Capture of *Bactrocera* Males (Diptera: Tephritidae) in Parapheromone-Baited Traps: A Comparison of Liquid versus Solid Formulations

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Abstract. *Bactrocera cucurbitae* (Coquillett) and *B. dorsalis* (Hendel) (Diptera: Tephritidae) are important agricultural pests of the Pacific region. Detection and control of these species rely largely on traps baited with male-specific attractants (parapheromones), namely cue lure for *B. cucurbitae* and methyl eugenol for *B. dorsalis*. Presently, these lures (plus naled, an insecticide) are applied in liquid form, although this procedure is time-consuming, and naled as well as methyl eugenol may pose human health risks. Recently, a solid formulation (termed a wafer) has been developed that contains both chemicals (plus DDVP, an insecticide), and here we present data from field tests that compare the effectiveness of liquid versus solid formulations of the lures in attracting males of these two *Bactrocera* species. Trapping in two areas of Oahu, Hawaii, yielded consistent results: Jackson traps baited with wafers captured (1) similar numbers of *B. cucurbitae* males and (2) significantly greater numbers of *B. dorsalis* males than Jackson traps baited with standard liquid lures. Additional tests examined the effect of ageing on the attractiveness of the wafers and revealed that Jackson traps baited with wafers aged for 6 or 8 weeks captured similar or significantly more males of both *Bactrocera* species than Jackson traps baited with fresh liquid lures.

Key words: Tephritidae, detection, male annihilation, trapping

Introduction

The genus *Bactrocera* contains approximately 500 species distributed primarily in tropical Asia and the South Pacific region (Drew and Hancock 2000). Most species are not economically important, but approximately 70 polyphagous species are serious agricultural pests (White and Elson-Harris 1992), attacking important crops, such as citrus (*Citrus* spp.), mango (*Mangifera indica* L.), squash and melons (*Cucurbita*, *Cucumis*, and *Citrullus* spp.), and papaya (*Carica papaya* L.) among others. Detection and control of *Bactrocera* pests rely heavily upon male-specific attractants, termed lures or parapheromones (Cunningham 1989). Males of most *Bactrocera* species respond to either raspberry ketone (RK, or the man-made analogue cue lure, CL) or methyl eugenol (ME) (Drew 1974, Drew and Hooper 1981, Hardy 1979). Traps containing these chemicals are widely used in detection programs (e.g., Verghese et al. 2004, Jessup et al. 2007), and fibrous (absorbent) boards, saturated with the appropriate lure and a toxicant and distributed widely, have been used successfully to eradicate invasive *Bactrocera* populations (Hancock et al., 2000; Seewooruthun et al. 2000).

At present, CL and ME are applied as liquids to cotton wicks positioned inside traps. Moreover, the insecticide naled is often added (also in liquid form) to the lures before application to the cotton wicks. Thus, the current procedure involves considerable handling time for measuring and applying the liquids as well as potential health risks resulting from accidental contact or ingestion of the insecticide. In addition, data derived from rodents suggest that ME itself may be carcinogenic, as subjects administered high doses showed increased incidence of liver cancer and mortality (National Toxicology Program 2000). This result does not necessarily confirm a parallel risk in humans: the doses tested were exceedingly high, the chemical was administered via oral gavage, and oral and percutaneous toxicities appear to be low (Robison and Barr 2006). In addition, ME occurs in many food products and essential oils without apparent health risk (Burdock 1995). Nonetheless, the data obtained from rodents have prompted concern over unnecessary exposure to ME and research for alternative lures, such as a fluorinated analog of ME (Khrimian et al. 2009).

Recently, Farma Tech International Corporation (North Bend, WA) has developed a "wafer" intended to serve as a solid dispenser for RK, ME, or both (the insecticide DDVP is also included in the wafers). The wafers are individually wrapped in a plastic envelope and can be removed and inserted in a trap body rapidly and safely. In a field study conducted in Hawaii, Vargas et al. (2009a) reported no difference in the number of captures of male *B. cucurbitae* (RK/CL-responder) or *B. dorsalis* (ME-responder) in traps baited with liquid CL or ME versus traps baited with wafers containing RK or ME (see also Suckling et al. 2008). More recently, Vargas et al. (2009b), working in a dry area of Kauai Island (annual rainfall < 60 cm) dominated by tropical mesquite (*Prosopis pallida* Humb. & Bonpl. ex Willd.), found that (after 24 h sampling periods) traps baited with wafers containing both RK and ME captured similar numbers of *B. dorsalis* males as traps baited with liquid ME and significantly more *B. cucurbitae* males than traps baited with liquid CL.

