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Source: Florida Entomologist, 103(1) : 103-111

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.103.0417

The effects of laboratory rearing diet on recruitment behavior of *Wasmannia auropunctata* (Hymenoptera: Formicidaea)

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Abstract

Wasmannia auropunctata (Roger) (Hymenoptera: Formicidae) is among the world's worst invasive species, and there is an increasing need for effective control methods for this species. Existing chemical treatments and baits used in managing other invasive ant species may not be as effective for managing *W. auropunctata.* Development of effective ant control treatments and baits depends on laboratory experiments to test the potential efficacy of a large number of products and control methods prior to implementation of large-scale field studies. However, anecdotal evidence suggests that laboratory-raised *W. auropunctata* may respond differently than their wild counterparts to bait types, and if this is the case, laboratory trials may not accurately predict results under field conditions. Here we report on experimental research investigating whether ant colonies raised in laboratories, and those in the field, show different patterns of recruitment to non-toxic baits. Laboratory and wild colony recruitment to non-toxic Hawaii Ant Lab gel bait, pureed tuna, and 50% gelled sucrose solution was measured via multi-choice and no-choice field recruitment studies. Secondly, we discuss experiments testing whether the bait preference of laboratory-raised *W. auropuncata* varies with their base diet. We tested 4 base diets: (1) lipid rich, (2) protein rich, (3) carbohydrate rich, and (4) a "complete" diet with lipid, protein, and carbohydrates offered as a buffet. Overall, we found that laboratory colonies differed from wild *W. auropunctata* in their foraging behaviors in no-choice and multi choice experiments, particularly in their levels of recruitment to the Hawaii Ant Lab gel bait. This contrast indicates that experimental trials may give misleading indications of potential outcomes of field trials. Further research is needed on optimal laboratory diets for laboratory-reared ant colonies. However, our results suggest that behavioral differences may be mitigated if colonies are maintained on a nutritionally limited diet while conducting laboratory experiments.

Key Words: little fire ant; bait response; bioassay; Hawaii

Resumen

Wasmannia auropunctata (Roger) (Hymenoptera: Formicidae) se encuentra entre las peores especies invasoras del mundo y existe una creciente necesidad de métodos eficaces para controlar esta especie. Los tratamientos químicos que existen y los cebos utilizados para el manejo de otras especies de hormigas invasoras pueden ser no tan efectivos para el manejo de *W. auropunctata*. El desarrollo de cebos y tratamientos eficaces para el control de hormigas depende de experimentos de laboratorio para probar la eficacia potencial de una gran cantidad de productos y métodos de control antes de la implementación de estudios de campo a gran escala. Sin embargo, la evidencia anecdótica sugiere que las *W. auropunctata* criadas en el laboratorio puede responder de manera diferente a sus contrapartes silvestres a las class de cebo, y si este es el caso, los ensayos de laboratorio no pueden predecir con precisión los resultados en condiciones de campo. Aquí informamos sobre investigaciones experimentales que investigan si las colonias de hormigas criadas en laboratorios, y aquellas de campo, muestran diferentes patrones de reclutamiento de cebos no tóxicos. El reclutamiento de colonias de esta especie criadas en el laboratorio y silvestres al cebo no tóxico de gel Hawaii Ant Lab, el atún en puré y la solución de sacarosa gelificada al 50% fueron medidas por medio de estudios de selección múltiple y de no elección en el campo. En segundo lugar, discutimos los experimentos que prueban si la preferencia de cebo de *W. auropuncata* criado en laboratorio varía con su dieta de base. Probamos dietas de 4 bases: (1) ricas en lípidos, (2) ricas en proteínas, (3) ricas en carbohidratos, y (4) una dieta "completa" con lípidos, proteínas y carbohidratos que se ofrecen como un buffet. En general, encontramos que las colonias de laboratorio de *W. auropunctata* difirieron de las colonias silvestres en su comportamiento de forrajeo en experimentos de no elección y de elección múltiple, particularmente en sus niveles de reclutamiento para el cebo de gel Hawaii Ant Lab. Este contraste indica que los ensayos experimentales pueden dar indicaciones engañosas de los resultados potenciales de los ensayos de campo. Se necesita más investigación sobre dietas de laboratorio óptimas para colonias de hormigas criadas en laboratorio. Sin embargo, nuestros resultados sugieren que las diferencias de comportamiento pueden mitigarse si las colonias se mantienen con una dieta nutricionalmente limitada mientras se realizan experimentos de laboratorio.

Palabras Clave: pequeña hormiga de fuego; respuesta al cebo; bioensayo; Hawaii

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Ants (Hymenoptera: Formicidae) are important terrestrial invertebrates in terms of biomass and filling niches essential for healthy ecological functioning (Andersen 1988; Abbott 1989; Holldobler & Wilson 1990; Porter & Savignano 1990; Folgarait 1998). The vast majority of the more than 12,000 described species (Ward 2007) are innocuous, but some are renowned for their destructive impacts on habitats to which they are introduced and regarded as pests (Zimmerman 1970; Beardsley 1980; Howarth 1985; Cole et al. 1992; Reimer 1994; Daly & Magnacca 2003; Krushelnycky & Gillespie 2008; Bleil et al. 2011; Fasi et al. 2013).

