Attraction of Bactrocera cucurbitae and Bactrocera dorsalis (Diptera: Tephritidae) to Beer Waste and Other Protein Sources Laced with Ammonium Acetate

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Source: Florida Entomologist, 100(1) : 70-76

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.100.0112

Attraction of *Bactrocera cucurbitae* and *Bactrocera dorsalis* (Diptera: Tephritidae) to beer waste and other protein sources laced with ammonium acetate

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Abstract

Adult tephritid fruit fly females require protein sources for adequate egg production, and ammonia and its derivatives serve as volatile cues to locate protein-rich food. The attractiveness of beer waste and the commercially available baits Nu-Lure® Insect Bait, Buminal®, and Bugs for Bugs® Fruit Fly Bait with and without ammonium acetate or ammonium carbonate to males and females of *Bactrocera dorsalis* (Hendel) and *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) was quantified in semi-field cage studies in Hawaii. Evaluations also compared the relative attractiveness of the baits to that of the standard bait GF-120® NF Naturalyte® Fruit Fly Bait. Our findings indicate that ammonium carbonate did not exert any effect on the response of *B. cucurbitae* or *B. dorsalis* to the protein baits evaluated and that the addition of ammonium acetate to beer waste and to the commercially available bait Bugs for Bugs® can improve bait attractiveness in particular to females of *B. cucurbitae*. Beer waste laced with ammonium acetate performed as well as the standard fruit fly bait GF-120®. There were variations in the level and type of response between *B. cucurbitae* and *B. dorsalis*, and such variability was dependent upon the type of bait being evaluated. For example, there were several instances where baits lacking ammonium acetate (e.g., beer waste and Nu-Lure® for *B. cucurbitae*; Buminal® for *B. dorsalis*) were as attractive as GF-120®. Results are discussed in light of potential applications associated with the use of beer waste as a low-cost, readily available material for fruit fly monitoring and suppression.

Key Words: low-cost bait; bait accessibility; monitoring; suppression; integrated pest management

Resumen

Se tiene conocimiento que hembras de moscas de la fruta requieren de fuentes de proteína para una adecuada producción de huevecillos, y que amoniaco y sus derivados sirven como señales volátiles para ubicar fuentes ricas de proteína. El potencial atractivo del desecho de cerveza y los cebos comerciales Nu-Lure® Insect Bait, Buminal®y Bugs for Bugs® Fruit Fly Bait en la presencia / ausencia de acetato de amonio o carbonato de amonio hacia machos y hembras de *Bactrocera dorsalis* (Hendel) y *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) fue cuantificado en jaulas de campo en Hawaii. Las evaluaciones también compararon la atracción relativa de los cebos con el cebo estándar GF-120® NF Naturalyte® Fruit Fly Bait. Nuestros resultados indican que el carbonato de amonio no produce efecto alguno en la respuesta de *B. cucurbitae* o *B. dorsalis* a los cebos proteicos evaluados y que la adición de acetato de amonio al desecho de cerveza y al cebo comercial Bugs for Bugs® puede mejorar la atracción en particular en el caso de hembras de *B. cucurbitae*. El desecho de cerveza con acetato de amonio se desempeñó tan bien como el cebo comercial GF-120®. En adición, hubo variaciones en el nivel y tipo de respuesta entre *B. cucurbitae* y *B. dorsalis*, y dicha variabilidad dependió del tipo de cebo evaluado. Por ejemplo, hubieron varias instancias en las cuales cebos que no contaban con acetato de amonio (e.g., desecho de cerveza y Nu-Lure® para *B. cucurbitae*; Buminal® para *B. dorsalis*) fueron tan atractivos como GF-120®. Los resultados se discuten en vista de las aplicaciones potenciales asociadas con el uso del desecho de cerveza como un material de bajo costo y de fácil disponibilidad para el monitoreo y supresión de moscas de la fruta.

Palabras Clave: cebos de bajo costo; disponibilidad de cebos; monitoreo; supresión; manejo integrado de plagas

Fruit flies (Diptera: Tephritidae) of the genus *Bactrocera* Macquart represent a highly invasive taxon, and collectively they pose a serious threat to the production and export of horticultural crops around the globe (Papadopoulos 2014; Vargas et al. 2016). The oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the melon fly, *B. cucurbitae* (Coquillett), are of particular importance given their pest severity, host range, invasiveness, and frequency of infestation (Vargas et al. 2015).

Application of protein baits mixed with a killing agent is a common and effective attract-and-kill approach to fruit fly management targeting female populations (Roessler 1989; Mangan 2014). This behavior-based approach has reduced the amount of pesticide needed for fruit fly control and has been used successfully in many integrated pest management and eradication programs, including those with *B. dorsalis* (Piñero et al. 2009) and *B. cucurbitae* (Vargas et al. 2010). Proprietary aqueous protein baits that incorporate ammonia derivatives also have been developed (Heath et al. 1997; Epsky et al. 1999, 2014). However, often commercial protein bait and lure materials are not accessible to farmers due to high cost and/or lack of availability in

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several regions of the world (Sookar et al. 2002). To circumvent these problems, evaluation and improvement of the effectiveness of locally produced baits may provide alternatives for small- and mid-scale growers for fruit fly population suppression and improved crop protection (Epsky et al. 2014). Cost considerations and accessibility are of paramount importance for small-scale growers who cannot afford expensive monitoring and management tools (Aluja & Piñero 2004).

