

Household and Structural Insects

Effect of Irrigation on the Control of Red Imported Fire Ants (Hymenoptera: Formicidae) by Water-Resistant and Standard Fire Ant Baits

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Subject Editor: Michael Rust

Received 29 September 2021; Editorial decision 22 November 2021

Abstract

The red imported fire ant, *Solenopsis invicta* (Buren), is an invasive pest of agricultural, urban, and natural areas. It is also considered a public health pest due to its painful stings. While it can be efficiently controlled by commercially available fire ant baits formulated with a corn-grit carrier, rain or irrigation is thought to degrade the carrier, compromising bait effectiveness. This study assessed the effect of irrigation on the efficacy of water-resistant and standard fire ant bait formulations, by comparing worker number, brood volume, and queen survivorship after access to water-soaked baits and to irrigated, bait-treated sod. In initial testing, wetted water-resistant and standard baits reduced fire ant colonies less than dry baits, both when baits were given to colonies directly and when colonies were given access to baits broadcasted (i.e., scattered) atop sod. Comparisons of the efficacy of piled versus broadcast applications of water-resistant and standard baits revealed reductions of >88% in adults and brood and no surviving queens for all bait treatments. This result was unexpected because piled baits were hypothesized to be better protected from irrigation than broadcast bait applications. In a field study, irrigated water-resistant and standard baits caused similar and significantly higher reductions in fire ant foraging activity relative to an untreated control. These results indicated that both the water-resistant and standard fire ant bait provided significant fire ant reductions even after irrigation.

Key words: invasive ant, control, bait, irrigation

The red imported fire ant, *Solenopsis invicta* (Buren) (hereafter, fire ant), is an extremely invasive ant that damages crops, injures livestock, reduces biodiversity, and is a public health threat due to aggressive stinging behavior (Wylie and Janssen-May 2016, US EPA 2002). The annual estimated economic cost of fire ant damage and control expenditures is over \$6 billion in the U.S. (Lard et al. 2006), and it has been the target of several regional eradication attempts both in the U.S. and internationally (Oi and Drees 2009). A primary method of fire ant control is the use of insecticidal fire ant baits. Fire ant baits typically consist of toxicant dissolved in a food attractant, such as soybean oil, that is then absorbed onto a corn-grit carrier (Lofgren et al. 1975, Williams et al. 2001). However, in wet conditions, the carrier absorbs water which reduces the effectiveness of

the bait (Markin and Hill 1971). This implies reduced efficacy of these types of baits in irrigated sites, for example, golf courses and plant nurseries, and in rainy climates (Greenberg et al. 2003, Souza et al. 2008, Hara et al. 2014). Indeed, instructions on the labels of commercial fire ant baits and fire ant treatment guides state that bait applications should avoid irrigation and/or rainfall (Barr et al. 2005, Oliver et al. 2010).

Prolonging the physical stability and palatability of fire ant baits exposed to water should advance the ability to control fire ants in wet conditions. Efforts have been made to decrease the effects of precipitation on fire ant baits. Shih and Kafle (2013) replaced the corn-grit carrier with a patented formulation containing dried distiller's grains solubles (DDGS). Another approach to improve the water

resistance is to protect the corn-grit carrier by spraying the water repellent corn protein, zein, on standard fire ant bait. The zein-coated bait carrier had decreased water absorption and improved bait performance (J.C., unpublished data).

Scheduling bait applications to avoid rainfall or irrigation can increase operational costs and inhibit the use of baits, which are an efficient and environmentally favorable method of fire ant control based on the minimal amount of active ingredient applied. In desert climates, irrigation is applied to golf courses and other landscapes several times a day and is suspended for 24–27 h when bait applications are scheduled (Greenberg et al. 2003, J.A.H., personal communication). Likewise in plant nurseries, irrigation schedules must be considered if fire ant baits are utilized (Oliver et al. 2010). In this study, we examined the effect of moisture on fire ant baits. Specifically, fire ant colony survivorship was compared among colonies given access to water-resistant and standard fire ant baits that were 1) soaked in water in the laboratory; and 2) applied to sprinkler irrigated sod. Lastly, fire ant foraging activity was compared among both types of bait applied to irrigated landscapes.

Materials and Methods

The laboratory efficacy study, sod study 1, and sod study 2 used laboratory-reared fire ant colonies. Colonies were reared from individual fire ant queens collected after mating flights in Alachua County, Florida, following procedures adapted from Banks et al. (1981). In each of these studies, pre- and post-treatment amounts of workers and brood were visually estimated by comparison to images of colonies in laboratory nest cells of known worker counts and brood volumes. All colonies were monogyne and queen survivorship was determined post-treatment.