Control of pest ants is difficult because feeding preferences, biology, and behaviors vary between species (Silverman & Brightwell 2008; Gentz 2009). Research on nesting habits, nutrient requirements, food preferences, and chemical sensitivity has resulted in species-specific control methods for some ant species, and formulation of baits for control of a variety of species within a feeding group, such as sugar-loving, lipid-loving, or protein-loving ants (Braness 2002). *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) is among the world's worst invasive species, and there is an increasing need for effective control methods for this species. The International Union of the Conservation of Nature's Invasive Species Specialist Group (Lowe et al. 2000) has listed this species as having negative impacts on agriculture, quality of residential life, and native ecosystems throughout their introduced range (B. M. Drees et al., unpublished; Davis & Van Schagen 1993; Abedrabbo 1994; Haines et al. 1994; Feener 2005; Cooper et al. 2008; Arakaki et al. 2009). This ant species has been reported to reduce biodiversity, farm phytophagous insects that vector plant disease (Smith 1929; Spencer 1941; Fabres & Brown 1978; Fowler et al. 1990; Delabie & Cazorla 1991; Delabie et al. 1994; Jourdan 1997; de Souza et al. 1998; Wetterer et al. 1999; Armbrecht & Ulloa-Chacon 2003; Le Breton et al. 2003, 2005; Walker 2006; Fasi 2009; Vonshak et al. 2009; Berman et al. 2013; Fasi et al. 2013; Vanderwoude et al. 2016), and are linked to the occurrence of tropical keratopathy (clouding of the cornea resembling cataracts) in wild and domestic vertebrates (Roze et al. 2004; Theron 2005; Rosselli & Wetterer 2017). In addition to ecological impacts, *W. auropunctata* is considered a major nuisance pest due to its painful stings in residential and agricultural environments (Spencer 1941; Fabres & Brown 1978; Fasi et al. 2016).

This species has gained little recognition as a pest in the continental USA. Although it has been established in Florida for almost a century, economic and ecological impacts appear to be minimal (Smith 1929; Spencer 1941). This is in contrast to the Pacific region, including Hawaii (Fabres & Brown 1978; Clark et al. 1982; Lubin 1984; De La Vega 1994; Lowe et al. 2000; Jourdan 2001; Holway et al. 2002; Armbrecht & Ulloa-Chacon 2003; Le Breton et al. 2003; Wetterer & Porter 2003; Vanderwoude et al. 2016) where impacts can be severe and widespread. Like *Solenopsis invicta* Buren (Hymenoptera: Formicidae), *W. auropunctata* respond primarily to lipid and protein baits, and it is generally assumed that commercial "fire ant" baits formulated for *S. invicta* also will be suitable for *W. auropunctata*. Despite this assumption, Montgomery et al. (2015) reported that this may not be the case given their observation that *W. auropunctata* is repelled by the insect growth regulator (S)-methoprene, an active ingredient used in some fire ant baits. Chemical sensitivity, biology, behavioral, and ecological differences between *S. invicta* and *W. auropunctata* are all factors influencing the efficacy of currently available baits against the latter species (Montgomery et al. 2015), thus necessitating species-specific bait development and laboratory trials.

As invasive ants continue to spread and new pest species emerge, the need for species-specific research grows. Laboratory experiments are essential to assess biology, behaviors, and screen efficacy of various pesticide formulations prior to implementing management plans for new pests. Generally, laboratory reared insects are frequently used as test subjects for pesticide efficacy trials against ants and other insects (Banks et al. 1983; Braness 2002), including post-harvest treatment studies (Follett & Armstrong 2004), biological control screening (Castillo et al. 2014), dietary and feeding studies (Bhatkar & Whitcomb 1970; Marchioro & Foerster 2012), as well as biological and behavioral observation studies (Adams & Traniello 1981; Howard et al. 1982; Abril et al. 2008; Kirschenbaum & Grace 2008; Rey et al. 2013). Laboratory colonies often are maintained for extended periods of time in controlled environments, often spanning many generations with little resemblance to the insects' natural habitat. This removal and disconnect from natural conditions raises questions regarding the biological and behavioral equivalence of laboratory-reared insects, their suitability as test subjects, quality of test results, and whether or not those results truly predict what should be expected under field conditions. Moreover, insects reared on artificial diets generally differ from their wild counterparts in feeding and foraging behavior (Herard et al. 1988; Propkopy et al. 1989; Ennis et al. 2015), predator evading capabilities (Hendrichs et al. 2007), and response to pheromone cues (Propkopy et al. 1989; Clark et al. 2011), all of which are evolutionary traits essential for species survival. For example, laboratory studies using *S. invicta* found that nutritional voids in diet caused foraging workers to feed on and bring back more food items that contained the limiting nutrient than others (Sorenson et al. 1985; Behmer 2009; Cook et al. 2010).

In addition, laboratory rearing diets are not analogous to natural diets (Marchioro & Foerster 2012; Ennis et al. 2015), and often are formulated to ensure an adequate supply of essential nutrients is available at all times. In contrast, wild populations are limited by irregular supply of at least some of these nutrients that, in turn, may alter feeding preferences when these nutrients become available. Dussutour and Simpson (2008) reported that ant foraging behavior is influenced by nutritional demand signals from their larvae. Cassill and Tschinkel (1999) and Portha et al. (2002) found that foraging workers adjust which resources are collected and shared to meet carbohydrate, protein, and lipid nutritional needs of the colony. In addition, nutrient allocation is regulated by nurse ants and foraging workers to promote and maintain optimal colony growth (Dussutour & Simpson 2008). Several studies have shown that insect rearing diets can influence directly the outcome of laboratory experiments and produce results not indicative of the behavior of that species under field conditions (Huettel 1975; Propkopy et al. 1989; Marchioro & Forester 2012; Ennis et al. 2015). This is especially important when evaluating insecticidal bait matrices and the attractants they may contain.

