ASSESSMENT OF EFFECTS OF *SOLENOPSIS PAPUANA* ON ARTHROPODS IN OAHU FORESTS

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Background

   Solenopsis papuana is the most widespread and abundant invasive ant species in the upland forests of both mountain ranges on Oahu. While other more conspicuous ant species often occur in exposed, drier microsites such as ridgetops with short-statured vegetation, *S. papuana* is the most common species that can be found under the canopy in the interior of mesic to wet forests, and appears to be nearly ubiquitous above elevations of roughly 1000 ft. Although concern about the ecological effects of this species has been raised for many years, almost no research has been done on any aspect of its biology or ecology. We are conducting a study of the ecological effects of *S. papuana* on the ground arthropod communities in forests under conservation management. A secondary goal is to attempt to measure effects of *S. papuana* on reproduction in native *Drosophila* flies in the field.

FY16 progress and results

   During fiscal year 2016, graduate student Sumiko Ogura-Yamada completed the majority of field work planned for the project. This included work in three general areas: developing methods for monitoring and controlling *S. papuana* in the field, conducting a field experiment to

assess effects of *S. papuana* on arthropod communities, and conducting a field experiment to assess the effects of *S. papuana* on native *Drosophila* reproduction.

A. Development of monitoring and control methods for *S. papuana*

Study Sites

A monitoring bait preference and a pesticide bait preference test were conducted in two forested sites on Oʻahu that supported high densities of *S. papuana*, as determined by prior distribution mapping (Ogura-Yamada & Krushelnycky, unpub. data). The first site was located within University of Hawaii's Harold L. Lyon Arboretum, in lowland, introduced wet forest in Mānoa Valley in the Koʻolau Mountain range (150 m elevation, 3836 mm annual rainfall (Giambelluca et al 2013)). The second site was located in mixed native and introduced mesic forest in Pahole Natural Area Reserve (NAR) in the Waiʻanae Mountain Range (480 m elevation, 1375 mm annual rainfall (Giambelluca et al 2013)). A pesticide bait efficacy test was conducted only at Pahole NAR.

Monitoring bait preference
Methods
 Four food baits containing varying amounts of sugar, oil and protein were chosen to compare relative attractiveness to *S. papuana*: 1) light corn syrup (Karo, ACH Food Companies), 2) peanut butter (Jif Creamy, J.M. Smucker Company), 3) SPAM (Hormel Foods), and 4) tuna/corn syrup blend (one can of tuna (Chicken of the Sea International) in water, drained, and blended with 1/3 cup light corn syrup in a food processor). Corn syrup (Eow & Lee, 2007), peanut butter (Lee, 2002; Causton et al. 2005; Hara et al. 2014), processed meats (Porter & Tschinkel 1987; Peck et al. 2015), and tuna/ corn syrup blends (Keeler, 1980; Krushelnycky et al. 2011) have been used in attracting a variety of ants in bait preference studies (Lee, 2002; Eow & Lee, 2007, Hara et al. 2014) and ant monitoring (Keeler, 1980; Porter & Tschinkel 1987; Causton et al. 2005; Krushelnycky et al. 2011; Peck et al. 2015)
Baits (approximately a 1.5 cm diameter quantity of corn syrup, tuna/corn syrup blend, and peanut butter, and one piece of SPAM approximately 1 x 1 x 0.5 cm) were placed in paper cupcake wrappers and presented next to each other at replicate stations, which were approximately 20 m apart, at each site. The cupcake wrappers prevented liquid baits from spilling, while allowing ants access to the baits both on the upper surface and underneath as the baits soaked through the paper. Ant numbers on each bait were recorded (top and bottom of wrapper summed) every hour for three hours. The preference test was conducted on June 18, 2015, at Lyon Arboretum, using 25 replicate stations, and on August 1, 2015, at Pahole NAR, using 24 replicate stations. Stations with fewer than 24 ants total across all bait types and hours (i.e., <2 ants/bait/hour on average) were removed from the data set; this left 16 replicate stations at Lyon Arboretum and 19 replicate stations at Pahole NAR. Due to unequal variances among groups, Welch's ANOVA followed by Games-Howell multiple comparison test was used to compare log-transformed numbers of ants among all bait types for each hour at each site. Numbers of ants were subsequently also compared across hours at each site for the two most attractive baits (peanut butter and SPAM, see Results). To compare relative detection rates for the four baits, we compared proportions of stations that attracted any *S. papuana* after one hour at each site, using a Chi-square contingency table. For peanut butter and SPAM, we also

compared proportions of stations attracting ants at one and two hours at each site, using Fisher's Exact Test. Statistical tests were performed using Minitab v. 17.1.