Using a comparative approach under semi-field conditions, we (1) assessed the effect of adding ammonium acetate and ammonium carbonate to beer waste and to the commercial baits Nu-Lure® Insect Bait, Buminal®, and Bugs for Bugs® Fruit Fly Bait, on the response of males and females of *B. dorsalis* and *B. cucurbitae*, and (2) compared the attractiveness of selected baits either having or lacking ammonium acetate versus that of the standard bait GF-120® NF Naturalyte® Fruit Fly Bait to males and females of *B. dorsalis* and *B. cucurbitae*. Our interest in beer waste stemmed from its local availability and comparatively low price, and our main goal was to determine whether the addition of ammonium acetate to commercial baits and to beer waste would result in increased bait attractiveness. Based on previous findings (Piñero et al. 2011), we hypothesized an overall stronger response of *B. cucurbitae* to baits compared with *B. dorsalis,* and a stronger effect of ammonium acetate on *B. dorsalis* than on *B. cucurbitae*.

Materials and Methods

STUDY SITE AND EXPERIMENTAL ARENA

This study was conducted from 25 Mar to 13 May 2014 at the University of Hawaii Agricultural Experiment Station, Kainaliu, Hawaii Island. All experiments were conducted under semi-field conditions in 6 Lumite® screened field cages (2 × 2 × 2 m) positioned inside a shade house. Each field cage contained 3 to 6 potted (11.3 L) guava (*Psidium guajava* L.; Myrtaceae) trees, arranged in a circle in the center of the cage to provide resting sites and fly habitat. Four equidistant hanging wires (30 cm in length) were positioned at each corner of the roof of the cage to hang treatment dishes, a standard method used in previous studies (e.g., Vargas & Prokopy 2006; Piñero et al. 2015).

INSECTS

Bactrocera dorsalis and B. cucurbitae adults were obtained from colonies established at the Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, United States Department of Agriculture (USDA) Agricultural Research Service (ARS), Hilo, Hawaii Island. Fruit fly rearing followed standardized procedures (Vargas 1989). Pupae (10 mL, ~500 flies) were allowed to emerge in 30 cm^3 rearing cages. Flies were fed a full diet of 3:1 sugar to yeast hydrolysate and water was provided ad libitum. Flies were tested when they were 12 to 15 d old, and protein was removed 19 h prior to fly release in the observation cages to elicit a moderate level of protein hunger (Piñero et al. 2011).

BAIT TREATMENTS

The baits evaluated were: (1) Nu-Lure® Insect Bait (44% corn gluten meal, hydrolyzed; Miller Chemical and Fertilizer Corp., Hanover, Pennsylvania) [= Nu-Lure]; (2) Buminal® (38.67% hydrolyzed protein; NABA GmbH, Gierstadt, Germany) [= Buminal]; (3) Bugs for Bugs® Fruit Fly Bait (50% yeast autolysate; Mauri Yeast, Camellia, NSW, Australia) (solids 50%, papain 0.2%, protein 55%, potassium sorbate 0.12%, no salt) [= Bugs for Bugs]; and (4) beer waste. Although the composition of beer waste was unknown, beer waste typically contains (in dry matter) proteins (50–70%), hops bitter substances non isomerized (10–20%), phenolic compounds (5–10%), carbohydrates (4–8%), and fatty acids (1–2%) (Mathias et al. 2015). Beer waste samples were provided by Qinge Ji, Fujian Agriculture and Forestry University, Fuzhou, China. For some of the evaluations, GF-120® NF Naturalyte® Fruit Fly Bait (Dow AgroSciences, Indianapolis, Indiana) [= GF-120], one of the most effective fruit fly protein baits commercially available, was used for comparative purposes. GF-120 contains approximately 1% ammonium acetate (= AA) in its formulation (Moreno & Mangan 2002), and therefore no AA was added to this bait in any of the experiments. None of the original bait formulations contained toxicants except for the spinosad-containing GF-120 bait. All baits were specified as ready to use on the product label except for the GF-120 bait, which was prepared at the recommended application rate, a 40% (vol/vol) solution.

BAIT APPLICATION

Each bait treatment was applied as thirty 10 µL droplets to the upper surface of circular discs made of coffee leaves that were rinsed, dried, and cut to cover the bottom of a Petri dish (10 mm in height, 9 cm in diameter) fastened with wire to hang inside the cages. Bait application was done with an Eppendorf™ repeater pipette (Brinkmann Instruments Inc., Westbury, New York), and this application procedure was intended to mimic a bait spray application (Piñero et al. 2015).

EXPERIMENTAL ARENAS AND OBSERVATIONS

For each observation day, approximately 500 adults of *B. cucurbitae* and *B. dorsalis* (mixed sexes) were released into separate field cages (3 cages for *B. cucurbitae*, 3 cages for *B. dorsalis*), at 8:30 AM and 12:30 PM in order to provide a relatively large supply of responding flies. Before each replicate, guava plant foliage in the cages was misted with water to ensure flies were not responding to the baits in order to obtain water. Unless indicated otherwise, for each experiment 4 different bait treatments were randomly hung by 1 of the 4 wires at the start of each replicate. Immediately thereafter, an observer recorded the number of male and female flies arriving at each station for a 20 min period. Each fly responder was removed with an aspirator. As ambient light varied at each of the 4 cage corners, the treatments were rotated 90° clockwise every 5 min so that each treatment was at every location during the 20 min test period. Experimental treatments were tested in a random order throughout each observation day to reduce any between-day bias. At the end of each day of testing flies, the trees and cages were flushed with water by using a garden hose to remove the flies. Range (mean ± SE) of temperature and relative humidity values during the observations were 22.51 ± 6.53 °C and 79.57 ± 11.33%, respectively.

EXPERIMENT 1

This series of tests evaluated the response of males and females of *B. cucurbitae* and *B. dorsalis* to 4 individual baits (Nu-Lure, Buminal, Bugs for Bugs, and beer waste) presented either alone or in combination with AA (1%, wt/vol) or ammonium carbonate (= AC) (1%, wt/vol). Water was used as a control. Trials were replicated 10 to 11 times.