Nonetheless, bait development requires laboratory experiments to assess the potential of new formulations, and evaluate the efficacy of currently available baits for new pest ant species. The advantages of artificial laboratory diets are numerous and include yr-round availability, and the ability for researchers to manipulate and control nutrient content (Ennis et al. 2015). Laboratory diets for *W. auropunctata*, as with many ant species, requires a mixture of carbohydrates, proteins, and lipids, along with some insect matter as a source of chitin to ensure prolonged colony health and survival (Williams et al. 1987; Porter et al. 2015). Several laboratory diets have been developed and reported as effective for rearing *S. invicta*, a species often used in laboratory studies. All such diets require nutrient supplementation with insect matter such as mealworms, or whole crickets, for continued brood production and colony growth (Bhatkar & Whitcomb 1970; Williams et al. 1987; Keller 1989; Porter et al. 2015). The disadvantage of nutritionally defined laboratory diets is that they are consistent and homogenous, which does not reflect the constantly changing nutritional needs of the colony or the temporal and spatial variability of the supply of these nu-

trients. Therefore, the objective of our study, reported herein, was to investigate whether rearing diet influenced *W. auropunctata* foraging behaviors on various non-toxic baits in the laboratory when compared with wild colonies.

Materials and Methods

2015 PILOT STUDY

Laboratory and field trials evaluating attractiveness and palatability of non-toxic protein, carbohydrate, and lipid rich baits were conducted between Feb and Jun of 2015. Both trials consisted of no-choice and multi-choice recruitment to assess bait preference. Laboratory experiments were conducted in a greenhouse with a glass roof and screen mesh walls (Hawaii Department of Agriculture, Hilo, Hawaii, USA (19.706494°N, 155.074455°W), where ambient temperature and relative humidity were not controlled, but was comparable to that of exposed outdoor environments. Laboratory colonies of *W. auropunctata* were used in these studies, and had been in continuous culture for approximately 4 mo prior to use. Ants from this colony were sourced originally by extraction from infested banana leaf litter collected from the University of Hawaii Experimental Farm in Pana'ewa, Hawaii Island (19.651408°N, 155.049938°W). To maintain a natural worker to queen ratio of 250 to 500 (Ulloa-Chacon & Cherix 1990), an average of 1,120 workers, 3 queens, and brood were transferred to 35 × 20 cm plastic Sterilite® containers (Sterilite Corporation, Townsend, Massachusetts, USA) with artificial nests made of a 16 × 150 mm glass test tube covered in black paper with approximately 10 mL of water and cotton wool inserted for moisture.

Prior to each experiment, colonies underwent a 4 wk acclimation period during which they were fed a standard diet that was a modified version of Keller's non-desiccating rearing diet (Keller 1989), where crickets were substituted for mealworms (referred to as Keller Cubes). This diet consisted of an oligidic (non-chemically defined) mixture of sugar, protein, lipid, mineral, and vitamins blended together for an "all-in-one" diet. The ingredients in this diet have been accepted among researchers studying laboratory ant colonies as a standard rearing medium. This standard diet was compared with 3 experimental dietary treatments (in no-choice tests) that consisted of (1) a high lipid diet (Great Value™ Creamy Peanut Butter, Walmart Apollo LLC, Bentonville, Arkansas, USA); (2) a high carbohydrate diet (unrefined honey); and (3) a "complete" diet comprised of unrefined honey, Great Value™ Creamy Peanut Butter, 1 quarter of an Up & Up™ brand Jumbo cotton ball (Target Brands Inc., Minneapolis, Minnesota, USA) soaked, but not dripping, with vegetable oil (Great Value™, Walmart Apollo LLC, Bentonville, Arkansas, USA) presented buffet fashion.

Brood production and colony growth of ants is known to cease without insect supplementation (Vogt 2003; Kay et al. 2010; Gavilanez-Slone & Porter 2014; Porter et al. 2015), whereas low carbohydrate diets are known to cause high worker mortality and reduced colony activity (Kay et al. 2006; Cook et al. 2010; Gavilanez-Slone & Porter 2014). Therefore, all colonies received whole dead crickets and 25% sugar water ad libitum in addition to their experimental dietary treatment. Colonies assigned to the standard diet did not receive whole dead crickets because crickets were already an ingredient in the Keller Cubes. Dietary treatments were randomly assigned to experimental colonies within each replicate. Experiments were completed at 28 d and replicated 6 times. Nutritional analyses for each dietary treatment are listed in Table 1. Laboratory experiments were separated temporally by 4 wk for ants to re-habituate to normal rearing conditions and ensure all colonies used in the second experiment were equally healthy. During this time any declining colonies were replaced.