Results and Discussion

Among the four foods evaluated as potential monitoring baits, SPAM and peanut butter generally attracted more ants than corn syrup and the tuna/corn syrup blend at most of the time intervals at both sites (Fig. 1). However, these differences were not always statistically significant (see Fig. 1) due to high variation in ant numbers among replicate stations. For SPAM and peanut butter baits, mean recruitment increased over time, but most of these differences were not statistically significant. Specifically, numbers of *S. papuana* at peanut butter baits did not differ among hours at either Lyon Arboretum (F = 0.34, p = 0.716) or Pahole NAR (F = 2.08, p = 0.140), nor did they differ among hours at SPAM baits at Lyon (F = 1.34, p = 0.278). On the other hand, ant numbers at SPAM baits at Pahole did differ significantly over time (F = 4.12, p = 0.025), with recruitment at hour 3 being significantly higher than at hour 1 (Games-Howell test, p = 0.022). Differences between hours 1 and 2 were marginally significantly different (p = 0.060) and differences between hours 2 and 3 not statistically significant (p = 0.881) for SPAM at Pahole.

SPAM and peanut butter also tended to attract *S. papuana* to a higher percentage of baits offered, relative to the other two baits (Fig. 2). Again, these differences were not always statistically significant. After one hour, an interval commonly used for ant monitoring and distribution mapping (Blachly & Forschler, 1996; Lee et al., 2003; Starr et al., 2008), there was a significant association between percentage of baits occupied and bait type at Pahole NAR (Chi-square = 10.556, p = 0.014), with SPAM and peanut butter baits exhibiting higher than expected occupancy, and corn syrup and tuna/corn syrup blend exhibiting lower than expected occupancy. At Lyon Arboretum, there was no significant association between percentage of baits occupied and bait type (Chi-square = 5.830, p = 0.120). For peanut butter baits, there was no significant difference in occupancy rates between hours 1 and 2 at either Lyon (Fisher's Exact Test, p = 1) or Pahole (Fisher's Exact Test, p = 0.693). Similarly, there was no significant difference in occupancy rates between hours 1 and 2 at SPAM baits at Lyon (Fisher's Exact Test, p = 0.172) or Pahole (Fisher's Exact Test, p = 0.232).