EXPERIMENT 2

We assessed the attractiveness of Nu-Lure, Buminal, Bugs for Bugs, and beer waste, either with or without AA (1%, wt/vol), to males and females of *B. cucurbitae* and *B. dorsalis* relative to the standard bait, GF-120. Given that only 4 baits could be evaluated per cage, 2 sub-experiments were conducted. The first series of trials (Experiment 2a) compared GF-120 versus Bugs for Bugs, Buminal, and water (control). The second series of trials (Experiment 2b) compared male and female response to GF-120 versus beer waste and Nu-Lure. The selection of baits being compared against GF-120 in each cage was done randomly. For each fly species, 11 to 12 replicates were conducted. Each semi-field cage was assigned a particular set of baits either, lacking or having AA.

STATISTICAL ANALYSES

Data on the number of males and females responding to the bait treatments were analyzed for each fly species by an analysis of variance (ANOVA). Data were transformed using √(x+0.5) prior to analysis to stabilize variances, and means were separated, whenever appropriate, by a Fisher's Least Significant Differences (LSD) test at the 5% probability level. All figures show untransformed data. Statistical analyses were conducted in STATISTICA (StatSoft Inc. 2013).

Results

EXPERIMENT 1

For *B. cucurbitae*, the response of males to the 4 types of baits was not influenced significantly by the addition of AA or AC. For this fly species, all bait treatment combinations were attractive to males when compared with water (ANOVA: Nu-Lure: $F_{3,36}$ = 3.56, P = 0.038; beer waste: $F_{3,40}$ = 3.37, P = 0.038; Bugs for Bugs: $F_{3,40}$ = 5.58, P = 0.003; Buminal: $F_{3,40}$ = 3.12, P = 0.040) (Fig. 1A).

For males of *B. dorsalis*, the addition of AA or AC to the various baits was not accompanied by an increase in response. The bait treatments involving beer waste, Bugs for Bugs, and Buminal were attractive to males when compared with water. In contrast, all Nu-Lure treatments were unattractive to males of *B. dorsalis* (ANOVA: Nu-Lure: $F_{3,36}$ = 0.64, P = 0.592; beer waste: $F_{3,28}$ = 4.81, P = 0.008; Bugs for Bugs: $F_{3,40}$ = 5.96, P = 0.002; Buminal: $F_{3,40}$ = 3.48, P = 0.038) (Fig. 1A).

Responses of *B. cucurbitae* females to baits were significantly increased by the addition of AA only in the case of beer waste and Bugs for Bugs, whereas the addition of AC did not exert a noticeable effect for any bait. All baits were significantly more attractive than the water control (ANOVA: Nu-Lure: $F_{3,36}$ = 9.37, P < 0.001; beer waste: $F_{3,40}$ = 3.11, P = 0.037; Bugs for Bugs: $F_{3,40}$ = 12.38, P < 0.001; Buminal: $F_{3,40}$ = 2.94, P = 0.044) (Fig. 1B). The response of *B. dorsalis* females was not significantly influenced by the addition of AA to baits. However, the addition of AA to Nu-Lure made this bait more attractive than water, a result not found with Nu-Lure alone. The response of *B. dorsalis* females to Nu-Lure with AC was significantly reduced when compared with Nu-Lure with AA (ANOVA: Nu-Lure: $F_{3,36}$ = 3.43, P = 0.027; beer waste: $F_{3,40}$ = 1.38, P = 0.267; Bugs for Bugs: $F_{3,40}$ = 2.99, P = 0.041; Buminal: $F_{3,36}$ = 1.68, P = 0.189) (Fig. 1B).

