Final Report for the Hawaii Invasive Species Council FY2021

Project Title
Biopesticides delivered with water-storing hydrogels for control of invasive yellow crazy ants

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$31,548

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1/1/2021 to 4/30/2022

Introduction

The yellow crazy ants (*Anoplolepis gracilipes*) cause severe impacts in natural and some agricultural systems. They also displace native insects, causing damage to biodiversity and ecosystem sustainability in natural settings (Hoffmann and Saul 2010; Plentovich et al. 2018). In addition to causing severe impacts on biological diversity, yellow crazy ants are considered agricultural pests in Hawaiʻi that damage crops (e.g., bananas) with their formic acid secretions (Nelson and Taniguchi 2012).

Broad spectrum insecticide sprays present a hazard to humans and environmental health. Liquid sucrose bait, infused with a low concentration of insecticide, is a highly effective alternative treatment option for invasive ants. However, current approaches require substantial economic investment in plastic bait dispensers and continual maintenance. Water-storing hydrogels provide the sucrose solution ants prefer in a no-spills, easy-to-deploy format, circumventing the need for bait dispensers. The deployable hydrogels individually act as micro-sized controlled-release bait dispensers, allowing sugar-feeding ants to feed directly on them (Tay et al. 2017, 2020). Recent experimental studies reported that hydrogel baits incorporated with small amounts of neonicotinoids successfully managed Argentine ant and yellow crazy ant populations in Hawaiʻi's natural areas (Krushelnycky 2019), as well as Argentine ant populations in California citrus (McCalla et al. 2020). However, neonicotinoids are under increasing scrutiny, because they can present a threat to pollinators. There is a critical need for effective and safe insecticidal compounds in ant baiting systems.

We initially proposed to use spinosad as an active ingredient in hydrogel baits. Although spinosad is a natural substance made by a soil bacterium and is classified as a reduced-risk pesticide by the US Environmental Protection Agency (EPA), we did not use spinosad in this study due to its toxicity to bees and other pollinators. Hence, we switched to using boric acid in the hydrogel baits. We chose boric acid as it is practically nontoxic to birds, fish, and aquatic invertebrates and relatively nontoxic to beneficial insects (US EPA 1993). According to the EPA toxicity rating standard (acute oral toxicity), boric acid is rated in the "toxicity category III," indicating it is only slightly toxic for vertebrates (US EPA 2006). With its sufficient solubility in water to be prepared in 10-25 percent sucrose solution, boric acid has shown to be a highly effective toxicant when it is incorporated in a liquid bait (Choe et al. 2021).

The specific objectives in this study are: (1) to determine the attractiveness of improved alginate hydrogel beads containing humectant towards yellow crazy ants; and (2) to determine the field efficacy of boric acid delivered with improved alginate hydrogels baits against yellow crazy ants.

Although we switched to a different insecticide and incorporated a field study, we did not need to make significant changes in the budget.

Technical Approach

Objective 1: The attractiveness of fresh and aged alginate hydrogels formulated with 25% sucrose and 0, 10, 20, or 30% humectant (we did not disclose the name of the humectant compound in this report as we are in the process of publishing this study) was tested with laboratory colonies of yellow crazy ants. In each trial, 150 worker ants were introduced into an experimental arena, which consisted of a polyethylene container (20 cm x 15 cm) whose inner sides were coated with a film of insect barrier to prevent escape. To prepare the aged hydrogels, fresh hydrogels were left exposed at ambient room conditions for 3, 7, 14 and 28 days. In each experiment, four individual beads of the same age formulated with 0, 10, 20 and 30% humectant were simultaneously placed 2 cm apart in a square configuration on a petri dish cover at the center of the experimental arena. Digital pictures of the arena were taken at 5, 10, 20 and 30 minutes after beads were introduced, and numbers of ants feeding on individual beads at each time interval were subsequently counted. The experiments were replicated four times using four different ant colonies. One-way ANOVA and Tukey's HSD test at the 0.05 level of significance were used to compare the ant counts among the different concentrations of humectant at each time point.

Objective 2: Each experimental site was treated once with ~500 g of improved or standard hydrogel baits at an application rate of 10 g/m^2. The hydrogel baits were hand-distributed in ~10 piles of ~50 g each, separated from each other by at least several meters and placed along multiple active ant trails on the soil. Field sites were monitored on day 0 (pre-treatment), and weeks 1, 2, 3, 4, 6 and 8 post-treatment using monitoring traps, which consisted of 50-ml centrifuge tubes containing a cotton ball soaked with 25% sucrose solution. At each site, 10 monitoring traps were placed along the foraging trails. After 30 minutes, the traps were collected, capped, and the number of ants in each trap was recorded. Numbers of ants were summed across the 10 monitoring traps at each site on each monitoring date. The difference in total ant counts among monitoring time points within the standard and improved bait treatments were assessed by Friedman test.