These results indicate that both SPAM and peanut butter should be effective baits for monitoring relative densities of *S. papuana* and for mapping *S. papuana* distributions. Temporal trends suggested that exposing baits for more than one hour may increase their performance to some degree, both in terms of higher recruitment and higher bait detection, but these trends were relatively weak and usually statistically non-significant. The cost of additional monitoring time may therefore not offset these benefits. Of the two baits, peanut butter is the more practical choice. It is much cheaper than SPAM, requires no preparation and is easy to use in the field, does not spoil after opening, and adheres to monitoring cards or other monitoring markers. The high attractiveness and ease of use of peanut butter has made it an effective bait for monitoring a variety of other ant species, particularly those in the myrmecine subfamily, such as *Wasmannia auropunctata*, *Monomorium pharaonis*, *Monomorium destructor*, *Pheidole* spp., *Solenopsis geminata*, and others (Lee 2002, Causton et al. 2005, Starr et al. 2008).
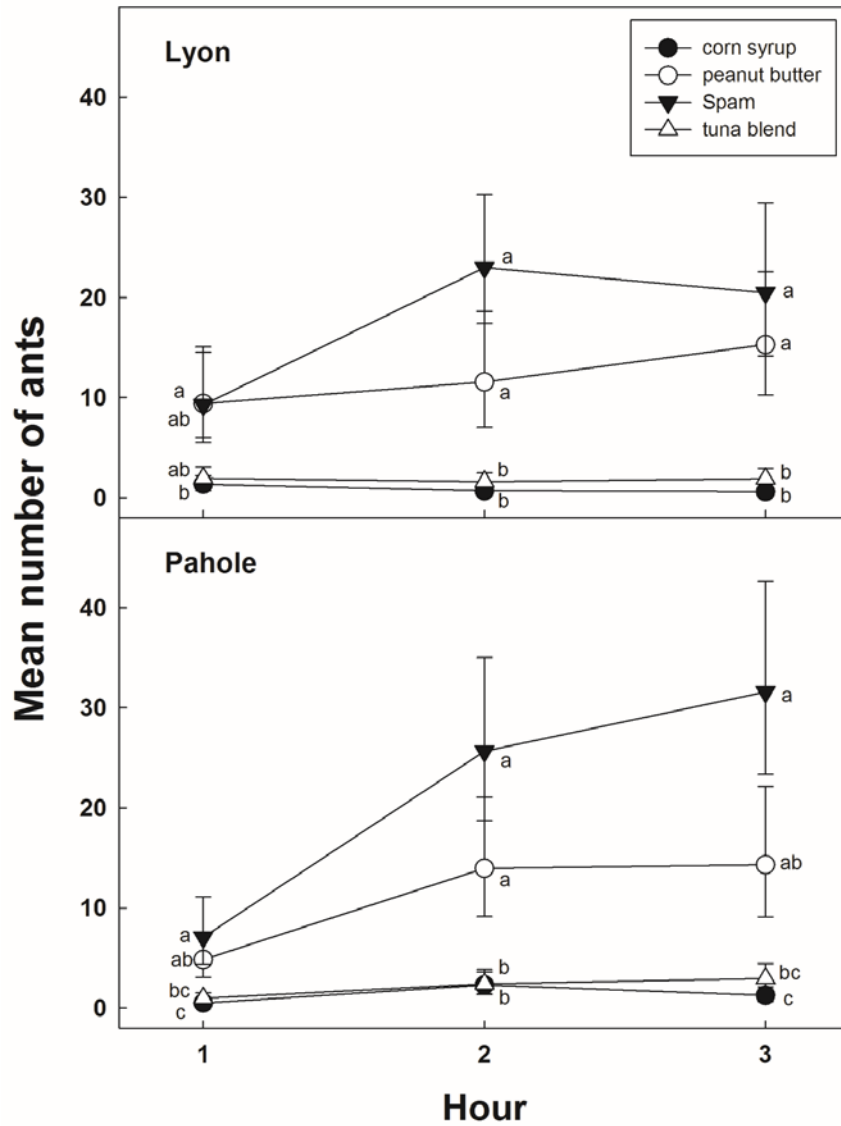
Figure 1. Mean number (±SE) of *S. papuana* attracted to food baits at Lyon (top) and Pahole (bottom) over the course of three hours. Means sharing the same letters within each hour at each site are not significantly different (Welch's ANOVA and Games-Howell posthoc test on log-transformed counts, α=0.05; depicted means and SEs are back-transformed).
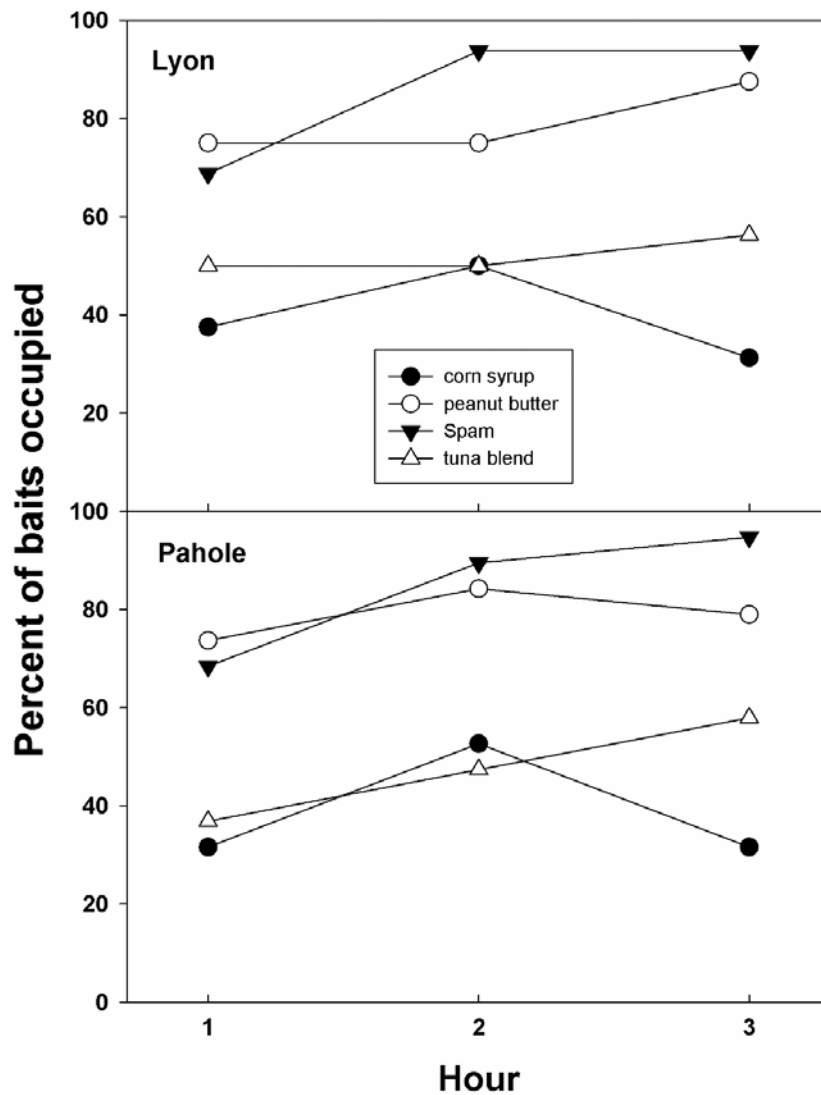
Figure 2. Percent of baits occupied by *S. papuana* at Lyon (top) and Pahole (bottom) over the course of three hours.