Laboratory Efficacy Study

This study evaluated colony decline in colonies given different moisture treatments of baits containing the insect growth regulator (IGR) pyriproxyfen. Pyriproxyfen effects include a reduction in egg production, and specifically in fire ants, a shift from worker caste brood to reproductive caste brood (Glancey et al. 1990, Banks and Lofgren 1991). Therefore, baits were evaluated primarily by the average percent reduction in the initial worker brood volume per colony.

Experimental Design

A randomized, complete block design was utilized, with three replicated blocks based on colony size. Colonies used in this study consisted of an average of $\approx 10,800$ workers (range, 3,000–30,000), 16 ml brood (range, 7–30), and 1 queen. Initial brood volume was determined 1 d before baits were provided and data were collected weekly for 6 wk.

Baits that were evaluated contained the IGR pyriproxyfen formulated on 1) the DDGS carrier (Erasant-Pyro, 0.5% pyriproxyfen, Chung Hsi Chemical Plant, Hsinchu, Taiwan); 2) the zein-coated, corn-grit carrier (0.1% w/w pyriproxyfen in once-refined soybean oil); 3) a standard fire ant bait (Esteem, 0.5% w/w pyriproxyfen, Valent USA, Walnut Creek, CA); and 4) a control bait of 20% (w/w) once-refined soybean oil on pregel defatted corn grit. The zein carrier was 10% zein (w/w) made by spraying pregel defatted corn grit with 20.5% zein (w/w) in 80% ethanol-water (v/v) followed by oven drying.

All the baits were exposed to a moisture treatment of either: 1) soaked in water for 30 min; 2) soaked in water for 30 min then allowed to air-dry overnight; or 3) left dry as a standard bait. Baits

were submerged in water using the methods of Kafle et al. (2010). For each wetted treatment, one g of bait was placed inside a 50 ml polypropylene centrifuge tube with 2 holes (3 mm diameter each) near the bottom of each tube for drainage. The tube was capped and then inverted such that the drain holes were on top, through which 15 ml deionized water was added. After 30 min the tube was set upright, allowing the water to drain for 10 min. Baits were scraped out of the tubes and provided to colonies within 20 min after draining. The water-soaked then air-dried baits were soaked in the same manner, then allowed to air-dry within a screened tray for 18–23 h. Colonies were starved for 1 day, followed by access to baits for about 24 h, after which baits were replaced with a laboratory diet of frozen crickets and 10% sugar solution.

Analysis

Effects of bait formulation, moisture treatment, and their interaction on percent brood reduction was compared on ranked data with a three-way analysis of variance (Friedman test) and Ryan-Einot-Gabriel-Welsch multiple range test (Conover 1980, Conover and Iman 1981; SAS Institute 2016). Percent reductions in brood were ranked among the combinations of main factors of bait formulations and moisture treatments within each block. Ranked data were analyzed because of the heterogeneity among variances of the percent brood reductions. Analyses were conducted on data obtained 6 wk after baits were introduced, which is when colony-level effects of pyriproxyfen are typically apparent (Banks and Lofgren 1991).

Because there was a significant interaction between baits and the moisture treatments, analyses of variance and Ryan-Einot-Gabriel-Welsch multiple range tests were conducted on the percent reductions in worker brood among the baits separately for each moisture treatment. Analyses were conducted on logarithmic ($\log_{10}(x + 1)$) transformed data for the dry and water-soaked baits, while rank data were used for the analyses of the water-soaked then dried baits.

Percent reductions in workers were compared among the moisture treatments and baits by three-way analysis of variance and Ryan-Einot-Gabriel-Welsch multiple range test. The number of surviving queens among the bait and moisture treatment combinations also were tabulated.

Sod Study 1

This study compared colony decline among broadcast applied water-resistant and standard fire ant bait on irrigated versus dry sod.

Experimental Design

The study followed a randomized, complete block design, with five replicated blocks based on initial colony size and irrigation scheduling. Colonies contained averages of $\approx 14,000$ workers (range, 4,000–29,000) and 15 ml of brood (range, 5–55). The following fire ant baits were used: 1) a standard corn-grit carrier fire ant bait containing 0.045% indoxacarb (Advion Fire Ant Bait, Syngenta Crop Protection, Greensboro, NC); the water-resistant bait formulation of 2) the Advion fire ant bait coated with zein; and 3) a control of zein-coated pregel, defatted corn grit with 20 % (w/w) soybean oil.