The objective of the present study was to compare the effectiveness of the Farma Tech wafers containing both RK and ME relative to liquid lures in two wetter habitats of Hawaii, one being primarily under agricultural and horticultural use and the other being a residential neighborhood. As in the aforementioned studies, the target species were *B. cucurbitae* and B. *dorsalis*, the two main pest *Bactrocera* species in Hawaii. In addition, we examined the effect of ageing on the attractiveness of the cue lure/methyl eugenol-containing wafers by comparing male captures in traps baited with wafers aged 6 or 8 weeks versus traps baited with fresh liquid lures.

Materials and Methods

Traps and lures. Jackson traps (Scentry Biologicals, Inc., Billings, MT) were used exclusively. These were triangular in shape, white in color, and made of thick, waxed paper (12.7 x 9.5 x 8.4 cm, l:w:h). A removable insert, made of the same waxed paper as the trap body and coated with "stickum," was placed on the bottom of the trap to catch the flies. Traps were suspended from tree branches using a metal hanger, with a straight rod that is positioned under the "roof" along the apex of the trap.

All lures, in both liquid and solid form, were obtained from Farma Tech International Corporation (North Bend, WA). Prior to use in the present study, the liquid lures were chemically analyzed by the USDA-APHIS-CPHST Laboratory, Gulfport, MS, and were found to exceed 95% purity. In all tests described below, we applied a total of 6 ml (1% naled) of a given lure to two cotton wicks (each 7.5 cm in length and 1 cm in diameter), which were then placed in a perforated, plastic basket. This basket, in turn, was fastened to the metal hanger and suspended in the middle of the Jackson trap directly above the sticky insert. In solid formulation, RK (1.8%), ME (2.7%), benzyl acetate (1.9%), and a toxicant (DDVP, 0.54%) were contained in a soft rectangular wafer (a polymer matrix, 7.5 by 6.3 cm, 0.1 cm thick), which was suspended in the Jackson trap by inserting the metal hanger through pre-made holes along one of the sides of the wafer.

Trapping wild populations. The relative effectiveness of liquid lures versus the lure-containing wafers was compared via field trapping in two low-lying (< 30 m elevation) locations on Oahu, Hawaii, in 2009. During July–August, trapping was conducted near Waimanalo, a rural area under agricultural and horticultural use with approximately 140 cm rain per year (R. Corrales, personal communication). Traps were placed in the canopy of non-host trees (e.g., fiddlewood, *Citharexylum spinosum* L., kiawe, *P. pallida*, and Norfolk pine, *Araucaria heterophylla* (Salisb.) Franco) along roads and were a minimum of 250 m apart. During September–October, trapping was conducted in Enchanted Lake, a residential area about 5 km from Waimanalo containing mostly single-family homes with scattered fruit trees and backyard gardens (approximately 120 cm rain per year, National Weather Service). Traps were placed on non-host trees along streets (e.g., plumeria, *Plumeria rubra*, L. and non-flowering shower trees, *Cassia* spp.) and were separated by at least 175 m. In both study areas, traps were placed 2-3 m above ground in shaded locations. Maximum and minimum air temperatures generally varied between 29–32°C and 23–25°C, respectively, over the entire July–October interval.