Pesticide bait preference

Methods

Pesticides formulated in attractive baits have the potential to be an effective ant control method because the delayed killing action allows the toxicant to be distributed through the colony (Stringer et al., 1964). Five granular commercial pesticidal ant baits were chosen to compare relative attractiveness to *S. papuana*: 1) Advion Fire Ant Bait (0.045% Indoxacarb, Syngenta Corporation), 2) Amdro Ant Block (0.88% Hydramethylnon, AMBRANDS), 3) Extinguish Plus (0.365% Hydramethylnon and 0.250% S-Methoprene, Wellmark International), 4) MaxForce Complete Brand Granular Insect Bait (1% Hydramethylnon, Bayer Environmental

Science), and 5) Siesta (0.063% Metaflumizone, BASF Corporation). These baits were chosen because they target *Solenopsis* fire ants, or because they have been found to be attractive or effective against other species in the subfamily Myrmicinae (Williams et al., 2001.; Oi & Oi, 2006; Warner et al., 2008; Hara et al., 2014). Advion Fire Ant Bait, Amdro Ant Block, Extinguish Plus, and Siesta are all based on a similar bait matrix composed of corn grit saturated with soybean oil. MaxForce Complete possesses two bait matrix types: a corn grit/soybean oil-based granule and a protein-based granule.

One half teaspoon of each bait was placed into paper cupcake wrappers and presented next to each other at replicate stations at both sites, and ant numbers were recorded every hour for three hours as described for the monitoring bait preference test. The pesticide preference test was conducted on September 18, 2015, at Pahole NAR, using a total of 25 replicate stations, and on November 6, 2015, at Lyon Arboretum, using 25 replicate stations. After excluding stations with fewer than 24 ants total across all bait types and hours, 10 stations at Pahole NAR and 23 stations at Lyon Arboretum remained for analysis. Numbers of ants (log transformed) were compared among bait types at each hour and site as described for the monitoring bait preference test.

Results and Discussion

The relative attractiveness of the five pesticidal ant baits differed somewhat by location, and large variation among replicate stations resulted in little consistent statistical separation between the baits (Fig. 3). Amdro Ant Block tended to attract the highest or second highest number of ants at both sites, but the relative positions of the other baits varied among sites. In particular, Siesta attracted a relatively high number of ants at Pahole, but the least number of ants at Lyon. The latter result was unexpected, because preliminary testing conducted at Lyon in February of 2015 suggested that Siesta was similar or greater in attractiveness than Amdro Ant Block (Ogura-Yamada, unpub. data). There may therefore be some variation in relative attractiveness tied to season or other unknown factors. Since pesticide baits are generally available to ants for longer periods of time, we did not assess using statistics whether bait attractiveness increased across the three monitoring hours.