Choice experiments used the same methods as mentioned earlier for no-choice experiments, and consisted of measuring recruitment to 3 non-toxic test baits: (a) Hawaii Ant Lab Gel Bait Matrix (Montgomery et al. 2015) consisting of 40% vegetable oil, 56% water, 0.8% xanthan gum, and 3.2% NOW® Argentine Beef Liver (NOW®, Bloomingdale, Illinois, USA); (b) gelled 50% sucrose solution; and (c) Star Kist® Chunk Light Tuna (Star Kist Co., Pittsburgh, Pennsylvania, USA) in water.

Food was withheld for 24 h prior to exposure to treatments offered in a buffet style by applying approximately 1 g of each diet on 9 × 3 cm plastic-laminated cards placed in the foraging area of each experimental colony. Cards were divided into 3 marked areas of 3 × 3 cm and bait placements randomized on the cards. Recruitment to each bait was measured once per wk for 5 wk by recording and examining high-resolution digital photographs of each card taken 60 min after exposure. Photographs were examined in the laboratory and ants on bait cards (within each of the 3 marked areas) were counted and recorded. By the end of the multi-choice experiment, 1 colony had died completely and several others had greatly declined.

Because *W. auropunctata* is typically controlled in the field through broadcast application of lipid based baits, the no-choice experiment focused only on recruitment to the Hawaii Ant Lab Gel Bait matrix. Experimental colonies were fed the same dietary treatments as in the multi-choice experiment, treatments again were randomly assigned within each replicate, and the experiment was replicated 6 times. Experimental colonies were maintained on their respective diets for 7 wk. Small amounts of Hawaii Ant Lab Gel Bait approximately 1 cm in diameter were applied to 4.5 × 4.5 cm square laminated cards and placed in the foraging area of each experimental colony. Recruitment was measured by recording high-resolution digital photographs of each card taken 60 min after exposure. Photographs were examined in the laboratory, and ants on bait cards were counted and recorded. As observed in the multi-choice experiment, by the end of the no-choice experiment many of the colonies had declined and were visibly unhealthy.

Table 1. Nutritional breakdown as the percent of lipid, carbohydrate, and protein for each dietary treatment for the 2015 pilot study's laboratory component. Sources where nutritional information was obtained for each diet and the ingredients for the Keller Cube diet are provided.

Diet	Percent lipid	Percent protein	Percent carbohydrate	Information source
Keller Cubes	4	7	2	USDA National Nutritional Database, Libby, Niell & Libby® Corned Beef product label, Food Insects Newsletter
Peanut Butter + crickets*	56	35	27	Great Value™ Creamy Peanut Butter product label, Food Insects Newsletter l
Honey + crickets*	6	13	87	USDA National Nutritional Database, Food Insects Newsletter
Buffet + crickets*	100	35	100	USDA National Nutritional Database, Food Insects Newsletter

*Total values for the some dietary treatments may total over 100% due to each element of the diet being offered separately and not being combined into an "all-in-one" diet, as is the case with the Keller Cubes diet.

Field trials were conducted concurrent with laboratory experiments at the University of Hawaii Hilo Experimental Farm in Pana'ewa, Hawaii Island, where the laboratory colonies were initially sourced. The same non-toxic baits tested in the laboratory experiments were placed in the field where forager recruitment to bait was measured at 60 min after exposure. Field experiments also consisted of multi-choice and no-choice experiments; however, the no-choice experiment compared all 3 non-toxic baits.

The field multi-choice experiments consisted of 15 replicates spaced 5 m apart throughout the study site. Replicates were comprised of three 4.5 × 4.5 cm square laminated bait cards arranged in a small 13.5 cm triangle, and bait placement was randomized. The field no-choice experiments were configured in a randomized block design with 10 replicates, where each bait treatment was represented only once per block and spaced 5 m apart to establish independence. Bait stations consisted of a single 4.5 × 4.5 cm laminated card, and treatments were randomly assigned within each block.

At the culmination of the experiment, 2 main issues were identified as detrimental to the experiment and results: (1) the nutritional composition of the dietary treatments overlapped, confounding analyses, and (2) colony health declined to the point that some colonies stopped foraging and 1 colony died during the experiment. These results were deemed to be unreliable and the 2015 experiments were treated as a pilot study and not used in subsequent analyses.

2016 STUDIES

In 2016, no-choice and multi-choice laboratory and field experiments were repeated with the laboratory component being conducted entirely under controlled conditions at the University of Hawaii College of Tropical Agriculture and Human Resources Waiakea Experiment Station (19.643402°N, 155.079969°W). Based on the findings by Galvinez-Slone and Porter (2014) a simple diet of 25% sucrose solution and dead crickets was more suitable for long-term rearing of fire ant colonies than the oligidic diet (Keller 1989) previously used as the standard treatment during the 2015 pilot study. Therefore, the dietary treatments consisted of (1) standard carbohydrate based diet (25% sucrose solution); (2) lipid based diet (small cotton wick saturated in Great Value™ vegetable oil); (3) protein based diet (pureed Star Kist™ Chunk Light Tuna in water); and (4) "complete diet buffet" (i.e., a mixture of diets 1 to 3).