For each bait treatment, 5 ml (1 tsp) of bait was scattered across a piece of St. Augustine grass, *Stenotaphrum secundatum* (Walter) Kuntze, sod (ca. 61 $\times$ 43 cm) to emulate a broadcast application of bait. Bait was evenly scattered by shaking the bait out of a plastic portion cup (1 fl. oz.) that was capped with a lid having 2–3 holes (4 mm diam each). Each replicate consisted of eight sod pieces, half of which were irrigated. Sod was irrigated outdoors with 1.3 cm (0.5 in.) of water, a recommended amount for turfgrass in Florida

(Trenholm et al. 2017), from an in-ground sprinkler (Rain Bird LG3HE, Azusa, CA). After irrigation, each of the sod pieces were placed in separate plastic swimming pools (91 or 114 cm diam.), returned to the laboratory, and held for 30 min to allow baits to absorb moisture from the wet sod before fire ant colonies were given access to the sod.

Fire ant colonies were starved (i.e., provided only water) for 24 h before being given access to the bait-treated sod. A tray containing a fire ant colony was supported above the sod with four plastic plant pots (6.35 L $\times$ 6.35 W $\times$ 5.37 cm H). The ants were given access to the sod by bridging the colony tray to the sod with a strip of fabric. The sides of the pools were dusted with talcum powder to prevent ant escapes. Frozen crickets and 10% (w/v) sucrose solution were provided 48 h after bait access. All sod was watered (ca. 500 ml) every 2–4 d to keep sod alive and provide additional moisture to the fire ants which always had access to water-filled test tubes. Fire ant colonies typically would move into the sod. After 2 wk, the sod was cut into small pieces and examined for living ants and pockets of brood. To facilitate the extraction of surviving ants, the cut sod was dried with lamps or by placing them into a bucket, and slowly flooding out ants with dripping water. Because indoxacarb causes relatively fast colony death (Oi and Oi 2006), the extraction process commenced as soon as 2 wk after baiting for sod where ant activity was not observed.

Analysis

The effects of bait treatment and exposure to irrigation, plus their interaction on percent reductions in worker numbers and brood volumes, from initial worker and brood levels per colony, were compared with a three-way analysis of variance and paired Tukey comparisons (Proc Mixed SAS Institute 2016). To reduce heterogeneity among variances of the percent worker and brood reductions, the square root ($\sqrt{(x + 0.5)}$) and the logarithmic ($\log_{10}(x + 1)$) transformations were applied to the worker counts and brood volumes, respectively, prior to calculating percent reductions. Queen survivorship also was tabulated among bait-irrigation treatment combinations.

Sod Study 2

In this study, we examined whether potential declines in fire ant bait performance from irrigation is due to bait inaccessibility from water washing away/burying bait particles. The effect of irrigation on the efficacy of baits against fire ant colonies was compared among water-resistant and standard fire ant bait that were either applied in piles or broadcasted. Applying bait in piles withstands the washing/burying effects of irrigation on bait particles (D.H.O., personal observation).

Experimental Design

Methods used in sod study 1 were repeated with the following modifications. The control bait was not zein-coated and baits (5 ml) were either scattered onto the sod or piled in two heaps (2.5 ml each), approximately 20 cm apart. Treatments were assigned according to a randomized, complete block design, with four replicated blocks based on initial colony size and irrigation scheduling. Colonies initially contained means of 23,000 workers (min–max, 11,500–39,000) and 28 ml of brood (min–max, 15–40).

Treatments consisted of the Advion and the zein-coated Advion—piled or broadcasted, then sprinkler irrigated (1.3 cm water) or left dry. There were two controls of piled, corn grit with 20% (w/w) soybean oil that were either irrigated or dry. Extractions of surviving

ants were initiated 2 wk after bait applications and took between 1–6 d.

Analysis

A two-way analysis of variance and the Ryan-Einot-Gabriel-Welsch multiple range test (Proc ANOVA, SAS Institute 2016) were used to evaluate the percent reductions in worker numbers and brood volumes per colony from initial worker and brood levels among treatments. Treatment was the main effect in the model, defined as the unique bait, bait application method (broadcast or pile), and irrigation (irrigated or dry) combination. Analyses were conducted on square root ($\sqrt{(x + 0.5)}$) and logarithmic ($\log_{10}(x + 1)$) transformed data to reduce heterogeneity among variances for workers and brood, respectively.

Field Study

A field study compared broadcast applications of the water-resistant, zein-coated Advion, the standard bait (Advion), and an untreated control in La Quinta and Palm Desert (Riverside County), California. This study was conducted in the desert climate of the Coachella Valley in May–June 2019 with average ($\pm$ SD, $n = 31$) daily minimum and maximum temperatures of 20.1 ± 3.9 and 34.7 ± 5.8 °C, respectively. Average ($\pm$ SD, $n = 29$) daily minimum and maximum relative humidity during the study were 21.3 ± 7.3 and 63.7 ± 8.3 %, respectively. The temperature and humidity data were obtained from the California Irrigation Management Information System station in La Quinta, CA.