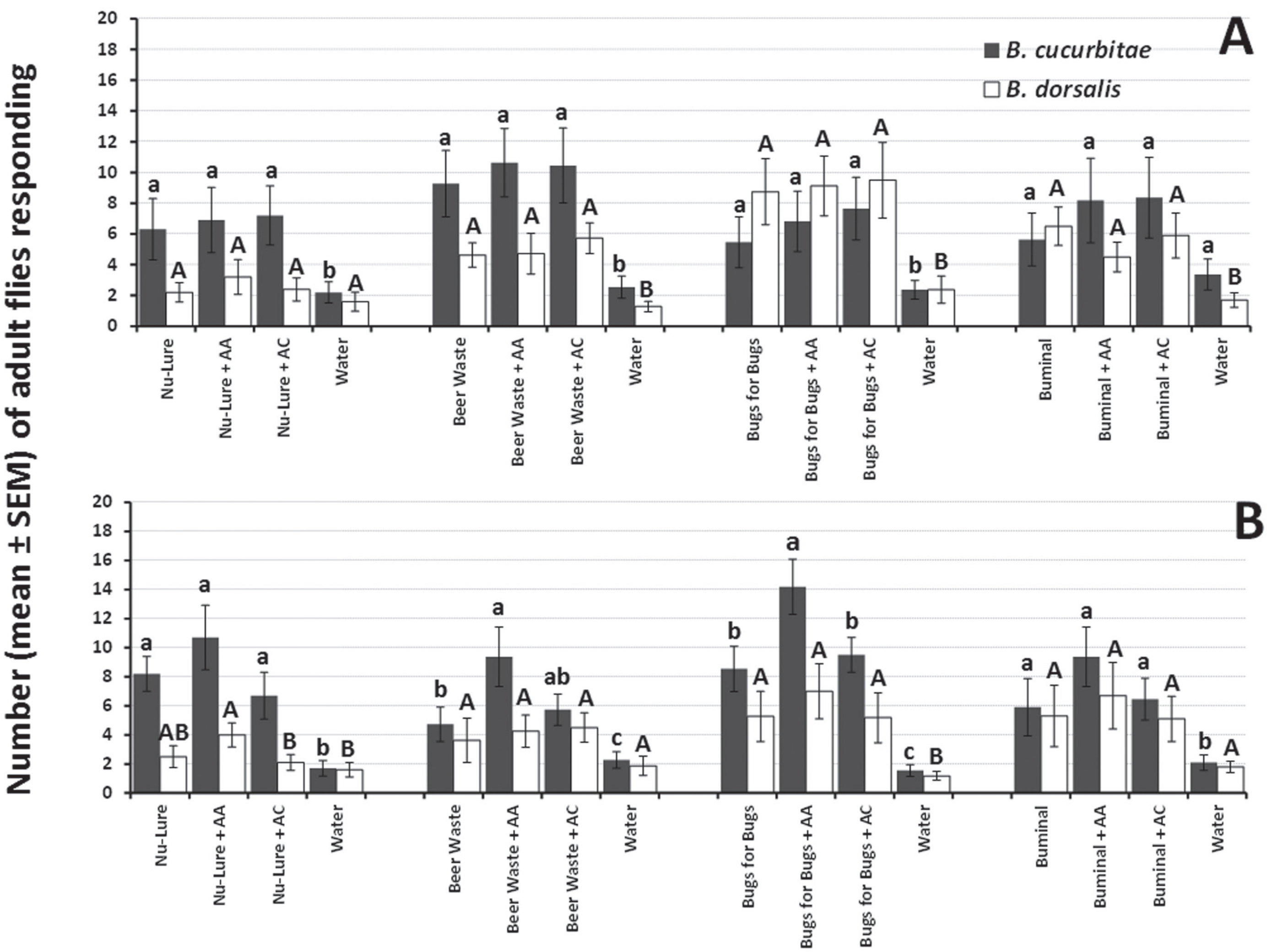

Fig. 1. Response of adult males (A) and females (B) of *Bactrocera cucurbitae* and *B. dorsalis* in field cages to Nu-Lure® Insect Bait (= Nu-Lure), beer waste, Bugs for Bugs® Fruit Fly Bait (= Bugs for Bugs), and Buminal® (= Buminal) either alone or with added ammonium acetate (= AA) or ammonium carbonate (= AC). Water was used as a negative control. For each fly species and sex, different letters (lowercase: *B. cucurbitae*; uppercase: *B. dorsalis*) indicate significant differences according to ANOVA and the Fisher LSD tests at $P \leq 0.05$.

Overall, the response of *B. cucurbitae* males was 2.9, 2.0, 0.6, and 0.9 times that of *B. dorsalis* males for Nu-Lure, beer waste, Bugs for Bugs, and Buminal, respectively, and 2.2, 2.3, 0.7, and 1.8 times for Nu-Lure with AA, beer waste with AA, Bugs for Bugs with AA, and Buminal with AA, respectively. The response of *B. cucurbitae* females was 3.3, 1.3, 1.6, and 1.1 times that of *B. dorsalis* females for Nu-Lure, beer waste, Bugs for Bugs, and Buminal, respectively, and 2.7, 2.2, 2.0, and 1.4 times for Nu-Lure with AA, beer waste with AA, Bugs for Bugs with AA, and Buminal with AA, respectively.

EXPERIMENT 2A

In the absence of AA, Bugs for Bugs and Buminal were as attractive to *B. cucurbitae* males as GF-120, and responses to all baits were significantly greater than to water (ANOVA: $F_{3,36} = 5.84$, $P = 0.002$) (Fig. 2A). The addition of AA to Bugs for Bugs made this bait as attractive to *B. cucurbitae* males as the standard GF-120 bait (ANOVA: $F_{3,40} = 2.99$, $P = 0.042$) (Fig. 2B).

Males of *B. dorsalis* preferred GF-120 over Bugs for Bugs in the absence of AA, and the response to Buminal was intermediate (ANOVA: $F_{3,36} = 3.71$, $P = 0.019$) (Fig. 2A). The addition of AA made Bugs for Bugs and Buminal as attractive as GF-120, and all baits were significantly more attractive than the control (ANOVA: $F_{3,40} = 2.99$, $P = 0.042$) (Fig. 2B).

For females of *B. cucurbitae* in the absence of AA, GF-120 was significantly more attractive than Bugs for Bugs, and Buminal was statistically as attractive as GF-120. All baits were significantly more attractive than the water control (ANOVA: $F_{3,36} = 4.37$, $P = 0.009$) (Fig. 3A). Addition of AA to baits resulted in a significant female preference for Bugs for Bugs compared with GF-120 (ANOVA: $F_{3,40} = 11.14$, $P < 0.001$) (Fig. 3B).