All colonies received whole dead crickets ad libitum in addition to the treatment in order to maintain brood production and maintain colony health. Nutritional analyses for each 2016 dietary treatment are listed in Table 2. Pureed tuna was replaced twice per wk due to rapid dehydration and desiccation. For multi-choice and no-choice experiments, ants were starved for 48 h prior to non-toxic bait exposure. During the multi-choice trial, ant recruitment was recorded at pre-treatment (0 d after treatment) and post-treatment (31 d after treatment). For the no-choice experiment, recruitment data were collected each wk from 0 d (pre-treatment) to 49 d after treatment. Ant recruitment rates to each non-toxic bait followed the same procedures as in the 2015 pilot study. Overall mortality at the end of each experiment was assessed by counting all dead and live ants and comparing between dietary treatments.

Statistical Analysis

Laboratory Multi-Choice Study. To determine if there are interactions between dietary treatment and ant recruitment rate to non-toxic baits, the difference between pre-treatment recruitment rates (0 d after treatment) and the final recruitment rates from each multi-choice experiment was analyzed. Data from each experiment were analyzed separately via Analysis of Covariance (ANCOVA, $P < 0.05$) (R© Statistical software v 3.2.3, R Development Core Team 2012) in order to accommodate negative values that could not be accommodated using a Poisson distributed generalized linear mixed effects model. An ANCOVA was more appropriate than a linear mixed model ANOVA ($P < 0.05$) due to our interest in overall effect of dietary treatment to bait response, as opposed to the change in response to bait over time after treatment (Dimitrov & Rumrill 2003; Knapp & Schafer 2009). Because the model compared the difference between pre- and post-treatment recruitment rates, a covariant was needed to account for variation in baseline recruitment rates between colonies. Therefore, mean pre-treatment recruitment to all 3 baits for each colony was calculated and included in the model as a covariant. Multiple pairwise comparisons were tested via Tukey's post hoc analysis, and the resulting covariant-adjusted means were used to determine whether laboratory ants showed a clear preference for a non-toxic bait when given a choice within each dietary treatment, and between all dietary treatments (Ramsey & Schafer 2002). Figures are presented using non-transformed means.

Laboratory No-Choice Study. Data were analyzed via a generalized linear mixed model using the log link function of Poisson distributed data where colony was a random variable nested in d after treatment and pairwise comparisons obtained via Tukey's post hoc analysis ($P < 0.05$). An observation-level random effect was included in the model to address over dispersion issues (Harrison 2014). Results were exponentially back transformed and interpreted as multiplicative outcomes (Ramsey & Schafer 2002). Overall mortality rate between dietary treatments was analyzed using a 1-way ANOVA ($P < 0.05$).

Field Multi-Choice and No-Choice Studies. To determine which non-toxic bait wild ants prefer, data from no-choice and multi-choice field experiments were analyzed the same way as the no-choice laboratory study. Results of the field experiments were used for qualitative comparison when interpreting the results from the overall bait preference of laboratory colonies in order to determine whether or not laboratory and wild colonies have similar preference for and recruitment rate to non-toxic baits.

Table 2. Nutritional breakdown as the percent of lipid, carbohydrate, and protein for each dietary treatment for the 2016 laboratory experiments are displayed in the table. Sources where nutritional information was obtained for each diet are provided.

Diet	Percent lipid	Percent protein	Percent carbohydrate	Information source
25% sucrose solution + crickets	0	13	30	USDA National Nutritional Database, Food Insects Newsletter
Pureed tuna + crickets	1	23	5	Star Kist™ Chunk Light Tuna in water product label, Food Insects Newsletter
Vegetable oil wick + crickets*	100	13	5	USDA National Nutritional Database, Food Insects Newsletter
Buffet + crickets*	100	32	30	USDA National Nutritional Database, Food Insects Newsletter

*Total values for the some dietary treatments may total over 100% due to each element of the diet being offered separately and not being combined into an "all-in-one" diet.

Results

2016 LABORATORY EXPERIMENTS

Results from the multi-choice experiment identified a significant interaction between dietary treatment and bait ($F_{6,32}$ = 4.01; *P* = 0.003). All colonies recruited to 50% gelled sucrose more than the other baits after 49 d of exposure to their respective dietary treatment (Fig. 1); however, these differences were not significant within the protein based dietary treatment (Sucrose-Hawaii Ant Lab t_{47} = 2.149; *P* = 0.091; Sucrose-Tuna t_{47} = 0.298; *P* = 0.952). Ants maintained on the complete buffet diet recruited to 50% gelled sucrose significantly more than the Hawaii Ant Lab gel bait with a mean difference in recruitment rate of 53.8 ants (t_{47} = 3.212; *P* = 0.007), but recruitment between 50% gelled sucrose and tuna was not significantly different (t_{47} = 1.158; *P* = 0.484). Recruitment to the Hawaii Ant Lab gel bait was also not significantly different compared with tuna (t_{47} = 2.053; *P* = 0.111). Recruitment patterns for ants maintained on the lipid based diet mirrored that of the complete buffet dietary treatment. Recruitment rate to 50% gelled sucrose was significantly greater than to the Hawaii Ant Lab gel bait with a mean difference in recruitment rate of 55.0 ants (t_{47} = 3.283; *P* = 0.005), and no difference in recruitment rate was detected between 50% gelled sucrose and tuna (t_{47} = 1.015; *P* = 0.571) or between the Hawaii Ant Lab gel bait and tuna (t_{47} = 2.268; *P* = 0.070). Recruitment rates to all 3 non-toxic baits were significantly different among ants maintained on the standard carbohydrate based dietary treatment. Ants recruited to 50% gelled sucrose more than both the Hawaii Ant Lab gel bait (mean difference in recruitment = 139.6 ants; t_{47} = 8.33; *P* < 0.0001) and tuna (mean difference in recruitment = 75.4 ants; t_{47} = 4.50; *P* = 0.0001). Greater recruitment rates also were recorded for tuna compared with the Hawaii Ant Lab gel bait (mean difference in recruitment = 64.2 ants; t_{47} =3.832; *P* = 0.001). Among ants maintained on the protein based dietary treatment, no significant differences in recruitment rate were detected between any of the baits (Sucrose-Hawaii Ant LabL t_{47} = 2.149; *P* = 0.091; Sucrose-Tuna t_{47} = 0.298; *P* = 0.952; Tuna-Hawaii Ant Lab t_{47} = 1.851; *P* = 0.165).

