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RELEASE-RECAPTURE OF *BACTROCERA* FRUIT FLIES (DIPTERA: TEPHRITIDAE): COMPARING THE EFFICACY OF LIQUID AND SOLID FORMULATIONS OF MALE LURES IN FLORIDA, CALIFORNIA AND HAWAII

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Abstract

Invasive species of *Bactrocera* fruit flies (Diptera: Tephritidae), particularly *B. dorsalis* (Hendel) and *B. cucurbitae* (Coquillett), pose serious threats to agricultural crops. Detection relies largely on traps baited with the male lures methyl eugenol (ME), which is attractive to *B. dorsalis*, and cue-lure (CL) or the related chemicals raspberry ketone (RK) and raspberry ketone formate (RKF), which are all attractive to *B. cucurbitae*. Currently, ME and CL are applied as liquids to cotton wicks (along with an insecticide), a procedure involving considerable handling time and exposure to pesticides. Recent studies have shown that traps baited with solid dispensers (plugs or wafers) of male lures, which arrive in sealed envelopes ready for use, catch at least as many *Bactrocera* males as traps baited with liquid lures. The present study compared captures of *B. dorsalis* and *B. cucurbitae* males in traps baited with liquid lures versus traps baited with lure-bearing solid dispensers in Florida, California, and Hawaii. In the first 2 locations, marked, irradiated males were released at street intersections, and captures were scored at 4 trapping stations 50 m distant along the intersecting streets with various lure/dispenser combinations. In Hawaii, trap catch of wild *B. dorsalis* and *B. cucurbitae* were compared among traps with liquid and solid formulations of the lures. Although several exceptions were observed, the overall finding was that the lure-bearing plugs and wafers captured as many or more *Bactrocera* males as the liquid application. Consequently, we suggest that solid dispensers could be adopted in area-wide fruit fly surveillance programs without lessening their detection sensitivity to incipient infestations. The use of solid dispensers that contain both ME and RK, in particular, could greatly reduce the number of traps required and result in considerable cost savings.

Key Words: fruit fly, detection, trapping, methyl eugenol, cue-lure

Resumen

Las especies invasoras de moscas de la fruta del género *Bactrocera* (Diptera: Tephritidae), en particular *B. dorsalis* (Hendel) y *B. cucurbitae* (Coquillett), constituyen una grave amenaza para los cultivos agrícolas. Su detección se basa en gran medida en trampas cebadas con el eugenol metíl (EM), un señuelo para los machos, que es atractivo para *B. dorsalis*, y un señuelo de señal (SS) o los químicos afines, cetona de frambuesa (KF) y formiato de la cetona de frambuesa (FCF), que son atractivos para *B. cucurbitae*. Actualmente, se aplican EM y SS en forma de líquido a mechas de algodón (con un insecticida), un procedimiento que requiere mucho tiempo de manipulación y la exposición a los pesticidas. Estudios recientes han demostrado que las trampas cebadas con dispensadores sólidos (tapones u obleas) de señuelos para los machos, que vienen en sobres sellados ya listos para el consumo, capturan por lo menos tantos machos de *Bactrocera* como las trampas cebadas con atrayentes líquidos. El presente estudio comparó las capturas de machos de *B. dorsalis* y *B. cucurbitae* en trampas cebados con atrayentes líquidos en comparación con las trampas de dispensadores sólidos cebadas con un señuelo en Florida, California y Hawai. En los dos primeros sitios, los machos irradiados fueron liberados en las intersecciones de las calles, y el número de machos capturadas fue anotado en 4 estaciones de captura con una distancia de 50 m entre ellas por las calles cruzadas con varias combinaciones de atrayentes/dispensadores. En Hawai, se compararon las capturas de moscas salvajes de *B. dorsalis* y *B. cucurbitae*

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en las trampas con las capturas de las trampas con formulaciones líquidas y sólidas de los señuelos. Aunque se observaron algunas excepciones, la conclusión general fue que las trampas cebadas con tapones y obleas cebadas con señuelos capturaron tantos o más machos de *Bactrocera* como la aplicación de líquido. Por eso, se sugiere que los dispensadores sólidos podrían ser adoptados en los programas de vigilancia para las moscas de la fruta en toda el área sin disminuir su sensibilidad de detección a las infestaciones incipientes. Palabras clave: mosca de la fruta, detección, captura, eugenol metíl, señuelo- señal

