food crops. Colonies were collected from the University of Florida site and from Winter Park, Orange County, Florida, USA. Before testing, colonies were maintained in the laboratory for 1 to 3 mo on a diet of frozen crickets (*Acheta domesticus* [L.]; Orthoptera: Gryllidae) and live house fly (*Musca domestica* L.; Diptera: Muscidae) larvae, 10% (w/v) sucrose solution, and water. Colonies were provided a nesting cell adapted from Williams (1990), where dental plaster (Castone®, Dentsply International, York, Pennsylvania, USA) was poured into the bottom of circular or square polystyrene Petri dishes (100 mm diam or 100 mm L × 100 mm W and both 15 mm H), and a depression formed by pressing a smaller circular Petri dish into the hardening plaster. The hardened plaster was moistened with water to increase humidity within the cell. Four access holes were melted through the sides of the dish and cover. The cell was darkened with red acetate over the Petri dish cover, or by covering the cell with a black, polypropylene plastic food container (17 cm L × 12 cm W × 6 cm H).

Baits were assigned to colonies according to a randomized complete block design with blocking based on the number of workers and queens per colony. Blocks were replicated 5 times. The following baits were used in the test: Advion, Amdro, Clinch, Siesta, and the control of soybean oil on the corn grit carrier (Table 1). To encourage bait feeding, food, but not water, was removed from the colonies for 1, 4, or 6 d before placing weighing dishes with 0.5 g of bait into plastic storage boxes (35.6 cm L × 20.3 cm W × 12.4 cm H) that contained the individual colonies. The interior sides of the boxes were coated with fluon (PTFE D-210, Daikin America, Inc., Orangeburg, New York, USA) to prevent ants from escaping. Baits were not removed for the duration of the study; however, 2 d after baits were provided, colonies were provisioned with the laboratory diet and colonies were fed 2 to 3 times per wk thereafter.

To determine bait efficacy, the number of living workers and queens were estimated by counting ants directly or from a magnified, digital image. Initially, test colonies contained a mean (min–max) of 1,740 (700–3,000) workers and 8 (1–23) queens. In this test, the presence or absence of brood was recorded. Brood was present in all colonies at the start of the study. Counts of the workers and queens, and determination of brood presence were conducted prior to bait introductions, 3 or 4 d (0.5 wk) later, and at weekly intervals from the initial bait introductions for 4 wk. Analysis of variance (ANOVA) and Tukey's HSD test were used to compare the reduction percentages in workers and the number of live queens among the bait treatments at the end of the test (wk 4). The general linear model procedure (SAS version 9.4, SAS Institute, Cary, North Carolina, USA) was used because the ANOVA was unbalanced due to unequal replication.

Wet Bait Colony Test

The efficacy of wet baits was determined on laboratory colonies of *W. auropunctata* that were collected from the University of Florida site and were maintained as described in the dry bait test. Advion, Amdro Pro, Clinch, Siesta, Erasant-Hydro, and control baits were soaked in water following the method used in the bait acceptance test. Erasant-Hydro was added to the test because it is a commercial red imported fire ant bait available in Taiwan that uses a hydrophobic carrier (Shih & Kafle 2013). The test used a randomized complete block design to assign bait treatments. Blocking was on colony size, which was based on the number of workers and queens as well as brood volume. Brood volume (eggs, larvae, and pupae combined) was estimated by visually comparing known volumes of brood to that in the nest cells. Colony sizes were determined before baits were provided to colonies (week 0), then at 3 d (0.4 wk), 1 wk, 2 wk, 3 wk, 4 wk, 10 wk, and 13 wk after initial access to the baits. Colonies initially contained means (min–max) of 2,580 (1,800–3,500) workers, 0.52 mL (0.2–1.5) of brood, and 9.5 (4–22) queens. Blocks were replicated 4 times. Colonies were starved for 3 d before baits (0.5 g each prior to soaking) were provided to the colonies. Baits were replaced with the laboratory diet after 24 h to emulate the potential for limited bait access under field conditions. Subsequently, colonies were maintained on the laboratory diet for the duration of the study.