Results from the laboratory no-choice experiment indicated diet also influenced recruitment rate to the Hawaii Ant Lab gel bait when no other non-toxic bait was available. Colonies maintained on the standard carbohydrate based diet had an 83% higher median recruitment rate to the Hawaii Ant Lab gel bait compared with colonies maintained on the buffet diet (z = 3.12; *P* = 0.01) and 84% higher median recruitment rate than colonies maintained on the lipid based diet (z = 3.15; *P* = 0.009). Colonies maintained on the protein based diet had a 138% higher median recruitment rate compared with colonies maintained on the buffet diet (z = 4.48; *P* < 0.001) and 140% higher median recruitment rate compared with colonies maintained on the lipid based diet (z = 4.51; *P* < 0.001). Colonies maintained on lipid based and the buffet diets did not differ in recruitment rate to the Hawaii Ant Lab gel bait (z = 0.029; *P* = 0.999). Recruitment rate between colonies maintained on protein and carbohydrate based diets differed only slightly and were not significantly different (z = 1.359; *P* = 0.525). Results from analysis of colony mortality data indicated final mortality rates did not differ significantly regardless of dietary treatment ($F_{3,16}$ 0.136; *P* = 0.937).

2016 FIELD EXPERIMENTS

Results from the field multi-choice experiment indicated significantly higher recruitment rates to the Hawaii Ant Lab gel bait over all other baits tested (Fig. 2) with an 11-fold greater (z = 9.07; *P* < 0.001) median recruitment rate to the Hawaii Ant Lab gel bait compared with tuna and 6-fold greater recruitment when compared with gelled