Palabras Clave: mosca de la fruta, detección, trampeo, methyl eugenol, señal- atracción

Invasive species of *Bactrocera* fruit flies (Diptera: Tephritidae) are important agricultural pests throughout tropical Asia and Australasia (White & Elson-Harris 1992), with several species having recently spread to Africa (White 2006) and South America (Sauers-Muller 1991). In particular, 2 polyphagous species, *B. dorsalis* (Hendel) and *B. cucurbitae* (Coquillett), pose serious threats to commercial crops and international trade, with the former species attacking many important fruits (e.g., mango, *Mangifera indica* L.; papaya, *Carica papaya* L.; and sweet orange, *Citrus sinensis* L.) and the latter attacking cucurbits primarily (e.g., cucumber, *Cucumis sativus* L.; zucchini, *Cucurbita pepo* L.; and watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai) (White & Elson-Harris 1992). Because of the potential economic impact of these species, several U.S. states, most notably California, Florida, and Texas, operate continuous monitoring programs to detect incipient infestations. In California, for example, approximately 25,000 traps are maintained specifically for *Bactrocera* detection in the Los Angeles area (IPRFFSP 2006).

Detection of *Bactrocera* species relies largely on traps baited with male lures or parapheromones (Cunningham 1989; Metcalf 1990). Among lure-responding *Bactrocera* species, males of a given species are attracted to either raspberry ketone (RK, or its artificial analog, cue-lure, CL) or methyl eugenol (ME) but not both (Drew 1974; Hardy 1979; Drew & Hooper 1981). Regarding the 2 aforementioned species, *B. dorsalis* males respond to ME, while *B. cucurbitae* respond to RK/CL, and accordingly surveillance programs operate both ME- and CL- baited traps. In U.S. detection programs, ME and CL are applied as liquids to cotton wicks positioned inside traps, with the insecticide naled added (also in liquid form) to the lures before application to the wicks. Thus, the current procedure involves considerable handling for measuring and applying the liquids as well as potential health risks resulting from pesticide exposure.

A series of field studies, most of which were conducted in Hawaii, has shown that traps baited with solid dispensers of male lures catch at least as many *Bactrocera* males as traps baited with liquid lures (Hiramoto et al. 2006; Jang et al. 2007; Vargas et al. 2009; Vargas et al. 2010; Shelly 2010; Shelly et al. 2011a,b; Leblanc et al. 2011; Vargas et al. 2012; see also Suckling et al. 2008). Collectively, these studies tested ME, RK, and CL as well as raspberry ketone formate (RKF), which, as the name implies, is chemically related to RK and CL and attracts RK/CL-responding species. Also, the solid dispensers used in these studies were polymerized matrices of 2 types, i.e., plugs (small cylinders) and wafers (larger, thin rectangles). These solid dispensers are appealing for large scale detection programs, because they arrive from the manufacturer in sealed envelopes and, with lure and insecticide already in place, they are immediately ready for use. Relative to liquid lures, therefore, the handling time and any possible health hazards associated with solid dispensers are much reduced.

The purpose of the present study was to refine similar work carried out previously, by comparing captures of *B. dorsalis* and *B. cucurbitae* males in traps baited with liquid lures versus traps baited with lure-bearing solid dispensers in residential areas of Sarasota, Florida, and Anaheim, California. This study expands upon previous research conducted in southern California (Shelly et al. 2011a) involving these same 2 *Bactrocera* species, which showed that males released equidistant from traps baited with liquid ME or CL and traps baited with ME/RK wafers were captured in equal or greater numbers in the wafer-baited traps. Here, we similarly report trap captures of released *Bactrocera* males but also compare liquid ME and CL, not only with ME/RK wafers (as in the previous California study), but also with plugs bearing ME, CL, or (in the case of Sarasota, FL) RKF. Ancillary field tests were also conducted in Hawaii on wild flies to assess the reliability of test results from the mainland locations. Thus, the results presented here allow assessment of the relative performance of liquid and solid dispensers in a variety of environmental conditions. The implications of the present findings for fruit fly detection programs are discussed.