In both Waimanalo and Enchanted Lake, we identified 24 trap sites (trees) and used these same sites over a 6-week period of trapping. During a given week, 12 sites contained two traps, one with liquid CL and the other with liquid ME, while the other 12 sites contained a single trap containing the wafer (with both RK and ME). On trees with liquid lure, the traps containing CL and ME, respectively, were separated by at least 3 m, because the lures may interfere with one another if placed too close together (≤ 1 m), which could influence trap catch (Hooper 1978, Vargas et al. 2000, Shelly et al. 2004). The type of lure formulation (liquid or solid) placed on particular trees was alternated weekly. Owing to the high number of captures (which over long intervals would have covered the sticky insert and blocked further captures), traps were placed in the field for only 1 d per week in Waimanalo. Preliminary trapping revealed much lower fly populations in Enchanted Lake, consequently traps were in place for 3 d per week in that area. At both sites, placement and removal of the traps occurred at midday. When not operating in the field, the traps (with their respective liquid or solid lures) were hung in an outdoor screen cage (and exposed to natural light and moisture conditions) on the grounds of the USDA-APHIS Fruit Fly Rearing Facility in Waimanalo. Neither the solid nor liquid baits were replaced over the course of the 6-week trapping period in either study site.

Attractiveness of aged wafers. In addition to the field trapping described above, we investigated the effect of ageing on the attractiveness of the wafers. Field trials were conducted at the Waimanalo Research Station, University of Hawaii, in a mixed fruit orchard (ca. 2 ha) that contained citrus (*Citrus* spp.), mango (*Mangifera indica* L.), and guava (*Psidium guajava* L.) trees. As these tests were conducted when wild flies were relatively scarce (March–May, 2009), mature (14–19 d old) males of *B. dorsalis* and *B. cucurbitae* from recently established laboratory colonies (< 1 year old) were released to augment wild populations. For a given trial, we placed Jackson traps at 16 sites (trees) arranged in a circle (40 m radius) about a central release point. Eight sites contained a single trap containing an aged wafer, while the remaining eight sites contained two traps, one baited with liquid CL and one baited with liquid ME (6 ml of lure [1% naled] per trap as above, traps on the same tree were separated by a minimum of 3 m as above). Wafers were aged for 6 or 8 weeks in the outdoor, screened cages noted above; the liquid lures were applied to cotton wicks immediately before the start of a trial. Traps were suspended 1.5–2 m above ground in shaded locations between 1000 and 1100 hrs, and flies were released immediately thereafter. Approximately 500 males of each species were released per replicate; the cubical, screened cages were placed on the ground, a side panel was removed gently, and flies exited on their own volition. Traps were collected and scored 24 h later. Both the wafers and wicks were used for a single replicate only. Replicates were separated by 3–5 d, and the type of bait at a given site (wafer or liquid) was alternated between successive trials.

Data analysis. Trapping data for wild populations in Waimanalo and Enchanted Lake were each subject to a two-way ANOVA, with time (week) and lure type (liquid or wafer) as the main factors. With one exception (see below), raw data or $\log_{10}$ transformed data (x + 1) met the assumptions of normality and equal variances. Neither raw nor transformed data for *B. cucurbitae* in Enchanted Lake met the parametric assumptions, consequently the effect of lure type on trap capture was analyzed using the Wilcoxin signed rank test following Zar (1996), where weeks were blocks and weekly means of captured males were compared between lure types (test statistic T). Temporal variation in trap capture of *B. cucurbitae* in Enchanted Lake was examined by pooling raw data across lure types in a given week and comparing these values across weeks using a Kruskal-Wallis test (test statistic H). Regarding the ageing tests, data were pooled over all wafer- or liquid-baited traps in a replicate, and the totals for each species were compared across replicates using a paired t-test (in all cases, raw data met assumptions of normality and equal variance).

Results

Trapping wild populations. In Waimanalo, both trap type ($F_{1, 133} = 7.7$, $P = 0.008$) and week ($F_{5, 133} = 3.4$, $P = 0.007$) had significant effects on numbers of trapped *B. dorsalis* males. The interaction term was not significant ($F_{5, 133} = 1.6$, $P = 0.18$). On average, traps baited with wafers caught more *B. dorsalis* males than traps baited with liquid lures during every sampling period (Fig. 1). In contrast, the data for *B. cucurbitae* in Waimanalo revealed that week ($F_{5, 133} = 3.9$, $P = 0.003$) had a significant effect on trap catch, whereas lure formulation did not ($F_{1, 133} = 0.2$, $P = 0.63$). The interaction term was significant ($F_{5, 133} = 3.1$, $P = 0.012$), which reflected the high variability in relative trap performance over time, as average values for the two trap types alternated rankings between successive weeks (Fig. 1).