The relatively weak and/or inconsistent differences in attractiveness among the baits is not very surprising given that they are all based completely or in part on similar corn grit and soybean oil granule matrices. However, each bait may have additional unknown proprietary ingredients that may influence attractiveness, and some active ingredients may exhibit repellency for certain ant species (Stringer et al. 1964; Reimer & Beardsley 1990; Montgomery et al. 2015)
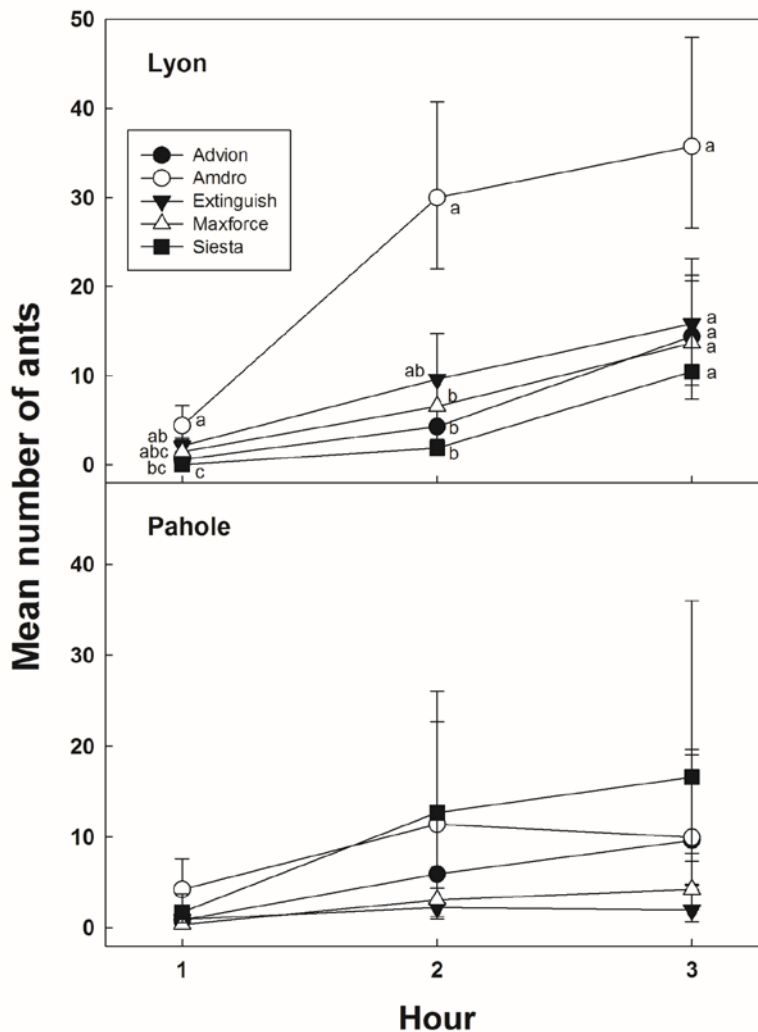
Figure 3. Mean number (±SE) of *S. papuana* attracted to pesticidal baits at Lyon (top) and Pahole (bottom) over the course of three hours. Means sharing the same letters within each hour are not significantly different (Welch's ANOVA and Games-Howell posthoc test on log-transformed counts, $\alpha=0.05$; depicted means and SEs are back-transformed). None of the means were significantly different at any hour at Pahole.

Pesticide Bait efficacy trial
    Methods
       We chose two baits, Amdro Ant Block and Siesta, to test efficacy of ant reduction over an eight-month period in field plots at Pahole NAR. Nine 5x5 m plots were established on July 3, 2015, and pre-treatment ant densities were determined in each plot: ants were counted on the top and bottom of 25 monitoring cards (one half of a 7.6 x 12.7 cm index card) baited with a smear of peanut butter. Cards were placed on the ground every 1.25 m in a grid pattern, and collected after 1.5 hours. The nine plots were subsequently randomly assigned to one of three treatments

(Amdro Ant Block, Siesta, or untreated control), with the exception that the two lowest-density plots were assigned to the control treatment to ensure that the pesticide baits were tested in plots with high ant densities. Mean pre-treatment ant densities were nevertheless fairly similar across the three treatment groups (see below). Nine bait stations, separated by 2.5 m in a grid pattern, were placed in each plot testing the two pesticide baits. The bait stations were constructed of 3.81 cm (1.5 in) long sections of 3.18 cm (1.25 in) diameter PVC tubing, fitted with PVC endcaps on the upper end. The open bottoms were screened with Amber Lumite Screen (530 µm mesh size, Lumite Inc.) fastened with PVC cement (Oatey SCS.), which allowed access to *S. papuana* workers but excluded most other non-target arthropods. Each station was supplied with 1.24 g of Amdro or 0.63 g of Siesta ant baits contained within a disposable polypropylene tea bag, which allowed ants to imbibe pesticide-laden oil from the baits while facilitating the periodic replacement of baits. Stations were staked to the ground to ensure that the endcaps shielded the bait from rain, and that contact between the screened opening and the ground was maintained.