Materials and Methods

Initial field work was conducted in Florida, and owing to a somewhat unexpected result (de-

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scribed below), follow-up studies were then performed in California and Hawaii. A similar protocol was adopted in Florida and California, and here we first describe the methods employed in Florida and then present a brief description of the California trials, noting only the pertinent differences from the Florida tests. Finally, we describe the supplementary field work in Hawaii, which differed from the other locations in monitoring trap capture of wild *Bactrocera* males with the different lure formulations.

Florida Trials

Study Site

Field work was conducted during Oct-Nov 2010, and Sep-Oct 2011, in residential neighborhoods of Sarasota, Florida. In general, the test sites contained single dwelling homes with many large shade trees planted in yards and along streets. Daily minimum and maximum air temperatures averaged 17.8 °C and 25.5 °C, respectively, during the 2010 study period and 21.1 °C and 26.7 °C, respectively, during the 2011 study period.

Study Insects

The flies used in this study derived from colonies maintained in Hawaii. For *B. dorsalis*, flies derived exclusively from a colony held at the USDA-APHIS facility, Waimanalo, Hawaii, that was started with 300-500 adults reared from field-collected papaya and mango. The colony, which was maintained at 2,000-3,000 individuals, was started ≈ 2 yr before the current study and was regularly "refreshed" by adding flies emerging from field-collected fruits. The colony was held in a screen cage (1.0 m × 0.9 m × 0.8 m) and provided with a mixture (3:1, wt:wt) of sugar (sucrose) and hydrolyzed protein (enzymatic yeast hydrolysate) and water ad libitum. Papayas, purchased in local supermarkets, were rinsed in water and introduced periodically for oviposition. Infested fruits were held over vermiculite, and pupae were sifted from the substrate. The colony was held at 23-25 °C and 60-90% RH and received artificial light under a daily photoperiod of 12:12 h L:D.

Unlike *B. dorsalis*, for which all trials were completed in 2010, tests involving *B. cucurbitae* were performed in both 2010 and 2011. In 2010, the flies derived from 2 sources: i) a colony held at the USDA-APHIS facility, Waimanalo, that was started at the same time and maintained in the same manner as the *B. dorsalis* colony described above and ii) a much larger and older colony maintained by the USDA-ARS laboratory in Hilo, Hawaii. Although our original intent was to use flies from the Waimanalo colony exclusively (as this colony was younger and presumably more 'wild' than the Hilo colony), a sudden decline in that population necessitated use of additional flies from the Hilo colony in 2010, such that all releases in that year included a mixture of males from the 2 colonies. Owing to the subsequent demolition of the Waimanalo facility in early 2011, *B. cucurbitae* were derived exclusively from the Hilo facility for trials performed in 2011.

The colony held at Waimanalo (used for the 2010 trials) was started with 600-800 adults reared from field-collected honeydew melons (*Cucumis melo* L.). Commercial zucchini were used for oviposition. The colony held at Hilo had been maintained at several million individuals for over 50 yr (based on Wong et al. 1991) following standard mass-rearing procedures (Spencer & Fujita 1997). While slight, but statistically significant, differences have been reported in the response of mass-reared and wild males of *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly) to the male lure trimedlure (Wong et al. 1982; Shelly & Edu 2009), cue-lure was found to have similar attractiveness to wild *B. cucurbitae* males as males from the same mass-reared strain used in the present study (Wong et al. 1991). Consequently, we assume the use of males from this mass-reared strain did not generate biased results regarding the relative attractiveness of the different lure formulations tested here.

To obtain males for release, we followed the procedures developed for sterile release programs against *Bactrocera* species (Orankanok et al. 2007). Two days before adult emergence, *Bactrocera* pupae were marked with dye (3 g of fluorescent dye per liter of pupae; see below), placed in transparent plastic bags, irradiated under hypoxia at 100 Gy by using a Co^{60} source (GammaCell 220 Excel; Nordion, Ottawa, Canada) and then shipped in insulated boxes (containing coolant) via express courier to the USDA-APHIS facility in Sarasota, Florida. The interval between irradiation and packing in Hawaii and arrival in Sarasota was approximately 48 h. With respect to marking, upon emergence the adults retain dye particles on the collapsed ptilinum, which may be viewed with a dissecting microscope under UV (blacklight) by crushing the head with a forceps. In nearly all cases, dye may also be visible on the exterior body surface.