Results and Deliverables

Results from Objective 1: For fresh hydrogel beads, no significant differences ($P > 0.05$) were found between numbers of yellow crazy ants attracted to standard hydrogel beads and any of the formulations containing humectant at 5 and 10 minutes post-introduction. At 20 minutes post-introduction, significantly fewer ants were attracted to beads containing 30% humectant compared to all other formulations. At 30 minutes post-introduction, however, numbers of ants attracted to 30% humectant were only significantly lower than numbers attracted to standard beads with no humectant (Figure 1A).

For hydrogels aged for three days, there were no significant differences in mean numbers of yellow crazy ants attracted to any of the formulations at any point in time (Figure 1B).

For hydrogels aged for seven days, significantly more yellow crazy ants were attracted to the hydrogel beads containing 10, 20, and 30% humectant compared to the standard hydrogel beads at 30 min post-introduction (Figure 1C). The standard beads aged for seven days appeared to be hardened and contain minimal moisture in contrast to the improved beads which were flexible, suggesting more water retention.

No yellow crazy ants were attracted to the standard hydrogel beads aged for 14 and 28 days whereas ant counts were significantly greater ($P < 0.05$) on all hydrogel beads containing 10, 20, and 30% humectant at all time intervals (Figures 1D & E). This further supports the inference that 10-20% humectant composition does not influence bait attractiveness. Improved hydrogel beads that were aged remained flexible and transparent in appearance, unlike the standard hydrogel beads which hardened and became opaque as they aged (Figure 2).

<u>Results from Objective 2:</u> After a single field application using the standard hydrogel baits (no humectant), yellow crazy ant numbers in the monitoring traps were significantly reduced compared to the pre-treatment data by two weeks post-treatment. However, ant numbers gradually rebounded and by four weeks post-treatment were not significantly different from pre-treatment levels (Figure 3A). In contrast, for a single treatment using the improved hydrogel baits (containing 20% humectant), ant numbers in the monitoring traps remained low and were significantly different from pre-treatment levels from week two through week eight post-treatment (Figure 3B), indicating considerable residual activity of the improved hydrogel bait. At three weeks post-treatment, ant numbers in the monitoring traps were reduced by 66%, the highest percent reduction observed during the study period (Figure 3B). Concurrently, numbers of ants in monitoring traps at the two untreated control sites did not show any decline during the study period, and instead exhibited a 3.2-22.8% increase during weeks 1-8 relative to pre-treatment levels.

From the results, we conclude that the improved boric acid alginate hydrogel baits (with a humectant) are more effective in yellow crazy ant management, as compared to boric acid hydrogel baits without a humectant. In the future, a variety of other sugar-loving ant species, including incipient invaders, can be similarly targeted with this approach.

Other deliverables from this project include oral presentations at the 2022 National Conference of Urban Entomology and 2022 Hawaii Pest Control Association annual conference. The relevant results and outcomes will also be shared and distributed through the Principal Investigator’s website (manoa.hawaii.edu/ctahr/urbanlab). Part of the data and results will be used for a peer-reviewed journal publication.