Similar results were obtained in Enchanted Lake. Here, both lure type ($F_{1, 133} = 28.4$, $P < 0.001$) and week ($F_{5, 133} = 33.6$, $P < 0.001$) had significant effects on numbers of trapped *B. dorsalis* males. The interaction term was not significant ($F_{5, 133} = 0.4$, $P = 0.88$). Traps baited with wafers caught, on average, more *B. dorsalis* males than traps baited with liquid lures during every sampling period (Fig. 2). As in Waimanalo, there was no significant effect of lure type on trap catch of *B. cucurbitae* males ($T = 5$, $P > 0.05$). This finding is evident through comparisons of weekly means of the two formulations: wafer- and liquid-baited traps caught essentially the same number of *B. cucurbitae* males in three of the six weeks (Fig. 2). There was no significant effect of time (week) on trap catch of *B. cucurbitae* males in Enchanted Lake ($H = 5.3$, $df = 5$, $P = 0.38$).

Attractiveness of aged wafers. Traps baited with wafers aged 6 weeks captured similar numbers of *B. dorsalis* males and significantly more *B. cucurbitae* males than traps baited with fresh liquid lure (Table 1). Traps baited with wafers aged for 8 weeks captured significantly more males of both species than traps baited with fresh liquid lure (Table 1).

Discussion

The present study shows that, in two areas of windward Oahu having very different densities of *Bactrocera* populations, Jackson traps baited with wafers containing RK and ME (plus DDVP) captured as many or significantly more males of *B. cucurbitae* and *B. dorsalis* as Jackson traps baited with liquid CL or ME (plus naled). Whereas previous studies (Hooper 1978, Vargas et al. 2000, Shelly et al. 2004) found mixtures of ME and CL reduced trap catch, no such effect was apparent with wafers. Coupled with the findings of Vargas et al. (2009a, b), the present results indicate that the wafers may be effective in both dry and moderately wet areas of Hawaii. In addition, wafers aged for 6 or 8 weeks