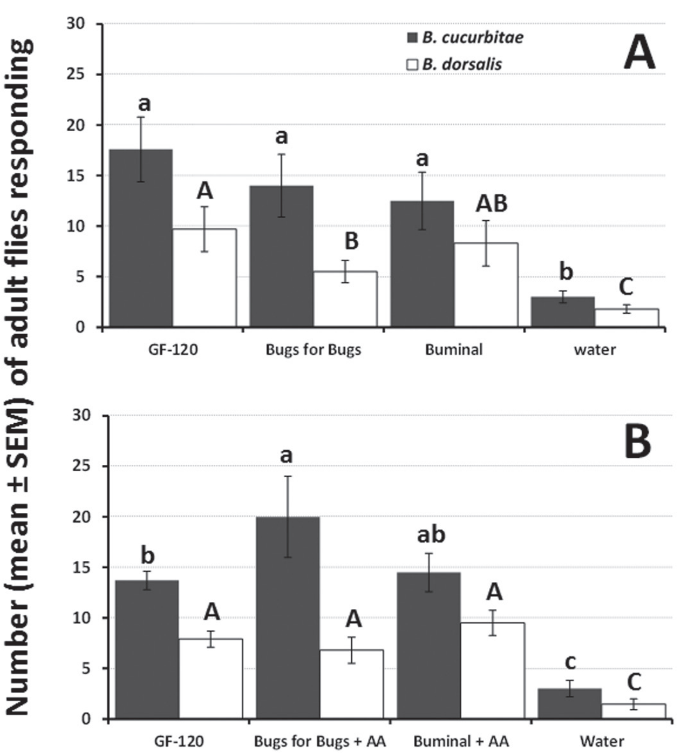

Fig. 2. Response of males of *Bactrocera cucurbitae* and *B. dorsalis* to GF-120® NF Naturalyte® Fruit Fly Bait (= GF-120), Bugs for Bugs® Fruit Fly Bait (= Bugs for Bugs), Buminal® (= Buminal), and water (negative control) either in the absence (A), or presence (B) of ammonium acetate (= AA). For each species, different letters (lowercase: *B. cucurbitae*; uppercase: *B. dorsalis*) indicate significant differences according to ANOVA and the Fisher LSD tests at $P \leq 0.05$.

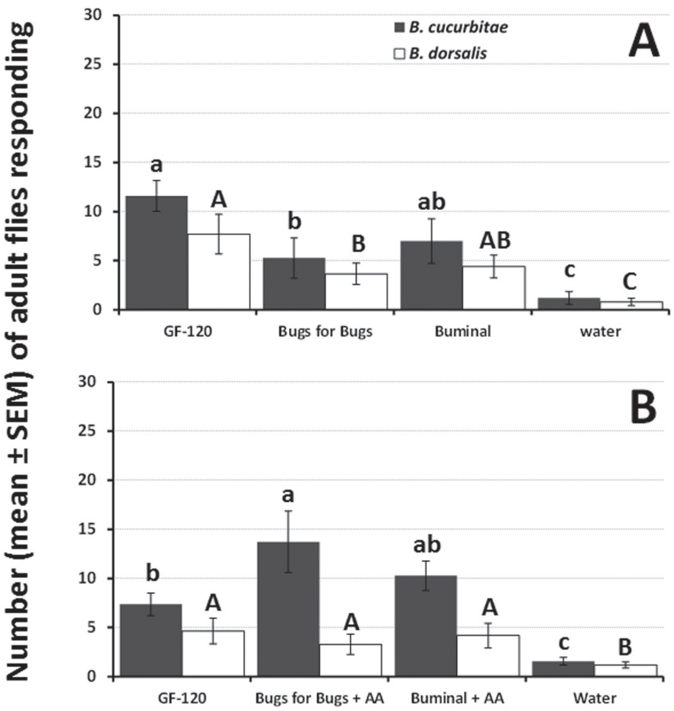

Fig. 3. Response of females of *Bactrocera cucurbitae* and *B. dorsalis* to GF-120® NF Naturalyte® Fruit Fly Bait (= GF-120), Bugs for Bugs® Fruit Fly Bait (= Bugs for Bugs), Buminal® (= Buminal), and water (negative control) either in the absence (A), or presence (B) of ammonium acetate (= AA). For each species, different letters (lowercase: *B. cucurbitae*; uppercase: *B. dorsalis*) indicate significant differences according to ANOVA and the Fisher LSD tests at $P \leq 0.05$.

In the absence of AA, *B. dorsalis* females showed a significant preference for GF-120 and Buminal compared with Bugs for Bugs, and all baits were more attractive than water (ANOVA: $F_{3,36} = 5.63$, $P = 0.003$) (Fig. 3B). The addition of AA improved the performance of Bugs for Bugs making it as attractive to *B. dorsalis* females as GF-120 and Buminal (Fig. 3B) (ANOVA: $F_{3,36} = 5.63$, $P = 0.003$).

EXPERIMENT 2B

As shown in Fig. 4A, all baits were similarly attractive to males of *B. cucurbitae* and were significantly more attractive than water (ANOVA: $F_{3,40} = 4.99$, $P = 0.005$). The addition of AA to beer waste and Nu-Lure did not increase the attractiveness of these baits to males of *B. cucurbitae*, relative to the level of response elicited by the spinosad-based protein bait GF-120 (Fig. 4B). All baits were attractive compared with water (ANOVA: $F_{3,44} = 5.09$, $P = 0.004$).

For *B. dorsalis*, GF-120 was significantly more attractive than beer waste and it was as attractive to males as Nu-Lure when the latter bait lacked AA (ANOVA: $F_{3,40} = 20.63$, $P < 0.001$) (Fig. 4A). Beer waste became significantly more attractive to males of *B. dorsalis* than GF-120 and Nu-Lure when AA was added (ANOVA $F_{3,44} = 16.90$, $P < 0.001$) (Fig. 4B).

Females of *B. cucurbitae* showed no significant preference for any specific bait regardless of whether AA was absent (ANOVA: $F_{3,40} = 6.18$, $P = 0.001$) or present (ANOVA: $F_{3,44} = 15.04$, $P < 0.001$) in the bait (Fig. 5A, B). In all instances, baits were significantly more attractive than the water control. Females of *B. dorsalis* preferred GF-120 over beer waste and Nu-Lure when AA was absent from these baits (ANOVA: $F_{3,40} = 6.56$, $P = 0.001$) (Fig. 5A). When AA was added to beer waste and Nu-Lure, the response of females to these baits was as high as that recorded for GF-120, and all baits were significantly more attractive than the water control (ANOVA: $F_{3,44} = 3.73$, $P = 0.021$) (Fig. 5B).