Experimental Design

This study took place in three, irrigated landscapes, 1) a golf course (PGA West [Jack Nicklaus course]), 2) a restaurant (Arnold Palmer Restaurant), and 3) grass medians of a shopping mall (bounded by Fred Waring Drive and Painter's Path).

Baits were broadcast with a battery-powered, hand-held seed/fertilizer spreader (Scotts Wizz Spreader, Marysville, OH) at the PGA West ($\approx$ 0.45 ha including control plot) and Arnold Palmer Restaurant ($\approx$ 0.49 ha) sites. The third site located in Palm Desert, consisted of grass median strips (ca. 4.6 m wide) between a parking lot and sidewalk, totaling approximately 0.33 ha. Because of the small amount of bait needed to treat the median strips, baits were applied by manually shaking a tennis ball container with 6–8 holes (4 mm diam. each) punched into the cover. This allowed the small volume (237–355 ml or 1–1.5 cups) of bait to be applied evenly over the median strips. Advion and the zein-coated Advion treated sites each received 1.8 kg of bait per hectare. Irrigation was turned on within 15 min after baits were applied at PGA West for 7–9 min (0.4–0.6 cm of water applied). At the other sites, baits were wetted with water from hand-held and backpack sprayers as soon as bait applications were completed. In addition, at the Arnold Palmer site, 30–45 min after baits were applied, irrigation was on for 7 min (irrigation amount not recorded). Normal irrigation occurred overnight at all sites with 0.2–1.0 cm of water recorded at PGA West, 0.4–2.0 cm at Arnold Palmer, 0.8–1.0 cm for the median strips. Irrigation water applied was estimated from rain gauges placed in plots at each site. The PGA West and Arnold Palmer sites each served as separate replicates, while the median strips were divided into three replicates. Thus, the study had a total of five replicates.

Fire ant populations were determined by counting the number of fire ants on dime-sized ($\approx$ 1–1.5 ml) dollops of peanut butter lures placed along transects within each plot. Lures were placed at $\approx$ 4.6–6.1 m ($\approx$ 15–20 ft) intervals and ants in contact with the lures were

counted 30–45 min after lures were dispensed. The number of lures per plot ranged from 5 to 22 with an average of 11 lures per plot. The most acceptable method to apply the peanut butter was directly onto the turf using syringes (60 or 100 ml). Sun exposed lures were shaded with wood placards (7.6 × 10 cm) supported by landscape staples (landscape fabric pins). Sampling was conducted at 0 (pre-treatment), 2, and 4 wk after bait application.

Analysis

The number of fire ants per lure was averaged for each treated and control plot within a replicate and the plot averages were compared among treatments by analysis of variance and Einot-Ryan-Gabriel-Welsch multiple range test (Proc ANOVA, SAS Institute 2016) for each sampling date.

Results

Laboratory Efficacy Study

Percent reductions in worker brood were not significantly different among moisture treatments of water-soaked, water-soaked then dried, and dry bait ($F = 0.22$; $df = 2, 22$; $P = 0.807$). Mean percent reductions for the moisture treatments over all the bait formulations (including controls) ranged from 54.7 ± 3.4 to 58.4 ± 3.3 ($\pm$ SEM, $n = 12$). Significant differences in percent worker brood reductions occurred among bait formulations ($F = 90.06$; $df = 3, 22$; $P < 0.0001$). Reductions in worker brood volume were significantly greater with the Esteem bait when compared to the other bait formulations across moisture treatments. The water-resistant bait formulations (Erasant-Pyro and zein) had significantly greater brood reductions than the control over all moisture treatments (Table 1).

The moisture by bait formulation interaction was also significant ($F = 9.01$; $df = 6, 22$; $P = 0.026$) (Fig. 1). Mean brood reductions were inconsistent between moisture levels for the Erasant-Pyro and zein baits, while all Esteem baited colonies were at or near 100% for each moisture level. Mean worker brood reductions per combination of pyriproxyfen bait and moisture treatment ranged from 70 ± 3.8 to 100 ± 0.0 % ($\pm$ SEM, $n = 3$). In contrast, mean brood volume increased 36.6 ± 6.0 – 60.4 ± 2.1 % ($\pm$ SEM, $n = 3$) for the control bait per moisture treatment.