Baits were replaced every four to seven weeks, for a total of five times, during the experiment, which ended on March 5, 2016. Ant densities in the plots were assessed on each of these dates using the peanut butter card monitoring methods described above. During each bait replacement event, bait stations were also systematically shifted such that every point located on a grid with 1.25 m spacing received a station by the second event in September, 2015. Bait stations were subsequently returned to their original positions for the remainder of the trial, except to target occasional localized surges in ant numbers in plots. Because we had only three replicate plots for each treatment, we present only descriptive statistics for trends in ant densities in the plots. To assess whether the bait station spacing interval (2.5 m grid) was effective in the Amdro and Siesta plots, we compared reductions in numbers of ants at the 25 monitoring stations in each plot (1.25 m grid) on the first monitoring event, 28 days after bait station placement, according to the distance of monitoring stations from the nearest bait station. The superimposed bait station and monitoring grids resulted in monitoring points that were either immediately adjacent to a bait station, 1.25 m from the nearest station, or 1.8 m from the nearest station. We compared reductions in ant numbers with a two factor ANOVA for each bait type, including the factors monitoring point position (n = 75) and plot number (n = 3) to control for individual plot differences. These tests were performed using Minitab v. 17.1.

Results and Discussion

Amdro was developed to combat the Red Imported Fire Ant, *Solenonpsis invicta,* after the banning of Mirex (Williams et al. 2001), and has been on the market since 1980. It is a widely used bait that has been highly effective against *Pheidole megacephala* (Reimer & Beardsley 1990; Hoffmann & O'Connor 2004; Plentovich et al. 2008, 2011), *W. auropunctata* in certain situations (Causton et al. 2005), and *S. geminata* to variable degrees (Hoffmann & O'Connor, 2004; Plentovich et al. 2008, 2011; Hoffmann et al. 2011). Siesta, a fairly newer product registered in 2007, has been shown to be effective against *P. megacephala* (Warner et al. 2008) and *S. invicta* (Thompson, 2008), and attractive to *W. auropunctata* (Hara et al. 2014). We chose to assess the efficacy of these two baits for controlling *S. papuana* in field plots in natural forest because both exhibited relatively high attractiveness to *S. papuana* at one or both of our bait preference test sites. Plots treated with Amdro generally had a greater reduction in ant densities than those treated with Siesta (Fig. 4). Ant counts in the Amdro plots dropped by 90.4 (± 4.5) % of pre-treatment levels by 28 days after bait station placement, and averaged 96.2 (±

1.1) % reduction from pre-treatment levels throughout the duration of the experiment (Table 1). Numbers of ants in the Siesta plots were very similar to those in the control plots, both of which exhibited a strong reduction from October through December 2015, possibly caused by seasonality or weather events, followed by a resurgence by February of 2016 (Fig. 4). In contrast, Amdro plots exhibited only a very small resurgence. The reason for the apparent lower efficacy of Siesta bait is unknown, but in preliminary tests with a different bait station design that made entry and exit more difficult, we observed many dead *S. papuana* workers after several hours inside stations containing Siesta, but many fewer inside stations containing Amdro. We therefore suspect that the lower efficacy of Siesta is related to the speed with which metaflumizone kills very small ants like *S. papuana*, rather than to issues with bait attractiveness.