In 2010, immediately upon receipt, the pupae were transferred to paper bags, which were placed inside screen cages (30 cm × 40 cm × 30 cm) with food (the same mixture noted above) and water. In 2011, pupae (*B. cucurbitae* only) were transferred to screened trays (the type used in eclosion towers, Salvato et al. 2004; 76 cm × 76 cm × 2.5 cm) with food and water sources resting on the outer surface. Emerged adults were separated by sex within 72-96 h of emergence by chilling the flies at 4 °C and selecting physically intact males (deformed males and females were killed by freez-

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ing). Chilling and sexing were accomplished in a walk-in, refrigerated room, and individual "sexing" sessions lasted 30-45 min. Males were then placed in screen cages with food and water. Pupae and adults were held at 21-22 °C and 60-70% RH and received natural and artificial light under a daily photoperiod of 12:12 h L:D. Adults of both *B. dorsalis* and *B. cucurbitae* from the Waimanalo colonies were held 14 days to achieve sexual maturation, when, in both species, male response to the lures is greatest (Wong et al. 1989, 1991; Shelly et al. 2008). Adults of *B. cucurbitae* from the Hilo colony were held only 10 days before release as long-term mass rearing has resulted in accelerated development, and the majority of males from this strain are sexually mature by 10 days (D. O. McInnis, personal communication).

Traps and Lures

Jackson traps (Scentry Biologicals Inc., Bozeman, Montana) were used exclusively following IAEA (2003) guidelines. In all traps, ME was the only lure used to attract *B. dorsalis* males and was presented separately i) as a liquid applied to cotton wicks or ii) in plugs or iii) in combination with RK in wafers. In contrast, 3 different lures, CL, RK, and RKF (all with very similar chemical structure) were used to attract *B. cucurbitae* males. CL was presented separately i) as a liquid applied to cotton wicks or ii) in plugs. RK was used only in combination with ME in wafers. RKF was used only separately in plugs.

Liquid ME and CL were obtained from Farma Tech International Corporation (North Bend, Washington) and met USDA's standards for purity. In all tests, 6 mL of liquid lure (containing 1% or 5% naled in ME and CL, respectively) were applied to a cotton wick; the specific gravity of the liquid lures is near unity, and thus approximately 6 g of a lure was used per wick. The wick was then placed in a perforated plastic basket, which, in turn, was suspended in the middle of the trap above the sticky insert. The plugs (Scentry Biologicals Inc.) were cylindrical and contained 6 g of ME, CL, or RFK (with 0.3 g of naled [5% active ingredient]). Dimensions (height/diameter in cm) of the plugs were: ME - 2.2/2.1; CL – 3.2/1.7; RKF – 3.8/1.7. Plugs were placed in perforated baskets as noted above for the wicks. The wafers (Farma Tech International Corporation) bearing both ME and RK were rectangular (7.0 cm × 5.0 cm × 0.32 cm) and contained 3.1 g of ME, 2.1 g RK, and 0.5 g of DDVP (an insecticide, dichlorvos; 16% and 24% of ME and RK, respectively). While the wafers contained less lure than the wicks or the plugs, traps baited with this same wafer captured an equal or greater amount of *Bactrocera* males as traps baited with 6 mL of liquid lure in various field experiments performed in Hawaii (Shelly et al. 2011b). It should also be noted that while blends of liquid ME and liquid CL repel *B. dorsalis* males (Shelly et al. 2004), the use of RK instead of CL in the wafer appears to eliminate this effect. The wafers were suspended in the Jackson traps by inserting the metal hanger through premade holes along 1 (long) side of the wafer.

Trap captures were compared using fresh and aged lures. Fresh lures were placed in the traps the day a trial started, while aged lures were used after 5 wk of weathering. To obtain aged lures, wicks, wafers, and plugs were placed in Jackson traps (without the sticky insert), which were then suspended approximately 2 m above ground in a shaded area at the USDA-APHIS facility in Sarasota under the same temperature conditions noted above.