Table 1. Laboratory efficacy study. Mean percent reductions in *S. invicta* worker brood volume and adult workers 6 wk after baits were provided to colonies (Also presented are the number of live queens 6 wk after bait introductions. Means are over all moisture treatments)

Bait	Mean % reduction ($\pm$ SEM) @ week 6 ($n = 9$)		No. queens alive @ week 6 ^b
	Worker brood	Adult workers	
Esteem	100.0 (0.9)a ^a	–17.4 (23.4)a	9
zein	89.4 (3.1)b	–26.7 (18.9)a	7
Erasant-Pyro	87.8 (2.4)b	–28.1 (21.3)a	8
control	–52.2 (2.3)c	–112.3 (20.3)b	9

Means followed by the same letter within a column are not significantly different ($P > 0.05$) by analysis of variance and Ryan-Einot-Gabriel-Welsch multiple range test on % reduction in adult worker ants.

^aAnalyses conducted on ranked % reductions in worker brood (Friedman Test) and Ryan-Einot-Gabriel-Welsch-multiple range test. Untransformed means are presented.

^bInitial number of queens = 9.

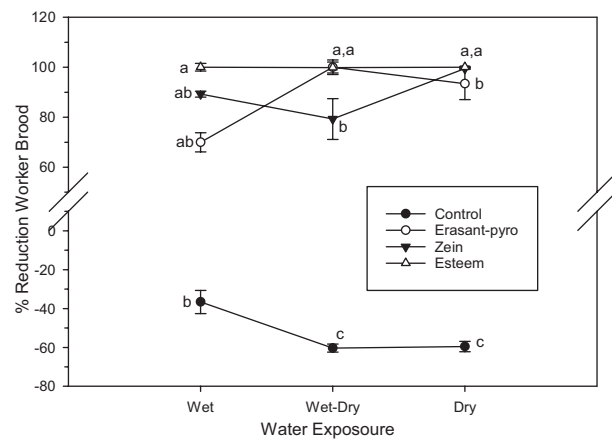


Fig. 1. Laboratory efficacy study. Average ($\pm$ SEM, $n = 3$) percent reductions in *S. invicta* worker brood 6 wk after colonies were given access to water-resistant (Zein, Erasant-pyro) and standard (Esteem, Control) fire ant baits. Baits were either water-soaked (Wet), water-soaked then dried (Wet-Dry), or not soaked (Dry) before being provided to the laboratory colonies. Identical letters adjacent to a bait symbol for a specific water exposure are not significantly different by analysis of variance and Ryan-Einot-Gabriel-Welch multiple range test. Logarithmically transformed data were used for analyses for the Dry and Wet baits, while rank data were used for Wet-Dry baits. Untransformed averages are presented.

The number of adult workers increased by the sixth week after bait introductions with a significantly larger increase in the controls than in the pyriproxyfen baited colonies (Table 1). Queen survivorship ranged from 78 to 100% among all baits and the control (Table 1). The increase in workers and the presence of live queens at week 6 are expected fire ant colony responses to pyriproxyfen baits (Banks and Lofgren 1991). The lower percent increase in workers in the pyriproxyfen baited colonies reflect reduced brood production and subsequent decrease in new workers.

Sod Study 1

There were significant differences between the irrigated and unirrigated (dry) sod over all baits (including controls) for percent reductions in workers ($F = 7.23$; $df = 1, 20$; $P = 0.0141$) and brood volume ($F = 10.62$; $df = 1, 20$; $P = 0.0039$). Percent reductions in workers averaged 63.9 ± 12.7 in the dry sod and 51.2 ± 10.1 ($\pm$ SEM, $n = 15$) in the irrigated sod. Percent reductions in brood volume averaged 75.9 ± 9.3 in the dry sod and 56.3 ± 11.7 ($\pm$ SEM, $n = 15$) in the irrigated sod. Percent reductions among the baits, over irrigated and dry sod combined, were also significantly different for workers ($F = 37.91$; $df = 2, 20$; $P < 0.0001$) and for brood ($F = 27.92$; $df = 2, 20$; $P < 0.0001$).

There was marginal interaction between irrigation and baits for percent worker reductions ($F = 3.29$; $df = 2, 20$; $P = 0.0583$) (Fig. 2), while the interaction for brood reductions was not significant ($F = 2.83$; $df = 2, 20$; $P = 0.083$). Comparisons between the irrigated and dry sod with the same bait revealed nonsignificant differences in percent worker reductions. However, there was significantly less brood reduction for the irrigated than the dry, standard Advion bait (Table 2). All queens died in colonies exposed to dry baits while one queen survived in the irrigated Advion bait treatments All queens survived in both the irrigated and dry controls (Table 2).