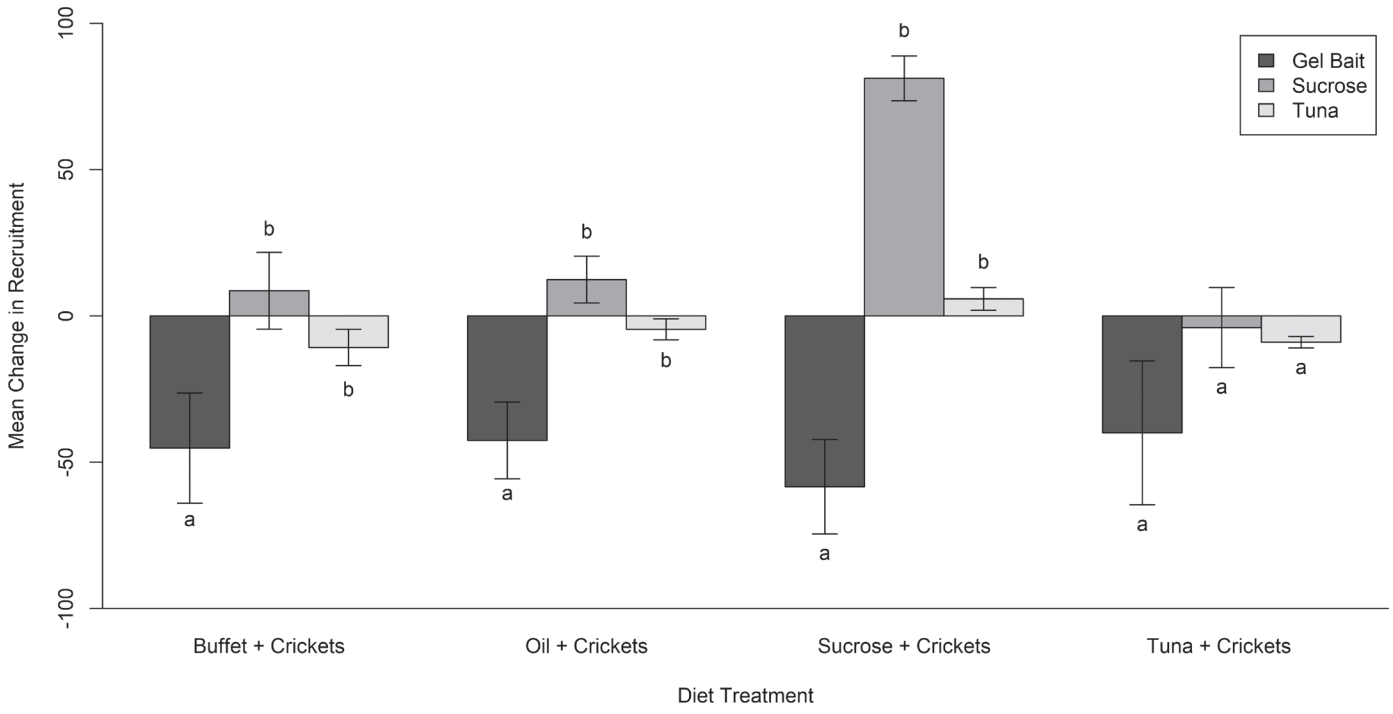

Fig. 1. Mean ± SE difference in recruitment rates of laboratory raised *Wasmannia auropunctata* to non-toxic baits: Hawaii Ant Lab gel bait, 50% gelled sucrose solution, and tuna between pre- and post-treatment measurements of the multi-choice laboratory experiment (n = 5 colonies). Bars in each group with different letters above have statistically different means (*P* < 0.05). Colonies were exposed to their respective dietary treatment (buffet plus crickets: n=5, vegetable oil wick plus crickets: n=5, 25% sucrose solution plus crickets: n=5, and pureed tuna plus crickets: n=5) for 49 d. Means represented in this chart are based on raw data for visualization and are not the reported marginal means.

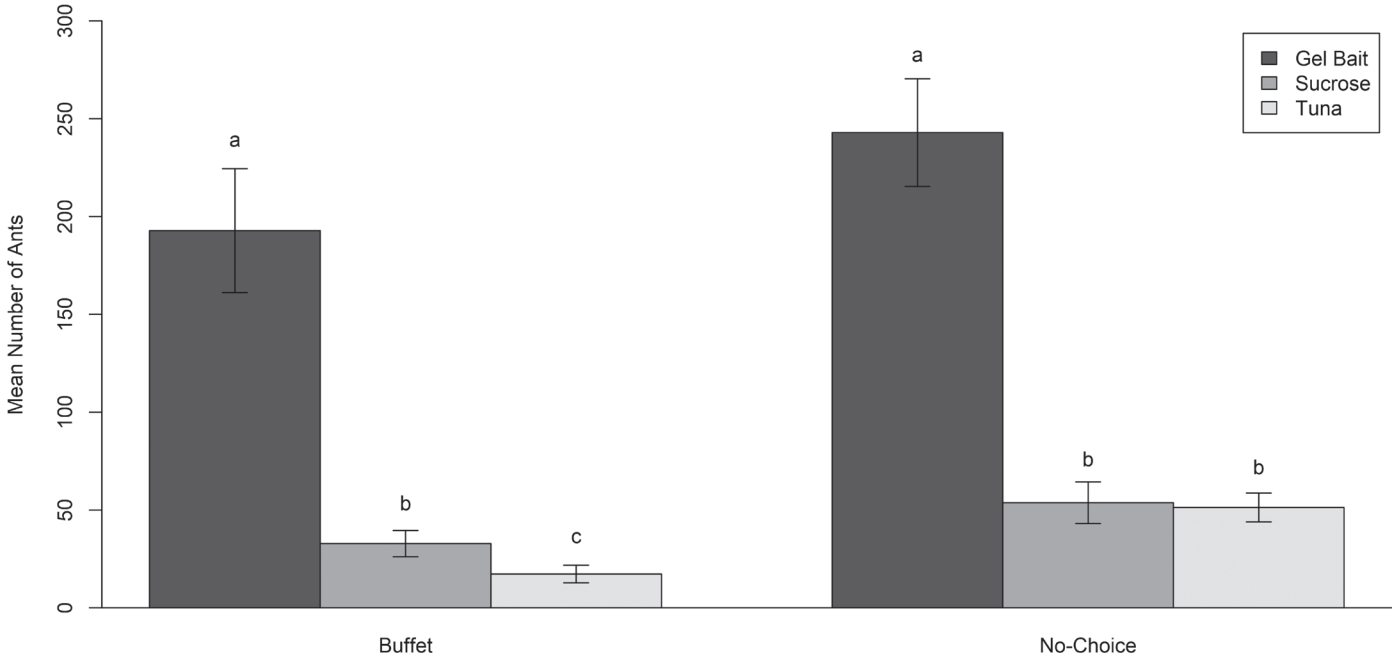

Fig. 2. Recruitment rates (mean number of ants ± SE) of wild *Wasmannia auropunctata* to the Hawaii Ant Lab gel bait, 50% gelled sucrose solution, and tuna for multi-choice (n = 6 per treatment) and no-choice (n = 6 per treatment) field experiments. Bars within clusters with different letters above have statistically different means ($P < 0.05$). Means represented in this chart are based on raw data for visualization and not proportional results from the Poisson distributed generalized linear mixed model as reported.

sucrose. Median recruitment rates to 50% gelled sucrose was 2-fold greater than to tuna (z = 2.41; $P = 0.042$).

Results from the no-choice experiment mirrored the multi-choice experiment. Recruitment rates to the Hawaii Ant Lab gel bait were significantly greater than to the other 2 baits (Fig. 2). Median recruitment rate to the Hawaii Ant Lab gel bait was nearly 5-fold (4.8 times) greater than both tuna (z = 6.39; $P < 0.001$) and 50% gelled sucrose solution (z = 6.64; $P < 0.001$). No significant difference in recruitment rate was detected between tuna and 50% gelled sucrose solution (z = 0.276; $P = 0.959$).