Release-Recapture Trials

The basic experimental design involved fly release at a central point (a street intersection) and trap placement in the 4 cardinal directions (i.e., N, S, E, and W) around the release point (i.e., along the intersecting streets). Thirteen test sites were selected based on the following: the streets crossed at right angles, one corner of the intersection had a large tree or bush (to serve as a release point), the intersecting streets and adjacent properties had ample shade trees and bushes (to provide resting sites for the released flies), and relatively large bushes or trees occurred at approximately 50 m from the designated release point along the intersecting streets in all 4 cardinal directions (to serve as trap locations).

At the start of a test, we placed traps in trees or bushes at 4 stations (N, S, E, and W) at 50 m from the designated release point along each of the intersecting streets. Among the 4 trap stations, 3 contained 2 traps baited with i) liquid ME and liquid CL, respectively, ii) ME and CL plugs, respectively, and iii) ME and RKF plugs, respectively. For these stations, the 2 traps were placed 2-3 m apart to avoid interference between the lures, which could influence trap catch (Vargas et al. 2000; Shelly et al. 2004). The 4th location contained a single trap baited with a wafer that contained both ME and RK. All traps were placed approximately 2 m above ground in a shaded site. Table 1A summarizes the different lure presentations among the 4 trapping stations per test site.

Immediately following trap placement, we released *Bactrocera* males at the central point. Based on an earlier study (Shelly et al. 2010), we used 200 *B. dorsalis* males and 300 *B. cucurbitae* males per release as these numbers were considered sufficient to provide adequate captures for statistical analysis. Flies were transported to the field in screen-covered plastic containers and were released by removing the screen and gently tapping the container. After several minutes, non-flying and dead males were counted

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Table 1. Description of lure presentations at the 4 trapping stations situated around a central release point in (a) Sarasota, Fl and (b) Anaheim, Ca, respectively.

A. Sarasota		
Lures presented	Manner of presentation	Number of traps per station
ME, CL	Separately, liquid on wicks	2, 1 for each lure
ME, CL	Separately, plugs	2, 1 for each lure
ME, RKF	Separately, plugs	2, 1 for each lure
ME/ RK	Together, wafer	1, containing both lures
B. Anaheim		
Lures presented	Manner of presentation	Number of traps per station
ME, CL	Separately, liquid on wicks	2, 1 for each lure
ME, CL	Separately, plugs	2, 1 for each lure
ME, CL	Separately, wafers	2, 1 for each lure
ME/ RK	Together, wafer	1, containing both lures

(and killed) and replaced with new individuals of the same age. Following the initial release, we re-visited the site 48 h later and i) replaced the sticky inserts from all traps, ii) rotated the traps between stations (i.e., N to E, E to S, etc.) to control for possible directional (i.e., wind) effects , and iii) released another set of males. This procedure was repeated until the different dispensers were presented at all 4 stations. Thus, a test (replicate) involved a total of 4 releases per species per site (i.e., on days 0, 2, 4, and 6), with traps remaining in place for 2 d following the final release (i.e., until day 8). This entire procedure was conducted twice for each species over all 13 test sites, once using fresh lures and once using aged lures. Tests using fresh or aged lures were run concurrently at different sites to avoid possible long-term temporal effects (from variable weather or fly quality) on trap captures for either age group. As noted above, all releases of *B. dorsalis* were completed in 2010. In that year, *B. cucurbitae* males were released simultaneously with *B. dorsalis* males at 4 sites in tests involving fresh lures and 6 sites in tests involving aged lures. In 2011, *B. cucurbitae* males were used exclusively to complete the releases for fresh and aged lures at all sites. Although releases often included a single species only, the full complement of dispensers was used in all cases. Trap inserts were delivered to the Florida Department of Agriculture and Consumer Services, Fruit Fly Identification Laboratory, where trapped *Bactrocera* flies were counted and checked for the presence of dye.