Sod Study 2

Percent reductions were significantly different among the treatments (combinations of application (piled or scattered), irrigation, and

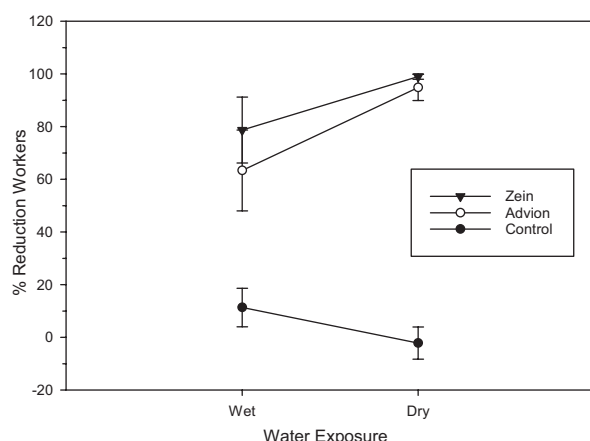


Fig. 2. Sod study 1. Average ($\pm$ SEM, $n = 5$) percent reductions in *S. invicta* workers 2–4 wk after colonies were given access to water-resistant (Zein, control) and standard (Advion) fire ant baits that were broadcasted atop sod that were either irrigated (Wet) or unirrigated (Dry).

Table 2. Sod study1 (Mean [$n = 5$] percent reductions in *S. invicta* workers and the total number of surviving queens, out of an initial total of 5 queens per bait, among water-resistant [zein+Advion], a standard fire ant bait [Advion], and the control bait [zein-coated corn grit + soybean oil] applied to irrigated [Wet] and unirrigated [Dry] sod)

Bait	Irrig. ^a	Mean % worker reductions	Mean % brood reductions	Total no. live queens
zein+Advion	Wet	78.7bc ^b	76.4bc ^c	0
	Dry	99.0c	100.0c	0
Advion	Wet	63.4b	63.2ab	1
	Dry	94.9bc	98.8c	0
control	Wet	11.3a	29.3a	5
	Dry	-2.1a	28.9a	5

Means followed by the same letter within a column are not significantly different ($P > 0.05$) by pairwise Tukey comparisons. Untransformed means are presented.

^aIrrigation.

^bAnalysis was conducted on square root transformed data.

^cAnalysis was conducted on $\log_{10}(x + 1)$ transformed data.

baits) for workers ($F = 22.94$; $df = 7, 21$; $P < 0.0001$) and brood volume ($F = 8.83$; $df = 7, 21$; $P < 0.0001$). The water-resistant zein+Advion and the standard Advion baits did not differ significantly in worker reductions regardless of application method or irrigation treatment (Fig. 3). Their mean percent reductions, which ranged from 88.8 ± 3.2 – 98.5 ± 0.9 % ($\pm$ SEM, $n = 4$), were significantly different from the control reductions of -4.2 ± 19.4 and 33.8 ± 21.8 which were both piled applications, with and without irrigation, respectively (Fig. 3). None of the queens from colonies exposed to baits with indoxacarb survived ($n = 24$) regardless of irrigation or application method. In contrast, all queens in the controls remained alive ($n = 8$).

Field Study

The zein-coated Advion and standard Advion baited plots had significantly less fire ants per lure than the untreated controls two ($F = 50.56$; $df = 2, 8$; $P < 0.0001$) and four ($F = 11.67$; $df = 2, 8$; $P = 0.0042$) weeks after baiting. The number of fire ants per lure

between the water-resistant zein bait and the standard Advion did not differ significantly throughout the study (Table 3). Two weeks after baiting, average fire ant counts were 62% and 43% less than