Bait efficacy was determined by comparing the reduction percentages from the initial number of workers and the initial brood volume per colony, and the number of living queens per colony. Analysis of variance and Tukey's HSD test were used to evaluate the reduction percentages in workers and the number of living queens among the bait treatments. Reduction percentages in brood were compared using the non-parametric Friedman test and Tukey's HSD on ranked data because of high heterogeneity among variances. Analyses were conducted on data obtained at the end of the test (13 wk) to allow treated colonies to recover if queens had survived.

Results

BAIT ACCEPTANCE TESTS

Most of the dry baits were foraged upon by *W. auropunctata* in the field acceptance tests. However, significantly ($P \leq 0.05$) fewer ants were counted on Esteem than Amdro, Complete, and Siesta in test 1, and on Seduce and Payback than Advion, Advance, and Erasant-Hydro in test 2 (Table 2). In addition, comparisons of the mean percentages of the total number of ants on the baits revealed the same results with less than 2% of the ants on the Esteem, Seduce, and Payback baits (Table 2). In a separate test, an average of 2,500 (± 413 SEM, $n = 4$) *W. auropunctata* foraged on the dry Clinch ant bait. There were no significant differences ($P > 0.05$) among the number and the percentages of *W. auropunctata* foraging on the wet baits, including the control and the standard bait of dry Amdro Pro (Table 3).

BAIT EFFICACY ON COLONIES

Dry Bait Colony Test

All the dry ant baits had significantly (≤ 0.05) greater reduction percentages in worker *W. auropunctata* than the control by the end of the 4 wk test. Amdro caused the fastest decline with > 90% reduction by 3 or 4 d (0.5 wk) (Table 4). Queen survivorship that averaged less than 1 queen per colony was significantly lower than the control, which had an average of 11 living queens. Clinch bait had an average of 3 queens per colony surviving at wk 4 and was the only treatment that did not differ significantly from the control (Table 5). By wk 4, brood was not present in any of the Amdro baited colonies, while the Advion and Siesta treatments each had 1 of 5 colonies with brood. Clinch had 2 colonies with brood and the control had brood in all 5 colonies (Table 5).

Table 2. Mean (± SEM, n = 4) number and percentages of *Wasmannia auropunctata* counted on dry ant baits at 15 min intervals and summed over a 1 h observation period.

Test no.	Dry ant baits[a]				
1	Amdro	Complete	Siesta	Esteem	
1	1582 ± 386 a	1389 ± 620 a	1289 ± 377 a	50 ± 13 b	
1[c]	39.2 ± 4.5% a	29.0 ± 4.5% a	30.5 ± 1.2% a	1.3 ± 0.3% b	
2	Advion	Advance	Erasant[b]	Seduce	Payback
2	913 ± 167 a	783 ± 229 a	661 ± 110 a	18 ± 7 b	8 ± 4 b
2[d]	38.7 ± 7.1% a	31.5 ± 6.9% a	28.7 ± 6.4% a	0.7 ± 0.3% b	0.3 ± 0.2% b

Means followed by the same letter within a row are not significantly different (P > 0.05) by analysis of variance and Tukey's HSD test (PROC ANOVA, SAS Institute 2016) on logarithmically transformed ($\log_{10}$ [x + 1]) ant counts and on arcsine transformed (arcsine√[x/100]) percentages. Non-transformed means are presented.
[a]Clinch ant bait was tested separately with a mean of 2,500 (± 413 SEM, n = 4) *W. auropunctata* that foraged on the dry bait.
[b]Erasant-Hydro.
[c]Percentages for test 1 were based on total counts of 5,148, 7,479, 2,944, and 1,664 feeding ants per replicate.
[d]Percentages for test 2 were based on total counts of 2,751, 2,795, 1,861, and 2,122 feeding ants per replicate.

Table 3. The mean (± SEM, n = 4) number and percentages of *Wasmannia auropunctata* counted on wet (water-soaked) fire ant baits at 15 min intervals and summed over a 60 min observation period. Dry Amdro Pro bait was included as a standard.