Data Analysis

For both species, variation in trap captures was first analyzed by linear mixed models using Restricted Maximum Likelihood (REML) algorithms. The impact of neighborhood (random effect) was blocked, and the effects of lure/dispenser (fixed effect, the 4 types listed in Table 1A) and lure age (fixed effect, fresh or aged) on the remaining variation were examined. This analysis revealed that neighborhood accounted for 0% and 4.5% of the total variation observed in male captures of *B. dorsalis* and *B. cucurbitae*, respectively. Thus, site differences were inconsequential, and their effect on the data was negligible. In light of this finding, we re-analyzed capture data for both species omitting neighborhood as a factor. For each replicate (n = 13 sites), trap captures for the different lure station types were added over the 4 releases to yield a single total capture for *B. dorsalis* and *B. cucurbitae*, respectively. These values were then used in a 2-way ANOVA, with lure/dispenser (the 4 types listed in Table 1A) and lure age (fresh or aged) as main factors (raw data were normalized by square root transformation). As will be shown, the interaction term was significant for both species; consequently, the impact of lure/dispenser could not be assessed independently of lure age and vice versa. Consequently, we present the results of the Tukey test, a multiple comparisons test, separately for each of the 2 main factors. Statistical analyses were performed using SigmaPlot 11.0.

California Trials

The tests conducted in California were similar to those described above for Florida but with the following chief differences.

Study Site

Field work was conducted in May-Jun, 2012, in residential areas in and near Anaheim, California. Daily minimum and maximum air temperatures averaged 16.1 °C and 27.7 °C, respectively, during the study period.

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Study Insects

Males of both *B. dorsalis* and *B. cucurbitae* derived from long-established colonies maintained by USDA-ARS, Hilo, Hawii, owing to the aforementioned demolition of the Waimanalo facility in 2011. The *B. dorsalis* colony had been maintained for approximately 22 yr (D. O. McInnis, personal communication). Males from this strain were found to respond to ME at the same rate as wild males (Wong et al. 1989), and thus we assume that the trials conducted with this strain did not produce biased results. The interval between irradiation and packing of the pupae in Hawaii and arrival at the CDFA-USDA Facility in Los Alamitos, California, was approximately 24 h. As with *B. cucurbitae* males derived from the Hilo colony, *B. dorsalis* males were held only 10 days before release, owing to their accelerated sexual maturation (McInnis et al. 2011).

Traps and Lures

As in Florida, ME was the only lure used to attract *B. dorsalis* males, but in California ME was presented separately i) as a liquid applied to cotton wicks, ii) in plugs, iii) in wafers or iv) in combination with RK in wafers. The liquid and plug dispensers were identical to those used in Florida, but when presented in separate wafers (ME-only) or in combination wafers (with 2.3 g RK, i.e., similar dose to that used in Florida), the ME dose was 6 g. Thus, unlike the Florida lures, the ME dose used was identical among liquid, plug, and wafer formulations. Regarding attractants for *B. cucurbitae* males, CL and RK were used as attractants, but RFK was not. CL was used separately i) as a liquid applied to cotton wicks, ii) in plugs or iii) in wafers. The liquid and plug formulations were the same as those used in Florida, and the separate wafer (CL only) contained 2.3 g of CL. Thus, in California, while ME loadings were equivalent among the different dispensers, CL (separate wafers) and RK (combination wafers) loadings were maintained at approximately the same doses used in Florida, which were substantially lower than the CL doses used in liquid or plug formulations (as will be shown, this reflects the high trap catch of RK-laden wafers in Florida). Fresh and aged lures were tested in California, with lure ageing taking place outside the CDFA facility in Anaheim (Jackson traps with lures were suspended approximately 2 m above ground from a chain link in full sunlight) under the same temperature conditions noted above.

Release-Recapture Trials

The experimental design was the same as that used in Florida and involved release-recapture in 9 sites in and around Anaheim. At a given site, 2 of the 4 trapping stations were the same as in Florida, namely the liquid lures (2 traps with separate liquid ME and liquid CL, respectively) and the plugs (2 traps with separate ME and CL plugs, respectively). However, in California the remaining 2 stations were separate wafers (2 traps with ME and CL wafers, respectively) or combination wafers (1 trap with a ME/RK wafer). Table 1B summarizes the different lure presentations among the 4 trapping stations per test site in California. Releases were performed as described for Florida, except that i) 200 *B. dorsalis* males and 200 *B. cucurbitae* males were used per release and ii) dead males were counted but not replaced. Trap inserts were delivered to CDFA's Insect Identification Section, where staff members examined trapped *Bactrocera* males for the presence of dye and provided counts.