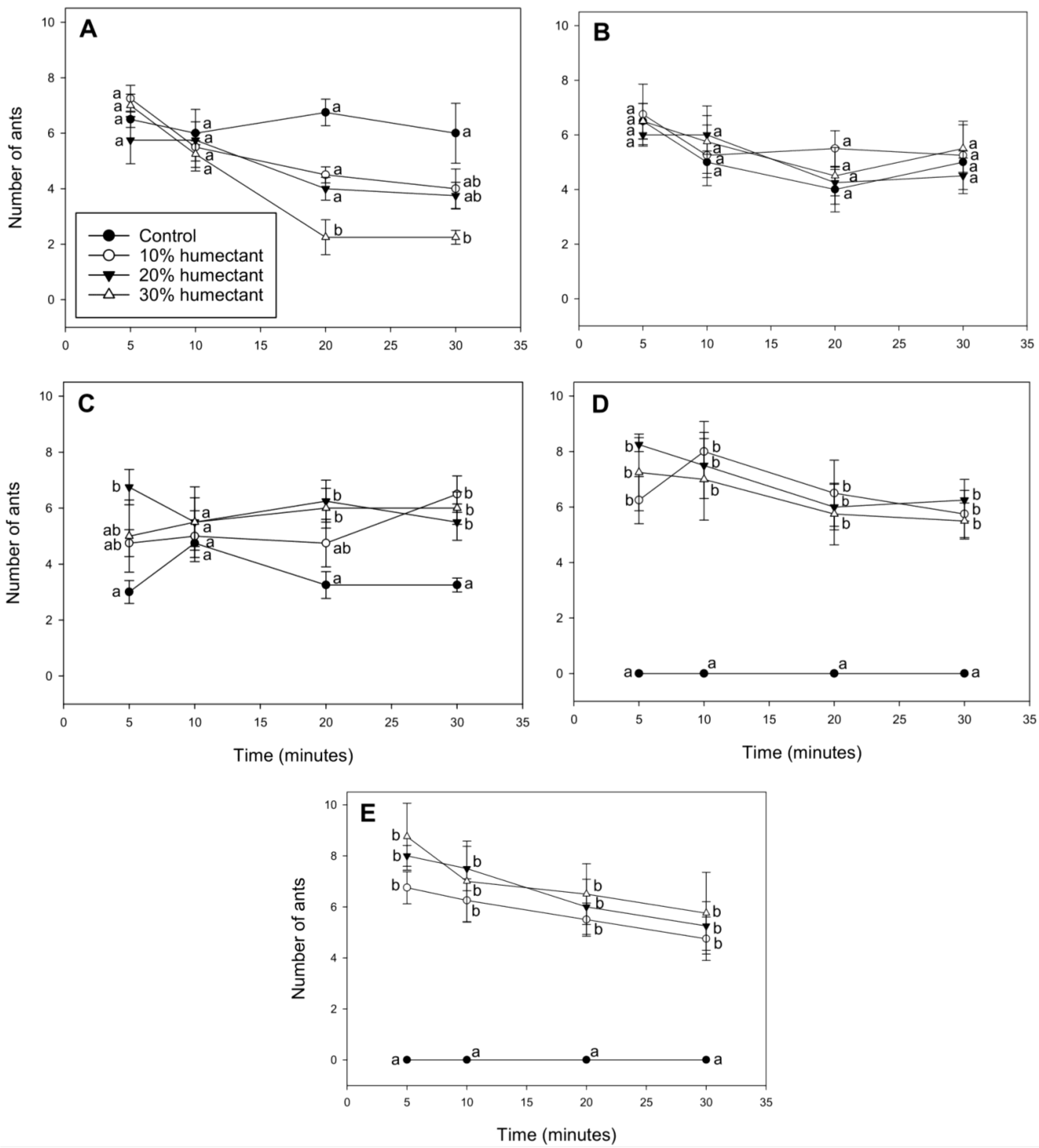

Figure 1: Number (mean ± SEM) of yellow crazy ant feeding on hydrogels conditioned in different concentration of humectant over time tested with (A) fresh hydrogel, (B) 3 days aging hydrogel, (C) 7 days aging hydrogel, (D) 14 days aging hydrogel, (E) 28 days aging hydrogel.

Figure 2: Attractiveness study with hydrogel beads aged for 28 days that contained sucrose solution and 0%, 10%, 20%, or 30% humectant at 5-minute post introduction into test arena.

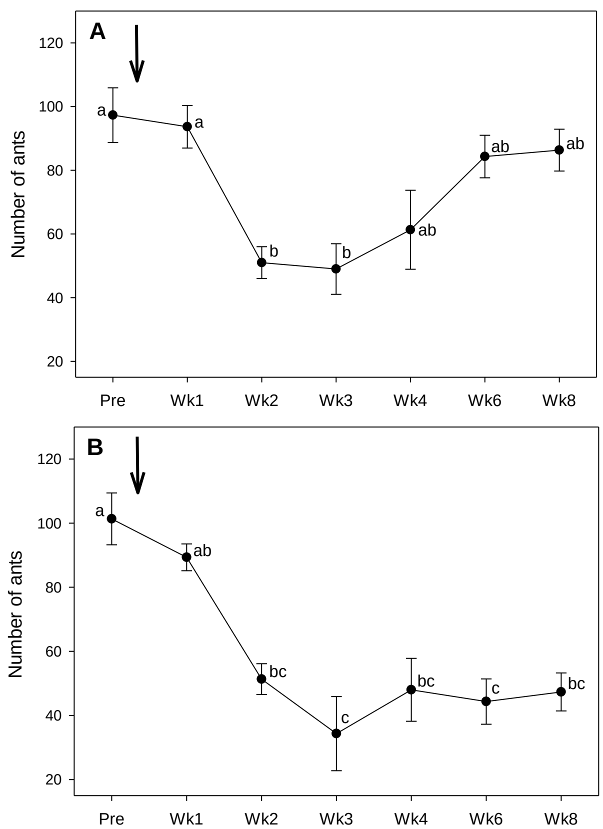

Figure 3: Pre- and post-treatment of yellow crazy ant visits (mean ± SEM) at three sites treated with (A) hydrogel baits containing 2% boric acid, (B) hydrogel baits containing 20% humectant and 2% boric acid.

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