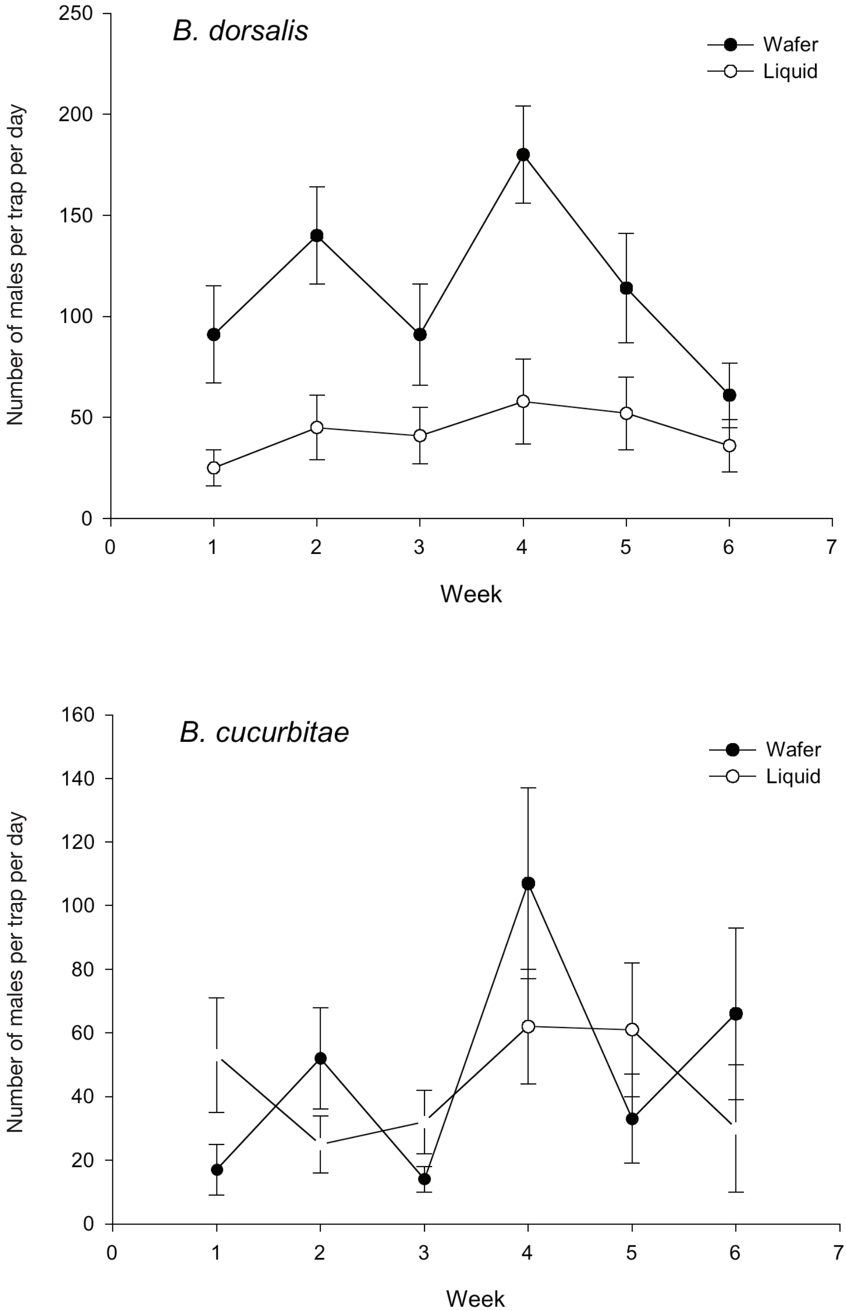

Figure 1. Trap catch of *B. dorsalis* (top) and *B. cucurbitae* (bottom) males in wafer- versus liquid-baited Jackson traps over a 6-week period in Waimanalo, Oahu. Symbols represent averages (± 1 SE) based on 12 traps of a given type. Traps were in place 1 d per week.