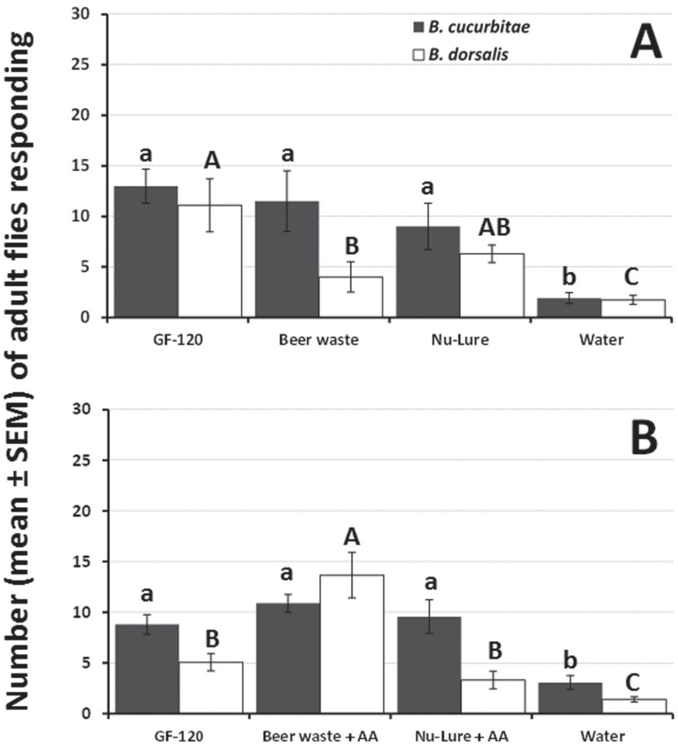

Fig. 4. Response of males of *Bactrocera cucurbitae* and *B. dorsalis* to GF-120® NF Naturalyte® Fruit Fly Bait (= GF-120), beer waste, Nu-Lure® Insect Bait (= Nu-Lure), and water (negative control) either in the absence (A), or presence (B) of ammonium acetate (= AA). For each species, different letters (lowercase: *B. cucurbitae*; uppercase: *B. dorsalis*) indicate significant differences according to ANOVA and the Fisher LSD tests at $P \leq 0.05$.

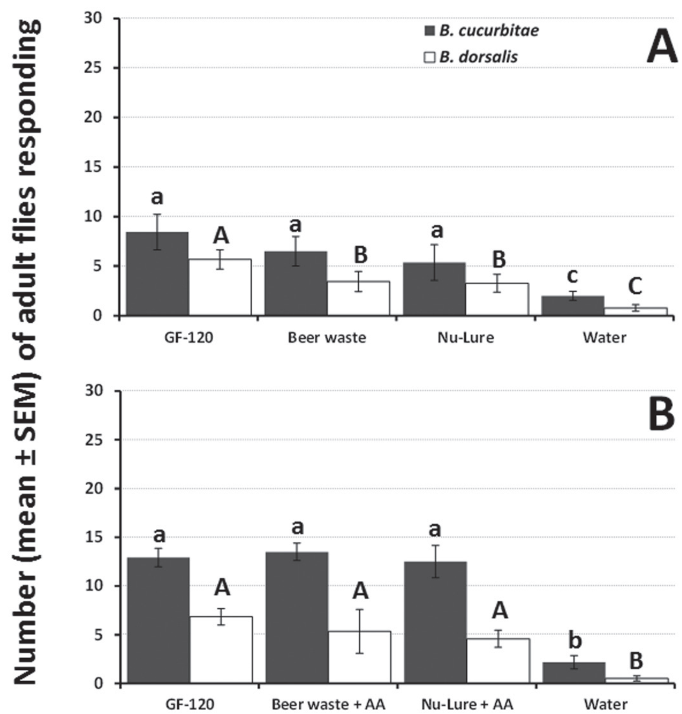

Fig. 5. Response of females of *Bactrocera cucurbitae* and *B. dorsalis* to GF-120® NF Naturalyte® Fruit Fly Bait (= GF-120), beer waste, Nu-Lure® Insect Bait (= Nu-Lure), and water (negative control) either in the absence (A), or presence (B) of ammonium acetate (= AA). For each species, different letters (lowercase: *B. cucurbitae*; uppercase: *B. dorsalis*) indicate significant differences according to ANOVA and the Fisher LSD tests at $P \leq 0.05$.

Discussion

The development and improvement of food-based baits targeting female fruit flies needs to take into consideration fly behavior, safety, cost, availability of ingredients, and performance under field conditions, among other factors. Attempts to modify beer waste, one of the materials of interest in the present study, have been made in other regions of the world, for example Australia (Lloyd & Drew 1997), Mauritius (Sookar et al. 2002), and China (Zhou et al. 2012).

The present study assessed the attractiveness of beer waste and 3 commercial baits, which are available in various regions of the world, to adults of *B. cucurbitae* and *B. dorsalis* and examined the role of AA in fruit fly response to baits. Overall, we found that (1) for both fly species, the effects of AA were more marked in females compared with males, (2) effects were more apparent when AA was added to beer waste and Bugs for Bugs than when added to Nu-Lure or Buminal, (3) responses of *B. cucurbitae* to single baits and baits laced with AA were consistently greater than those for *B. dorsalis*, and (4) in both species, in those instances where GF-120 was more attractive to females than the other protein baits the addition of AA made protein baits as attractive to females as the standard bait GF-120. Findings are discussed emphasizing these central points.