	Water-soaked ant baits						
Amdro Pro (dry)	Clinch	Amdro	Advion	Erasant-Hydro	Amdro Pro	Siesta	Control[a]
242 ± 108 a	385 ± 163 a	240 ± 133 a	200 ± 128 a	129 ± 50 a	102 ± 24 a	69 ± 31 a	181 ± 112 a
19.6 ± 7.1%[b] a	24 ± 3.6% a	14.7 ± 3.3% a	8.7 ± 3.8% a	10.7 ± 6.2% a	8.8 ± 2.1% a	4.1 ± 1.2% a	9.3 ± 6.1% a

Means followed by the same letter within a row are not significantly different (P > 0.05) by analysis of variance (PROC ANOVA, SAS Institute 2016).
[a]Control bait consisted of 20% (w/w) soybean oil in pregel defatted corn grit.
[b]Percentages were based on total counts of 826, 502, 1,784, and 3,079 feeding ants per replicate.

Table 4. Mean (± SEM, n = 5) number of initial workers and reduction percentages in live workers of *Wasmannia auropunctata* per colony at 0.5 to 4 wk after access to dry imported fire ant baits.

		Reduction percentages in workers at wk after access to dry bait				
Bait	Initial no. workers	0.5 wk[a]	1 wk	2 wk	3 wk	4 wk[b]
Amdro[c]	1767 ± 433.3	92.3 ± 2.9	97.7 ± 0.3	98.5 ± 0.8	100 ± 0.0	100 ± 0.0a
Advion	1660 ± 213.5	84.7 ± 7.5	90.2 ± 7.7	90.8 ± 7.8	94.7 ± 5.3	94.7 ± 5.3a
Siesta	1680 ± 359.7	73.5 ± 17.6	90.2 ± 4.3	94.8 ± 2.5	93.3 ± 6.7	94.7 ± 5.3a
Clinch	1580 ± 400.5	48.8 ± 16.5	63.0 ± 13.0	74.9 ± 4.2	75.8 ± 9.1	84.3 ± 6.7a
Control	1760 ± 237.9	1.7 ± 1.7	1.7 ± 1.7	5.5 ± 7.8	1.8 ± 5.7	15.8 ± 13.0b

[a]3 to 4 d
[b]Means followed by the same letter within a column are not significantly different (P > 0.05) by analysis of variance and Tukey's HSD test. Analysis was conducted only for wk 4.
[c]n = 3 colonies for the Amdro treatment.

Table 5. Mean (± SEM, n = 5) number of living *Wasmannia auropunctata* queens per colony and the number of colonies with brood at specified wk after access to dry imported fire ant baits.

		Wk after initial dry bait access					
Bait	Initial no. queens	0.5[a]	1	2	3	4[b]	No. colonies with brood @ 4 wk[c]
Amdro[d]	10 ± 2.7	5 ± 0.7	1 ± 1.0	0 ± 0.0	0 ± 0.0	0 ± 0.0a	0
Advion	8 ± 2.7	4 ± 1.8	3 ± 2.0	2 ± 1.2	0.8 ± 0.8	0.4 ± 0.4a	1
Siesta	5 ± 1.3	3 ± 1.2	1 ± 0.9	1 ± 0.9	0.4 ± 0.2	0.4 ± 0.2a	1
Clinch	7 ± 1.3	8 ± 1.5	8 ± 1.5	7 ± 1.5	5 ± 1.5	3 ± 1.7ab	2
Control	10 ± 4.2	10 ± 4.2	13 ± 4.2	13 ± 3.9	14 ± 4.1	11 ± 4.4b	5

[a]3 to 4 d
[b]Means followed by the same letter within a column are not significantly different (P > 0.05) by analysis of variance and Tukey's HSD test on logarithmically transformed ($\log_{10}$ [x + 1]) data. Non-transformed means are presented. Analysis conducted only at 4 wk.
[c]Brood was initially present in all colonies.
[d]n = 3 colonies for the Amdro treatment.