Discussion

Our results from the laboratory experiments suggest diet may affect recruitment rates to resources containing different nutritional profiles; however, the differences in recruitment rates did not appear to be driven entirely by nutritional voids, as described in previous studies conducted on *S. invicta* (Sorenson et. al. 1985; Cassill & Tschinkel 1999; Behmer 2009). When only a single bait option (Hawaii Ant Lab gel bait) was provided to laboratory colonies during the no-choice experiment, a marked difference in recruitment rate was observed. We also found that colonies maintained on diets limited in lipids, such as carbohydrate based (25% sucrose) and protein based diets (tuna), recruited to the Hawaii Ant Lab gel bait significantly more than colonies maintained on diets that included vegetable oil soaked cotton ball (lipid based and complete buffet diets). This supports the hypothesis that nutritional voids influence foraging behavior and bait preferences in laboratory colonies. Conversely, results from the multi-choice experiment indicated a strong preference for 50% gelled sucrose regardless of whether or not carbohydrates were included in their respective dietary treatment.

Results from our field studies indicate *W. auropunctata* are attracted to lipids above other nutrients (Williams & Whelan 1992; Ndueze et al. 2013; Montgomery et al. 2015). This is consistent with historical observations and past studies (Williams & Whelan 1992) and re-affirms the use of lipid-rich lures and baits for survey, detection, and control of this species.

Our studies further suggest a disconnect between the manner in which laboratory *W. auropunctata* and wild *W. auropunctata* behave toward food baits. These results have profound implications in regard to results collected from laboratory bait preference, bait efficacy, and dietary studies. Laboratory reared insects do not behave similarly to wild populations; therefore, the results from laboratory experiments are not necessarily relevant in the context of projecting possible outcomes of large field studies and efforts focused on laboratory experiments may, in fact, be counterproductive.

We were able to elicit a reliable recruitment rate to the Hawaii Ant Lab gel bait from ants maintained on lipid-limited diets during the no-choice laboratory experiment. This suggests reasonably reliable results may be obtained from bait palatability and efficacy experiments on *W. auropunctata* laboratory colonies provided they are maintained on a diet limited in lipids and that all baits tested are formulated with similar nutrient composition. For example, reasonably reliable results can be expected when testing preference between peanut butter, Hawaii Ant Lab gel bait, and various proprietary fire ant baits, because all baits being evaluated are formulated with high lipid content as the primary food attractant. Results from such experiments are more likely to reflect results of future field trials. Experiments comparing baits formulated with carbohydrates as the primary food attractant should not be compared against baits formulated with lipids as the primary food component. Additionally, laboratory data should be paired with field experiments for result validation whenever possible.

Although providing all essential nutrients to laboratory colonies in a buffet, not as an "all-in-one" diet such as the Keller Blocks, is beneficial for colony maintenance and growth (Gavilanez-Slone & Porter 2014), it appears to confound the results of feeding experiments with *W. auropunctata* as test subjects. Since wild *W. auropunctata* prefer-

entially recruit to baits high in lipids, our results suggest it is important to limit the amount of lipids in the rearing diet prior to conducting a laboratory experiment.

Past studies have shown other ant species raised on high protein and low sugar diets can have high mortality rates in laboratory colonies (Dussutour & Simpson 2008; Cook et al. 2010). Our observations, combined with the findings of past research, suggest high protein with low sugar diets also are not appropriate for maintaining laboratory colonies, regardless of the results from the studies reported here. Despite no significant difference in mortality rate between dietary treatments and no significant difference in recruitment rates compared with colonies maintained on carbohydrate rich diets, colonies maintained on a protein-rich diet without carbohydrates generally appeared to be less active, and exhibited slightly higher mortality and lower brood production than treatments where a carbohydrate resource was included. It is our recommendation that laboratory colonies of *W. auropunctata* be maintained on a diet consisting primarily of carbohydrates and crickets with occasional protein supplements in order to maintain healthy colonies while maintaining the integrity of data collected from laboratory experiments and reliability of results. Additional lipids may be supplemented occasionally but should be withheld entirely for 1 to 2 wk prior to conducting a laboratory experiment in order to elicit a reliable recruitment response to lipid based baits.

In conclusion, laboratory experiments are a critical component of managing invasive pest ants and limiting their economic and environmental impacts. Evaluation of suitable rearing diets is an essential aspect of determining the best means of maintaining experimental colonies while also maintaining the integrity of data from experimental research. In addition, the need for species-specific bait development and laboratory trials is an important consideration in developing the best, most effective approach to managing invasive species. Though we were able to identify an interaction between rearing diet and recruitment to non-toxic baits from our statistical model, there appear to be other factors influencing bait preference which we were unable to identify during this study. Studies of various other ant species have indicated foraging preferences change seasonally. This could be due to external factors (e.g., temperature, humidity, type of available resources) or factors within individual colonies (e.g., amount of brood, queen fecundity), or any combination of these factors. Given that invasive *W. auropunctata* are polydomous, it is also possible that the mere separation of a bulk rearing colony into multiple individual experimental colonies could influence colony behavior. Further research is needed to test other factors which could potentially influence laboratory colony behaviors, such as foraging preferences.

Acknowledgments

This work formed part of the Ph.D. research by the senior author at the University of Canberra, Australia, and was supported by funding from the Hawaii Invasive Species Council, Honolulu, Hawaii, USA, and the Hawaii Department of Agriculture.

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