Several studies have reported the use of beer waste for fruit fly control in various regions of the world. For example, beer waste has been used in Tonga to suppress populations of *B. melanotus* (Coquillett) (Heimoana et al. 1997). In Mauritius, Sookar et al. (2002) evaluated formulations of waste brewer's yeast that contained either papain enzyme powder, pawpaw juice, or pineapple juice. These formulations were found to be as effective in controlling *B. cucurbitae*, as estimated by infestation of ridge gourd, *Luffa acutangula* (L.) Roxbdata (Cucurbitaceae), as the standard protein hydrolysate used in Mauritius for fruit fly control (Sookar et al. 2002). Chinajariyawong et al. (2003) evaluated the Australian protein bait, Pinnacle® (Mauri Yeast, Camellia, NSW, Australia) and brewery waste from Thailand, and found that both baits significantly reduced fruit fly infestation when compared with controls. Yeast autolysate baits supplied from Mauri Yeast, such as Pinnacle® and Bugs for Bugs, have been an important component in battling *Bactrocera* species as part of integrated pest management programs in Australia (Lloyd et al. 2010).

Only a few studies have attempted to increase the attractiveness of beer waste with AA. In one example, Zhou et al. (2012) evaluated a new bait containing an enzymatically hydrolyzed protein produced by the industrial processing of beer yeast, feeding stimulants, orange juice, brown sugar, and AA. This new bait, termed H-protein bait, outperformed GF-120 in citrus orchards in China (Zhou et al. 2012). In another recent example, Piñero et al. (2015) documented that the addition of AA to protein baits, including beer waste, led to significant increases in response of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) females to 7 out of 8 protein baits tested.

The amount of AA present in protein baits has been shown to influence the response of various fruit fly species, including *B. cucurbitae*, *B. dorsalis*, and *C. capitata* (e.g., Mazor 2009; Piñero et al. 2011). The present study documented greater responses of males and females of *B. cucurbitae* to Nu-Lure, beer waste, Bugs for Bugs, and Buminal than males and females of *B. dorsalis*. These findings, when combined with a previous report by Piñero et al. (2011), which also tested the attractiveness of GF-120 on the same species, confirm an inherent stronger response to protein baits by *B. cucurbitae* when compared with

B. dorsalis. The stronger overall response of *B. cucurbitae* females to food-based baits compared with *B. dorsalis*, and the stronger effects of AA added to baits is expected to result in more effective control of the former species.

Variations in the level and type of response between *B. cucurbitae* and *B. dorsalis* were documented here. Such variability was dependent upon the type of bait being evaluated. For example, there were several instances where baits lacking AA (e.g., beer waste and Nu-Lure for *B. cucurbitae*; Buminal for *B. dorsalis*) were as attractive as GF-120. This finding differs from the more consistent positive effects documented with *C. capitata* when AA was added to protein baits (Piñero et al. 2015). Such variations in response might reflect interspecific differences in nutritional requirements, variability associated with proteinaceous sources as they relate to processing, and effects of the flies' physiological status (Piñero et al. 2011). Variability associated with the use of protein baits for monitoring purposes has been reported (for a review, see Epsky et al. 2014). Our findings pertain to the particular beer waste that was used for this study and may not necessarily apply for a different source of beer waste. It would be interesting to evaluate the effect of AA added to various types of beer waste–based baits on the level of fruit fly attraction.

The end goal of this type of research is to develop low-cost technologies for farmers who are not able to monitor or manage fruit fly populations because commercially produced baits are too expensive or are unavailable. We postulate that the addition of AA to beer waste can result in (1) increased attractiveness of beer waste to various fruit fly species including *B. dorsalis*, *B. cucurbitae*, and *C. capitata* (Piñero et al. 2015), (2) reduced variability that may exist among beer waste sources, and (3) elimination of the time and effort required to modify beer waste to produce more effective materials. Importantly, AA is inexpensive, quite accessible and, if properly stored, can last for several years. As discussed by Sookar et al. (2002), if farmers are able to produce their own bait from locally available materials then the costs associated with fruit fly monitoring and suppression could be reduced, thereby enhancing the sustainability of fruit fly control activities in various regions of the world.

In conclusion, our findings indicate that *B. cucurbitae* shows an overall strong response to protein baits, and that for both *B. cucurbitae* and *B. dorsalis* the effects of AA were more marked in females than males. The addition of AA, but not AC, to beer waste and to the commercially available bait Bugs for Bugs improved bait attractiveness, in particular to females of *B. cucurbitae*, to a level comparable to the standard bait GF-120. These findings can potentially increase the effectiveness of protein baits for fruit fly monitoring and suppression.

Acknowledgments

We express gratitude to Mike McKenney and Keith Shigetani (Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, USDA-ARS, Hilo, Hawaii) for rearing *B. cucurbitae* and *B. dorsalis* flies and to Benedikt Billig and Anthony Heinrich for providing samples of Buminal® and Bugs for Bugs® Fruit Fly Lure, respectively. We thank Russell Ijima for assisting in experimental setup and data collection and Marc Meisner and Kelly Asai (University of Hawaii Kona Experiment Station at Kainaliu) for logistic support. Funding for this research was provided by Bob Dowell and Kevin Hoffman of the California Department of Food and Agriculture. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use. USDA is an equal opportunity employer.

References Cited

Aluja M, Piñero JC. 2004. Testing human urine as a low-tech bait for *Anastrepha* spp. (Diptera: Tephritidae) in small guava, mango, sapodilla and grapefruit orchards. Florida Entomologist 87: 41–50.

Chinajariyawong A, Kritsaneepaiboon S, Drew RAI. 2003. Efficacy of protein bait sprays in controlling fruit flies (Diptera: Tephritidae) infesting angled luffa and bitter gourd in Thailand. The Raffles Bulletin of Zoology 51: 7–15.

Epsky ND, Hendrichs J, Katsoyannos BI, Vásquez LA, Ros JP, Zümreoglu A, Pereira R, Bakri A, Seewooruthun SI, Heath RR. 1999. Field evaluation of female-targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. Journal of Economic Entomology 92: 156–164.

Epsky ND, Kendra PE, Schnell EQ. 2014. History and development of food-based attractants, pp. 75–118 *In* Shelly T, Epsky ND, Jang EB, Reyes-Flores J, Vargas RI [eds.], Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications. Springer, Netherlands.