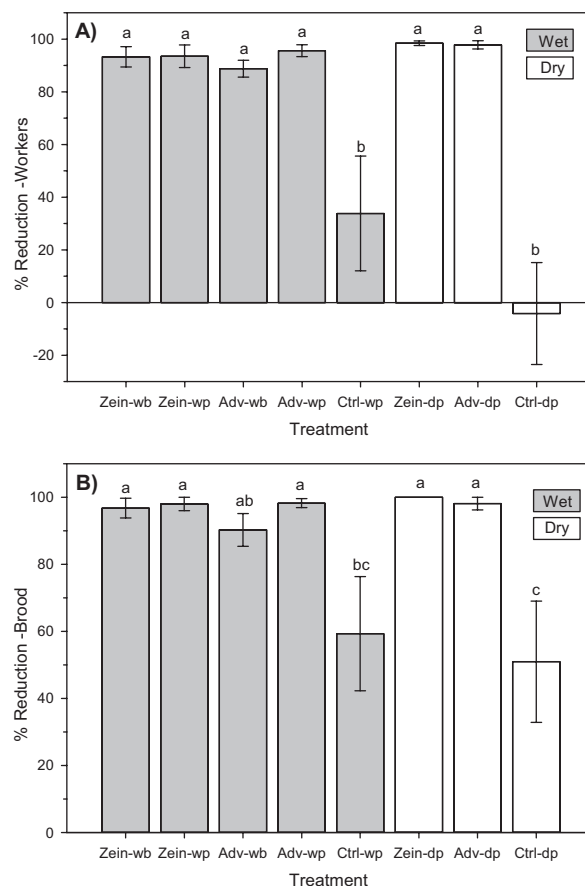


Fig. 3. Sod study 2. Average ($\pm$ SEM, $n = 4$) percent reductions in *S. invicta* workers (A) and brood (B) 2–3 wk after colonies were given access to the water-resistant zein+Advion (Zein), standard Advion (Adv), and control (Ctrl) baits that were either broadcasted or piled atop sod and were either irrigated (Wet) or unirrigated (Dry). The same letters above the bars are not significantly different by analysis of variance and Ryan-Einot-Gabriel-Welch multiple range test on square root and logarithmic transformed data, for the worker and brood reductions, respectively. Untransformed averages are presented. (wb, wet-broadcast; wp, wet-pile; dp, dry-pile).

Table 3. Field study (Average [$n = 5$] number of red imported fire ants, *S. invicta*, per lure of peanut butter dispensed along transects within each plot. Sampling was conducted at 0 [pretreatment], 2, and 4 wk after bait applications)

Treatment	Average ($n=5$) No. red imported fire ants per lure		
	Week 0	Week 2	Week 4
zein+Advion	48.9a ^a	18.5a	25.7a
Advion	30.5a	17.4a	25.6a
control	35.0a	64.2b	53.2b

Averages followed by the same letter within a column are not significantly different ($P > 0.05$) by analyses of variance and Ryan-Einot-Gabriel-Welch multiple range test.

^aWeek 0 analysis on $\log_{10}(x + 1)$ transformed data; non-transformed averages are presented.

pretreatment averages for the zein and Advion baits, respectively, while the control increased 84%. After 4 wk, percent reductions from pretreatment average counts were 48% and 16% for the zein and Advion, respectively, while the control increased by 52%. Thus, bait efficacy of the water-resistant formulation of zein-coated Advion was not significantly different from the standard Advion even after the baits were wetted soon after baits were applied.

Discussion

The laboratory comparison of water-resistant and standard fire ant baits resulted in a significant interaction between bait formulation and exposure to moisture (Fig. 1). Interestingly, the standard Esteem had > 99% reductions in worker brood across all three moisture treatments while the water-resistant formulations had less consistent brood reductions when either water-soaked or water-soaked then dried, with mean brood reductions of 70–100%. Fire ants were observed feeding on all water-soaked bait formulations even when they were still wet and continued feeding on them as they dried. These results demonstrated that the water-soaked baits tested will be fed upon by fire ants and can cause significant negative impacts to colonies. However, the laboratory study allowed fire ant colonies unfettered access to the water-soaked baits for a day.

The sod studies added more complexity to fire ant access to the baits by emulating bait applications to lawns exposed to sprinkler irrigation. In sod study 1, analysis of the main factors indicated irrigation had a significant effect on both percent reduction in workers and brood with lower reductions in irrigated than dry baits. If controls were excluded, mean worker reductions ranged from 63–79% for the irrigated baits in contrast to 95–99% for the dry baits. Similarly, brood reductions were 63–76% and 99–100% for irrigated and unirrigated baits, respectively. Notably, for the irrigated baits, only the water-resistant zein+Advion treatment had significantly higher reductions than the controls for both workers and brood, and none of the queens survived (Table 2). Pairwise comparisons also revealed nonsignificant worker reductions between dry and irrigated baits within the same bait formulation (Table 2). In general, irrigated baits, including the standard Advion, did result in colony reductions, but these reductions were variable and not as consistent as the dry baits (Fig. 2).

The inconsistent control associated with irrigation may be attributed to the inaccessibility of scattered bait granules to fire ant foragers when granules may be washed away or buried into the soil by water droplets from the sprinkler. Sod study 2 sought to lessen the impacts of the water on the bait granules by applying bait in piles. Comparisons among piled and scattered baits exposed to irrigation did not reveal significant differences in worker and brood reductions between the standard Advion and the water-resistant, zein-coated Advion. For all non-control treatments, percent reductions for workers and brood were $\geq 89\%$ (Fig. 3) and none of the queens survived. In this study, irrigated baits were being foraged regardless of the application method or bait formulation. It should be noted that colonies used in this study were larger than that of sod study 1, and presumably would be more efficient at finding bait granules in the limited area of a piece of sod. Nevertheless, irrigation did not diminish the effectiveness of the baits in this study.