Wet Bait Colony Test

All water-soaked ant baits caused significantly ($P \leq 0.05$) greater reduction percentages in *W. auropunctata* workers than the control by the study termination at wk 13. All workers were dead in the Amdro Pro and Erasant-Hydro treatments by wk 3. The remaining baits had reductions of 74 to 88% at wk 13 while the control colonies grew larger with a negative reduction percentage of −19% (Fig. 1A). Reduction percentages in brood of 96 to 100% for all baits, except Advion, were significantly ($P \leq 0.05$) greater than the control, which had a 36% in-

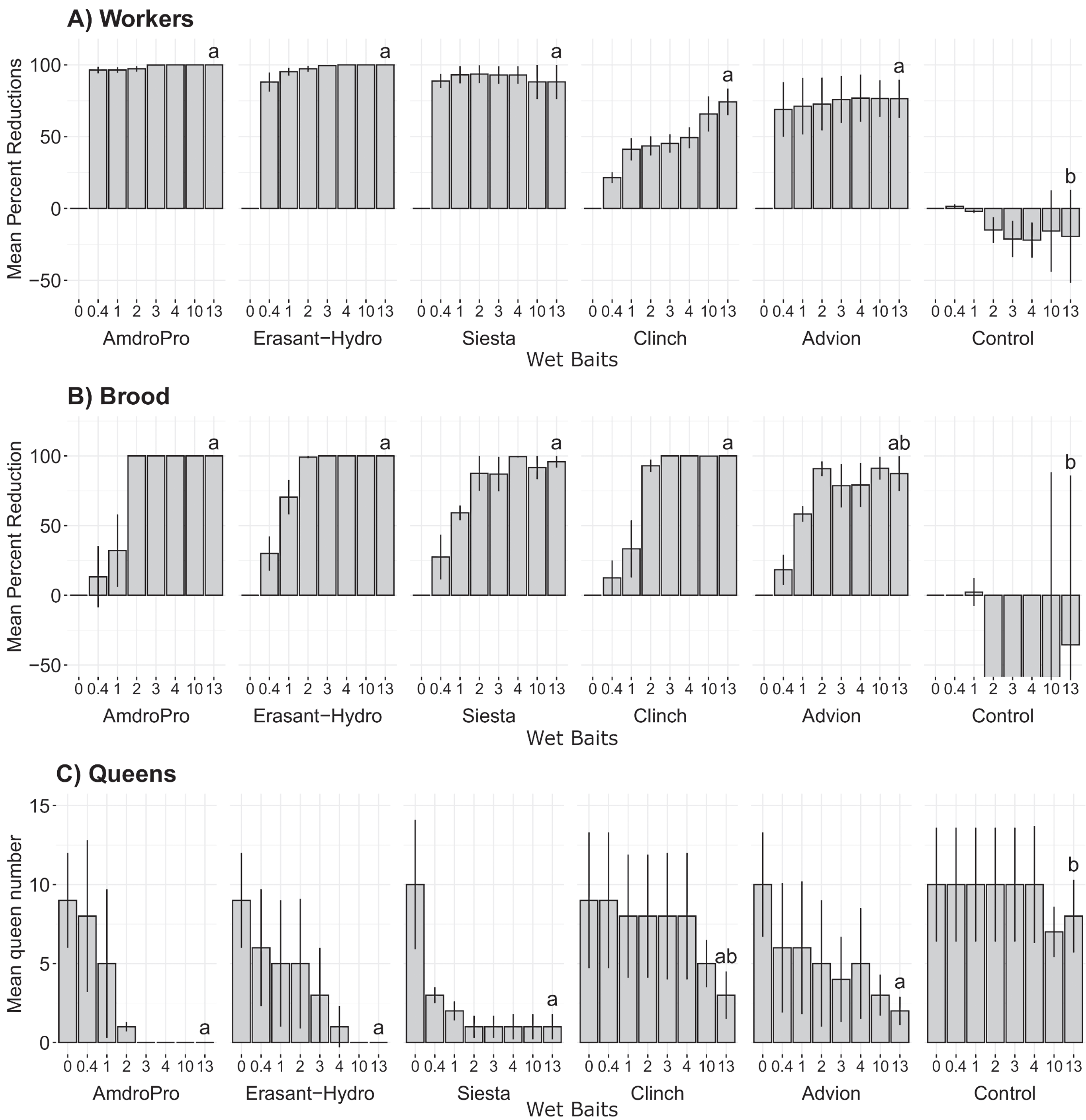

Fig. 1. Wet Bait Colony Test. Mean (± SEM, *n* = 4) reduction percentages per colony of (A) live *Wasmannia auropunctata* workers and (B) brood volume; (C) Mean (± SEM, *n* = 4) number of live queens per colony at specified wk after providing sole access to wet imported fire ant baits. Reduction percentages in brood volume for the control were truncated at wk 2 to 4 and wk 10, with reduction percentages of −156, −192, −220, and −59 indicating colonies grew larger. Means for workers and queens with the same letter above wk 13 bars within a graph are not significantly different ($P > 0.05$) by analysis of variance and Tukey's HSD test. Means for brood with the same letter above wk 13 bars are not significantly different ($P > 0.05$) by the Friedman test and Tukey's HSD test on ranked data. Non-transformed means are presented.

crease in brood by the end of the study. Advion had inconsistent brood reductions and was not significantly different from the other baits or the control (Fig. 1B). Averages of 0 to 2 living queens per colony among all baits, except Clinch, were significantly ($P \leq 0.05$) less than the average of 8 queens per colony surviving in the control at wk 13. Clinch had an average of 3 queens surviving per colony and was not significantly different from the control or the other baits (Fig. 1C).

Discussion

The dry bait acceptance test results were consistent with other *W. auropunctata* bait acceptance studies where ant baits containing the active ingredients pyriproxyfen (Esteem) and spinosad (Seduce and Payback) had significantly fewer *W. auropunctata* foraging counts than other baits (Hara et al. 2014; Hara & Niino-DuPonte 2017). Baits containing abamectin, metaflumizone, indoxacarb, and various concentrations of hydramethylnon were all accepted, each having means of 29 to 39% of the total number of ants that foraged among all the baits within a test. *Wasmannia auropunctata* exhibited a robust acceptance of baits that used carriers