Heath RR, Epsky ND, Dueben BD, Rizzo J, Jeronimo F. 1997. Adding methyl-substituted ammonia derivatives to a food-based synthetic attractant on capture of the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). Journal of Economic Entomology 90: 1584–1589.

Heimoana V, Nemeye P, Langi T, Allwood AJ. 1997. Assessment of protein bait sprays for the control of fruit flies in chilli and *Capsicum* crops in Tonga, pp.179–182 *In* Allwood AJ, Drew RAI [eds.], Management of Fruit Flies in the Pacific. ACIAR Proceedings No 76. Australian Centre for International Agricultural Research, Canberra, Australia.

Lloyd A, Drew RAI. 1997. Modification and testing of brewery waste yeast as a protein source for fruit fly bait, pp. 192–198 *In* Allwood AJ, Drew RAI [eds.], Management of Fruit Flies in the Pacific. ACIAR Proceedings No 76. Australian Centre for International Agricultural Research, Canberra, Australia.

Lloyd AC. Hamacek EL, Kopittke RA, Peek T, Wyatt, PM, Neale CJ, Eelkema M, Gu H. 2010. Area-wide management of fruit flies (Diptera: Tephritidae) in the Central Burnett district of Queensland, Australia. Crop Protection 29: 462–469.

Mangan RL. 2014. History and development of food-based attractants, pp. 423–456 *In* Shelly T, Epsky ND, Jang EB, Reyes-Flores J, Vargas RI [eds.], Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications. Springer, Dordrecht, Netherlands.

Mathias TRS, Alexandre VMF, Cammarota MC, de Mello PPM, Servulo EFC. 2015. Characterization and determination of brewer's solid wastes composition. Journal of the Institute of Brewing 121: 400–404.

Mazor M. 2009. Competitiveness of fertilizers with proteinaceous baits applied in Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera: Tephritidae) control. Crop Protection 28: 314–318.

Moreno DS, Mangan RL. 2002. Bait matrix for novel toxicants for use in control of fruit flies (Diptera: Tephritidae), pp. 333–362 *In* Hallman G, Schwalbe CP [eds.], Invasive Arthropods in Agriculture: Problems and Solutions. Science Publishers, Enfield, New Hampshire.

Papadopoulos NT. 2014. Fruit fly invasion: historical, biological, economic aspects and management, pp. 219–252 *In* Shelly T, Epsky ND, Jang EB, Reyes-Flores J, Vargas RI [eds.], Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications. Springer, Dordrecht, Netherlands.

Piñero JC, Mau RFL, Vargas RI. 2009. Managing oriental fruit fly (Diptera: Tephritidae), using spinosad-based protein bait sprays in papaya orchards in Hawaii. Journal of Economic Entomology 102: 1123–1132.

Piñero JC, Mau RFL, Vargas RI. 2011. A comparative assessment of the response of three fruit fly species (Diptera: Tephritidae) to a spinosad-based bait: Effect of ammonium acetate, female age, and protein hunger. Bulletin of Entomological Research 101: 373–381.

Piñero JC, Souder SK, Smith TR, Fox AJ, Vargas RI. 2015. Ammonium acetate enhances the attractiveness of a variety of protein-based baits to female *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 108: 694-700.

Roessler Y. 1989. Insecticidal bait and cover sprays, pp. 329–335 *In* Robinson AS, Hooper G. [eds.], Fruit Flies: Their Biology, Natural Enemies and Control, *In* Helle W [ed.], World Crop Pests, Volume 3A. Elsevier Science Ltd., Amsterdam, Netherlands.

Sookar P, Facknath S, Permalloo S, Seewoorothum SI. 2002. Evaluation of modified waste brewer's yeast as a protein source for the control of the melon fly, *Bactrocera cucurbitae* (Coquillet), pp. 295–299 *In* Proceedings of 6th International Fruit Fly Symposium. Stellenbosch, South Africa, 6–10 May 2002.

StatSoft, Inc. 2013. STATISTICA (data analysis software system), version 12. Available online: www.statsoft.com

Vargas RI. 1989. Mass production of tephritid fruit flies, pp. 141–152 *In* Robinson AS, Hooper G. [eds.], World Crop Pests, Volume 3B. Elsevier Science Ltd., Amsterdam, Netherlands.

Vargas RI, Prokopy RJ. 2006. Attraction and feeding responses of melon flies and oriental fruit flies (Diptera: Tephritidae) to various protein baits with and without toxicants. Proceedings of the Hawaiian Entomological Society 38: 49–60.

Vargas RI, Piñero JC, Mau RFL, Jang EB, Klungness LM, McInnis DO, Harris EB, McQuate GT, Bautista RC, Wong L. 2010. Area-wide suppression of Mediterranean fruit fly, *Ceratitis capitata*, and oriental fruit fly, *Bactrocera dorsalis,* in Kamuela, Hawaii. Journal of Insect Science 10: 1–17.

Vargas RI, Piñero JC, Leblanc L. 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific Region. Insects 6: 297–318.

Vargas RI, Piñero JC, Leblanc L, Manoukis NC, Mau RFL. 2016. Area-wide management of fruit flies (Diptera: Tephritidae) in Hawaii, pp. 673–693 *In* Ekesi S, Mohamed S, Meyer M [eds.], Fruit Fly Research and Development in Africa: Towards a Sustainable Management Strategy to Improve Horticulture. Springer International Publishing, Switzerland.

Zhou XW, Niu CY, Han P, Desneux N. 2012. Field evaluation of attractive lures for the fruit fly *Bactrocera minax* (Diptera: Tephritidae) and their potential use in spot sprays in Hubei Province (China). Journal of Economic Entomology 105: 1277–1284.