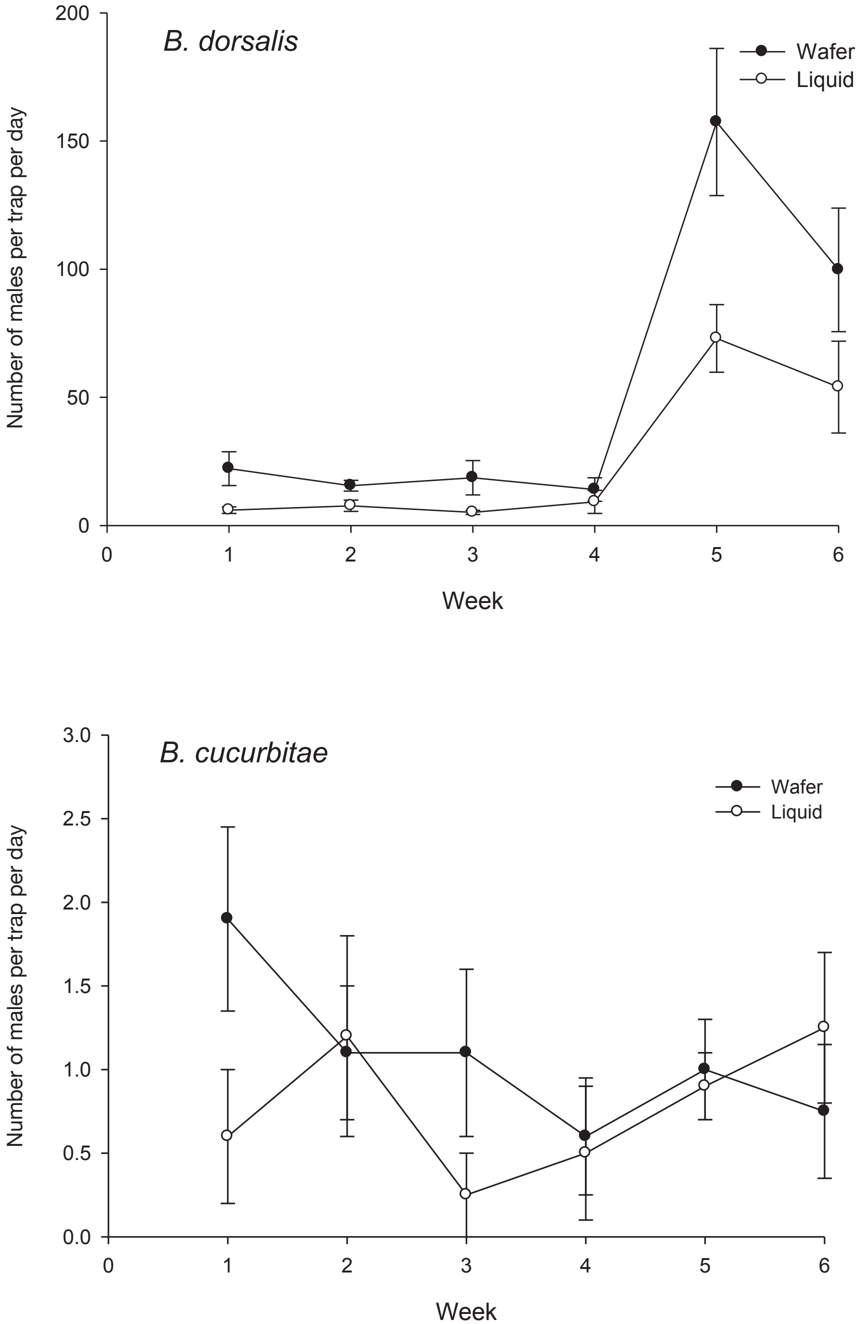

Figure 2. Trap catch of *B. dorsalis* (top) and *B. cucurbitae* (bottom) males in wafer- versus liquid-baited Jackson traps over a 6-week period in Enchanted Lake, Oahu. Symbols represent averages (± 1 SE) based on 12 traps of a given type. Traps were in place 3 d per week.

Table 1. Captures of *B. dorsalis* and *B. cucurbitae* males (including both released and wild individuals) captured in Jackson traps baited with aged wafers (6 or 8 weeks old) containing both methyl eugenol and cue lure or fresh liquid methyl eugenol or cue lure applied to cotton wicks. The values represent the average number (± 1 SE) of males captured in all traps of a given type (i.e., wafer or liquid, n = 8) in a given replicate (n = 8 for 6 weeks ageing and n = 6 for 8 weeks ageing). Results of paired t-tests are provided.

Species	Wafer	Liquid	t	Significance
Wafers aged 6 weeks				
B. dorsalis	660.2 (83.9)	545.6 (57.8)	2.13	P = 0.071
B. cucurbitae	325.7 (81.1)	183.2 (26.8)	2.39	P = 0.048
Wafers aged 8 weeks				
B. dorsalis	267.3 (36.2)	175.2 (29.3)	2.90	P = 0.034
B. cucurbitae	123.3 (14.5)	103.5 (10.1)	2.79	P = 0.039

were equally or even more attractive to both *B. cucurbitae* and *B. dorsalis* males as fresh liquid lures. These are the first data specifically addressing the effects of weathering on the RK/ME wafers, and they show no decrease in their attractiveness under the climatic conditions found in Waimamanlo.

The lure and insecticide-bearing wafers appear to be a viable replacement for the liquid formulations now in use. Use of the solid dispensers would presumably reduce the time spent preparing traps as well as the health risks associated with measuring and applying the liquid lure-insecticide mixture. In addition, because the wafers attracts both CL- and ME-responding *Bactrocera* males, they would eliminate the need for separate sets of LC and ME traps and thus reduce the number of traps placed in the field by 50%. This could result in considerable cost savings for large-scale surveillance programs, as in California, that continuously operate tens of thousands of *Bactrocera* detection traps (IPRFFSP 2006). The results from Hawaii notwithstanding, it is, of course, necessary to compare wafer- and liquid-baited traps in California as the relative performance of the two formulations may differ between locations. In California, this can be accomplished via releases of dyed, irradiated males equidistant from the two formulations, with releases conducted in various grids to include potential spatial heterogeneity in trap performance. In addition, tests should involve both fresh and aged formulations, with the ageing conducted under local conditions in California.

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