that differed from the standard imported fire ant bait formulation of corn grit and soybean oil. The Erasant-Hydro has a water-resistant carrier (Kafle et al. 2010; Shih & Kafle 2013), and Complete and Advance baits have proprietary formulations that target a variety of pest ants. Based on our results and those of Williams and Whelan (1992), Hara et al. (2013, 2014), and Montgomery et al. (2015), the bait matrix and the active ingredient and its concentration are important factors in *W. auropunctata* bait acceptance. For example, *W. auropunctata* foraged less on baits with high concentrations of the active ingredient (S)-methoprene (Montgomery et al. 2015).

Overall, there were fewer ants foraging on the wet baits in the acceptance tests compared to the dry bait tests. However, this difference could be attributed to the wet bait test being conducted in the spring when *W. auropunctata* populations probably were smaller and less active than in the summer when the dry baits were tested. While the dry Amdro Pro had more foragers than the wet Amdro Pro, it was not significantly different and it had less foragers than the wet Clinch (Table 3). The bait with the lowest mean percentage of ants foraging at 4% (Siesta), had a mean count of 69 ants indicating that *W. auropunctata* would still feed in moderate numbers on the least accepted wet bait. Thus, the palatability of the wet baits seemed to be maintained.

All the dry imported fire ant baits significantly reduced worker numbers (84–100%) in *W. auropunctata* colonies. However, queen reductions and brood absence were less consistent with 1 to 4 queens and brood present in at least 1 colony per bait, except with the Amdro treatment, where none of the colonies survived. Of the 4 baits tested, Amdro and Siesta had similar reductions in workers to those reported in other laboratory colonies studies (Williams & Whelan 1992; Hara et al. 2011). Until this study, Clinch and Advion lacked laboratory colony efficacy data for *W. auropunctata*.

Water-soaked imported fire ant baits caused significant reductions in *W. auropunctata* colony size. The baits containing hydramethylnon (Amdro Pro, Erasant-Hydro) eliminated all colonies in 4 wk. Siesta had a single surviving colony comprising 3 queens, 0.05 mL brood, and 950 workers. Three of the Clinch treated colonies had 2 to 7 queens but only 1 colony had brood after 13 weeks. The active ingredient in Clinch is abamectin, which sterilizes red imported fire ant queens (Glancey et al. 1982; Lofgren & Williams 1982), and thus it seems to have a similar effect in *W. auropunctata*.