This experiment also confirmed that our bait station design and spacing interval are effective for controlling *S. papuana* when using Amdro Ant Block. The interior of the bait stations remained fairly dry provided that the stations were not dislodged, the design made it relatively easy to replace bait, and we observed very few ants or other arthropods trapped inside them. The strong reduction in *S. papuana* numbers at monitoring stations indicated that this ant was able to easily access the bait, which was not the case in preliminary trials with a bait station design used for Argentine ants (Krushelnycky et al. 2011). The latter bait station allows entry into a PVC tube only through small holes in caps on both ends, which appears to be too restrictive to entry and exit for *S. papuana* (Ogura-Yamada unpub. data). There was also no strong evidence that the bait station spacing interval (2.5 m) was too large to achieve effective bait coverage: the magnitude of reduction in ant numbers at monitoring stations 28 days after station placement was not significantly related to distance from the nearest bait station for either Amdro Ant Block (F = 1.79, p = 0.174) or Siesta (F = 2.30, p = 0.107). In Siesta plots, however, there was a non-significant pattern suggesting potentially weaker reduction at greater distances from bait stations, which was absent in Amdro Ant Block plots (Fig. 5). It is possible that a greater spacing interval may remain effective with Amdro Ant Block bait, although some observations in preliminary trials suggest that *S. papuana* forages relatively short distances and may not effectively retrieve baits located more than several meters away from nests.

Although the attractiveness of Amdro Ant Block was not overwhelmingly stronger than the other baits tested (Fig. 3), it was consistently attractive to *S. papuana*, and has other characteristics that make it a good option for controlling *S. papuana* in natural areas. It is widely available, relatively inexpensive, and has the broadest label language regarding allowable uses, including in forested areas. The EPA (1998) considers hydramethylnon, the active ingredient in Amdro Ant Block, to be of low acute toxicity, unlikely to contaminate ground water, of low concern to birds, and to have minimal effects on terrestrial non-target organisms when used for insect control. Hydramethylnon degrades quickly when exposed to light (Vander Meer et al., 1982), so presenting the bait in stations can not only reduce non-target exposure but also prolong the potency of the bait (Taniguchi et al. 2003).
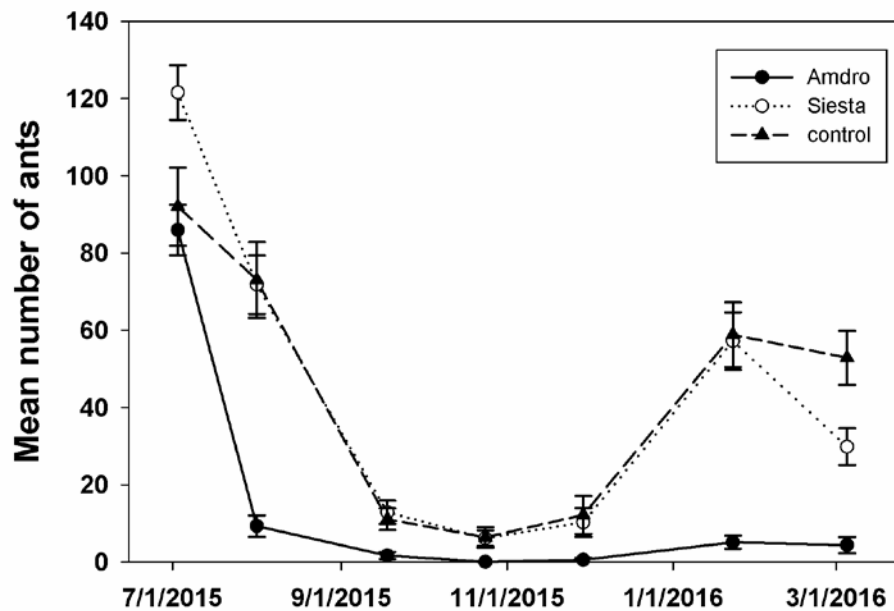
Figure 4. Mean (±SE) number of *S. papuana* in field plots treated with Amdro Ant Block and Siesta baits.
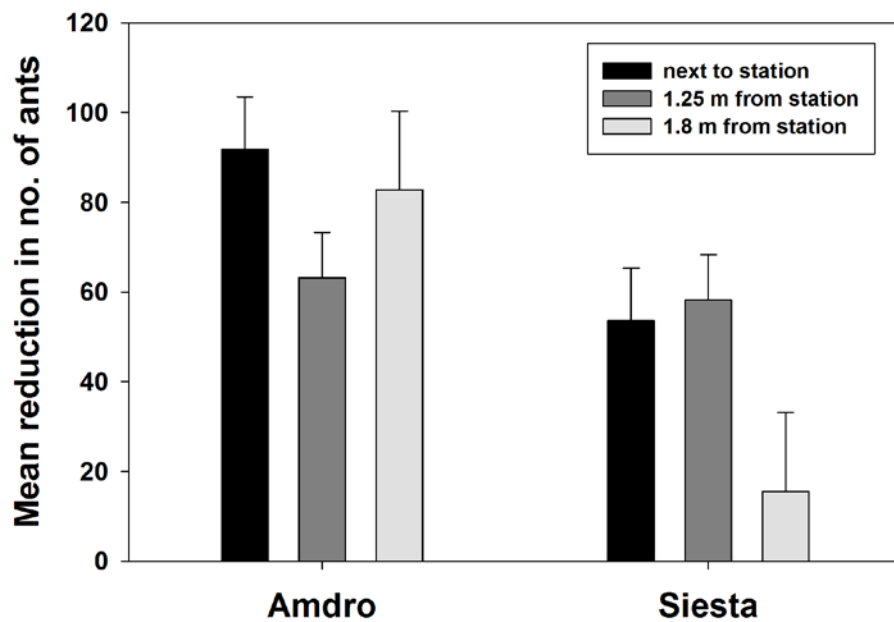