In the field study, reductions in fire ant activity were the same in both the water-resistant and standard bait treatments, with both being significantly less than the untreated control (Table 3). Thus, both baits were adequately accessible to fire ant foraging even when wetted soon after application as well as receiving the regularly scheduled irrigation the same day. Granules of both bait types were

visible after irrigation and presumably available to foraging fire ants. This suggested that irrigation did not have to be turned off after bait applications were made under the conditions of this study.

During the initial development of fire ant baits, Lofgren et al. (1963) indicated that the effect of rainfall on the corn-grit carrier needed further evaluation. They reported that fire ants actively forage baits during the “...first several hours after application and appears to be negligible after 24 h.” It was thought that if rainfall were to affect bait treatments, it would need to occur soon after baits were applied (Lofgren et al. 1964). In contrast, in the desert climate of the Coachella Valley, where our field study was located, fire ants were observed feeding on bait immediately after sprinklers were turned off, in hot (42°C), sunny conditions, presumably because of the high humidity at the surface of recently irrigated turfgrass. Fire ants also were observed foraging on wet bait at dawn after irrigation during the night (D.H.O., personal observation).

Our evaluations of water-resistant and standard fire ant bait demonstrated that fire ants will feed on both types of water-soaked baits, as evidenced by detrimental impact on colonies, including the death of queens. This is consistent with reports of heavy dew and rainfall not reducing the efficacy of standard fire ant baits if the dew dried or there was adequate foraging time before there was significant rainfall (Collins et al. 1993, Barr et al. 2000). Results of our studies with water-resistant and standard (corn-grit carrier) fire ant baits indicated that irrigation did not completely compromise the efficacy of either bait type against fire ant colonies. While water-soaked/irrigated baits were generally less detrimental to fire ant colonies than dry bait, both water-resistant and standard baits were discovered and fed upon by the ants. Thus, the practice of suspending irrigation after applications of standard fire bait may not always be necessary.

Water-resistant bait formulations may still improve efficacy of fire ant baiting under more extreme conditions such as periods of prolonged wetness. The DDGS bait particles dried faster than the corn-grit carrier and the DDGS bait was readily foraged by fire ants soon after being irrigated (D.H.O., personal observation). Vander Meer and Milne (2017) attributed better performance by DDGS formulated bait (Erasant, 0.5% pyriproxyfen) to its water-resistant properties when applied in the presence of heavy dew. However, Kafle et al. (2010) reported significantly less fire ant mortality from DDGS bait containing cypermethrin that was water-soaked than when the bait remained dry. Bait formulated as a gel, which can adhere to vegetation and withstand moderate rainfall, have been utilized in tropical climates to control little fire ants, *Wasmannia auropunctata* (Roger), (Vanderwoude et al. 2010, Montgomery et al. 2015). Hydrogel baits tested against various invasive ants, can rehydrate with rainfall which may prolong the palatability of the baits to foraging ants (Tay et al. 2020, D.H.O., personal observation). While low moisture absorption and fast-drying properties of water-resistant baits can initially allow more efficient bait foraging of wet bait, the best control is associated with the key factor of sufficient time under conducive foraging conditions before and/or after baits are exposed to water. Fire ants should collect bait before the decline in palatability because of effects related to weathering such as mold growth (Hara et al. 2014), or active ingredient breakdown by UV light (Vander Meer et al. 1982, Webb and Jovic 2019).

While our studies revealed less consistent efficacy to fire ant colonies when water-resistant and standard baits were wetted compared to dry baits, both bait formulations still provided significant fire ant control despite being irrigated. Knowing that standard fire ant baits can withstand sprinkler irrigation should offer more flexibility with scheduling bait applications. Improving water-resistant

formulations to consistently obtain high bait efficacy through extended periods not conducive to bait integrity or fire ant foraging, for example, high humidity, rain, wet soil, and cool temperatures, would be vital to containment and eradication programs where 100% efficacy is desired.

Acknowledgments

We thank S. Steininger, K. Lawson, M. Ratajczak, E. Schuchman (all formerly with the USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology) and I. Sheffield (Oak Ridge Institute for Science Education) for their technical assistance. Our gratitude is extended to R. Dieckmann and M. Martinez (Coachella Valley Mosquito and Vector Control District) for locating sites used in the field study. We greatly appreciate the cooperation and generosity of Chung Hsi Chemical Plant Ltd. and Valent USA for providing baits. This work was supported by a grant from the Coachella Valley Mosquito and Vector Control District and through an interagency agreement with the United States Department of Agriculture, Animal and Plant Health Inspection Services, Plant Protection and Quarantine, Science & Technology. Mention of trade names or commercial products in this article are for information and convenience of the reader and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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