There is evidence that imported fire ant baits exposed to rain and high humidity still can suppress *W. auropunctata*. Imported fire ant bait containing metaflumizone, which was weathered outdoors under tropical conditions of intermittent sunlight, rainfall, and > 75% relative humidity, was foraged upon by laboratory colonies of *W. auropunctata* resulting in substantial mortality of workers and queens similar to that of fresh bait (Hara et al. 2011). However, the weathered baits in these tests were available to the colonies for the duration of the 3 wk study and presumably had time to dry. In our study, the baits were accessible for 24 h, which limited the drying time.

In this study, after the baits were soaked in water, the corn grit carrier had a wet, mushy consistency. The wet bait particles would form a glob that could easily break apart. Ants would feed around the edges of the glob or on small, pulpy granules that separated from the glob. The hydrophobic Erasant-Hydro bait particles became soft and clumped together in irregular pieces when water-soaked. It did not form a mushy glob like the corn grit baits. Erasant-Hydro dried faster and crumbled apart as it dried. Similar to the standard imported fire ant baits, *W. auropunctata* would feed principally around the edges of the wet, Erasant-Hydro pieces. The presumption that imported fire ant baits formulated with a corn grit carrier have poorer control when exposed to rain or dew (Lofgren et al. 1964; Collins et al. 1993) possibly could be attributed to the degradation of the carrier when wetted, thus making the bait unpalatable to imported fire ants. This does not seem to apply to *W. auropunctata* based on our observations of ants feeding on wet baits, and colonies declining or dying at similar levels to the water resistant Erasant-Hydro bait. However, it should be noted that in the bait acceptance and colony efficacy studies, the baits were dispensed in piles and were very accessible to foraging ants.

Vander Meer and Milne (2017) reported the water-resistant bait formulation Erasant (0.5% pyriproxyfen, Chung Hsi Chemical, Hsinchu, Taiwan) caused greater red imported fire ant mortality than standard commercial fire ant bait when broadcasted or scattered around nests in the presence of heavy dew. Interestingly, a comparison of standard imported fire ant bait broadcasted with and without heavy dew present resulted in nonsignificant differences in the control (> 85%) of red imported fire ant colonies (Collins et al. 1993). Oi et al. (2022) also reported control of red imported fire ants with water-soaked and irrigated fire ant baits in laboratory and field test, respectively. The results of these studies suggest that (1) wet corn grit baits are palatable, and (2) there is a possibility that hydrophobic baits could increase control under high moisture conditions by improving the accessibility of broadcasted or scattered baits to foraging ants in the field. An important factor of the successful control of *W. auropunctata* has been the use of paste bait formulations that adhere to tall vegetation, tree trunks, and branches, which facilitates bait accessibility to arboreal nesting colonies (Vanderwoude & Nadeau 2009; Vanderwoude et al. 2010).

Our emphasis on testing imported fire ant baits registered for food crops is due in part to the limited number of ant baits currently labeled for vegetable production sites. Only Amdro Pro is registered for both imported fire ants and *W. auropunctata*, as well as for some crop sites, including tropical fruit and nut orchards. Clinch, whereas not as effective as other baits, is registered for many terrestrial food crops including vegetables. In addition to *W. auropunctata*, red imported fire ants also are present at the Gainesville sites. During a bait application for red imported fire ants where *W. auropunctata* co-occurred, the faster moving imported fire ants collected most of the bait (DHO personal observation). This interspecific competition should be considered when developing a treatment strategy for 1 or both species.

The Gainesville *W. auropunctata* infestations are in a humid subtropical climate of mild winters and hot, humid summers with frequent thunderstorms. The apparent efficacy of wet baits to control *W. aruopunctata* must be validated using broadcast applications under these field conditions. The adaptive management approach (Hoffman & Abbott 2010) should be used to develop and refine site specific treatment strategies relative to seasonal reproductive phenology and population abundance cycles of *W. auropunctata* within the framework of the farm and garden operations.

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