Figure 5. Mean (±SE) reduction in number of *S. papuana* 28 days after bait stations were deployed in the field plots, categorized by distance of monitoring points from bait stations. There was no significant difference (based on ANOVA, α=0.05) in degree of reduction among distances for either ant bait.

B. Effects of *S. papuana* on arthropod communities

During fiscal year 2016, S. Ogura-Yamada completed field work on this aspect of the project. Ants were suppressed for one year in the six treatment plots established in FY15 at four field sites (Ekahanui, Puu Hapapa, Pahole and Kahanahaiki), five of which were 20 x 20 m in size, and the sixth was 10 x 10 m due to restrictive topography. Ants were controlled using Amdro Ant Block bait in the same bait stations and using the same application protocol described above for the bait efficacy trial. These methods proved to be similarly effective in the larger plots: numbers of *S. papuana* were reduced on average by 83.63 (± 2.79) % over the course of one year in the treated plots, compared to an average increase of 58.73 (± 15.63) % from pre-treatment levels (with the exclusion of one extreme plot) in paired, untreated plots. At the end of this year, post-treatment arthropod sampling was completed in the plots in April-May of 2016. Sorting and identification of the arthropod samples is nearing completion, after which changes in arthropod communities following ant suppression will be compared to changes in untreated control plots.

C. Effects of *S. papuana* on native *Drosophila* reproduction

This aspect of the project plans to compare emergence rates of adult *Drosophila* flies from pieces of larval host plant material in the presence and absence of *S. papuana* in the field. This realistic experimental test of *S. papuana* impacts on *Drosophila* reproduction requires the successful oviposition of adult females on suitable host plant material, the development of larvae on the host plant material, and the capture of emerging adults in the field. Each of these steps is challenging, but progress was made during FY16 to advance this goal.

The relatively common, non-listed species *Drosophila crucigera* is being used as a surrogate for listed *Drosophila* species, since it uses the same, relatively common host plant (*Pisonia* spp.) as some of the listed species. A captive lab colony of *D. crucigera* was established in Dr. Ken Kaneshiro's *Drosophila* rearing lab in FY15 using wild-caught individuals provided by Dr. Karl Magnacca. This colony crashed several times for unknown reasons, and eventually perished. However, a successful colony was finally re-established in FY16 from additional wild flies captured by K. Magnacca. In this latest attempt, separate iso-lines were maintained from individual females, and currently several of these lines are highly productive.

Several methods were also tested for collecting, inoculating, and promoting the rotting process for pieces of *Pisonia umbellifera*, in order to create suitable oviposition and larval feeding substrate. Adult flies have been found to readily oviposit on the branch pieces, and in one test, several flies successfully emerged following development in branch pieces. Further discussions with Drs. Kaneshiro and Magnacca have led to a finalized plan for preparing the host plant material for the field trial. A cage design was also developed for capturing emerging adults in the field trial.

The upcoming experiment will install these cages in the same field plots that were used to investigate effects of *S. papuana* on the wider arthropod community (section B, above). Several months ahead of the trial, we will redeploy bait stations in the treated plots of each pair, in order to suppress ant numbers again. Subsequently, host plant branch pieces will be exposed to adult *D. crucigera* in the lab, and will then be placed inside the cages in both the treated and control

field plots, and emerging adult flies will be captured in the cages using baited fruit fly traps and yellow sticky traps. This experiment is planned for the fall to early winter of 2016.

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