Data Analysis

Data were analyzed in the same manner as described above for Florida. Although non-flying males were not replaced, these males represented only small proportions of the release totals and were nearly identical for the 2 species. For a given replicate, on average, only 6.1% (49/800) of *B. dorsalis* males and 7.0% (56/800) of *B. cucurbitae* males were non-fliers, and consequently, we performed the analysis using absolute numbers of captured flies despite slight variation in release numbers among replicates. The initial analysis in which the neighborhood effect was blocked showed that between-site variation accounted for only 2.7% and 4.3% of the total variation observed in male captures of *B. dorsalis* and *B. cucurbitae*, respectively. In light of these findings, we again re-analyzed capture data for both species using a 2-way ANOVA omitting neighborhood as a factor. As shown below, the interaction term was significant for *B. dorsalis*, and as with the Florida data, we present the results of the Tukey test separately for each of the 2 main factors.

Hawaii Trials

Field work in Hawaii was conducted during Jun-Aug 2012, to provide supplementary data on lure effectiveness from wild populations of *B. dorsalis* and *B. cucurbitae*. At 4 locations on Oahu, we compared male captures among Jackson traps baited with fresh or aged lures of the following types: i) liquid ME and CL applied to cotton wicks placed separately in 2 traps, ii) ME and CL plugs placed separately in 2 traps, and iii) ME/RK wafers placed singly in traps. Lure doses were the same as those used in California for all formulations. The field sites included Mililani Agricultural Park, Waimanalo, Kapolei, and Waialua, descriptions of which are available in Shelly et al. (2012a). At each site, we placed traps

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with fresh lures in a particular dispenser type at 12 sites separated by a minimum of 50 m. Sites were non-host trees, and traps were placed in the canopy 1.5-2 m above ground. Traps operated for 48 h, captures were then scored at the laboratory, and the traps (without the sticky inserts) were suspended outdoors in the shade to allow ageing. After 6 wk, the field protocol was repeated with the aged lures in each of the 4 study areas.

Because wild populations may have varied in size between trials involving fresh and aged lures and because description of such variation was not the focus of the study, we performed separate analyses comparing the 3 lure/dispenser types for fresh and aged treatments, respectively, using a 1-way ANOVA (following square root transformation of the raw data), with the Tukey test again used to identify significant differences among treatments.

Results

Florida Trials

Bactrocera dorsalis

Lure/dispenser ($F_{3, 96}$ = 6.7, P < 0.001) had a significant effect on captures of *B. dorsalis* males, but lure age did not ($F_{1, 96}$ = 0.1, P = 0.99; Table 2). The interaction term was significant ($F_{3, 96}$ = 6.4, P < 0.001). For fresh lures, the Tukey test revealed no significant differences between traps of any of the different lure/dispenser combinations. For aged lures, captures were significantly lower for traps baited with wafers than for any other lure/dispenser presentation, and it is this result, which was unexpected based on previous studies (Vargas et al. 2009; Vargas et al. 2010; Shelly 2010; Shelly et al. 2011a,b; Leblanc et al. 2011), that prompted the California and Hawaii tests with enhanced ME levels in the wafers. While lure age did not have a significant effect overall, the interaction term was significant, which reflected pronounced differences in the relative performance of fresh vs aged lures among different lure/dispenser combinations. In particular, trap catch was significantly greater for aged than fresh lures for liquid ME, whereas the converse was true for traps baited with wafers. No significant effect of lure age was observed for the plugs.

Bactrocera cucurbitae

Lure/dispenser ($F_{3, 96}$ = 8.0, P < 0.001) had a significant effect on captures of *B. cucurbitae* males, but lure age did not ($F_{1, 96}$ = 3.5, P = 0.07; Table 2). The interaction term was significant ($F_{3, 96}$ = 3.0, P = 0.04). For fresh lures, the Tukey test revealed that traps baited with wafers captured significantly more males than traps baited with liquid or RKF plugs. For aged lures, captures were not significantly different between traps baited with liquid lures and wafers, but both of these had significantly higher trap catch than traps baited with CL or RKF plugs. While lure age did not have a significant effect overall, the interaction term was significant, which reflected pronounced differences in the relative performance of fresh vs. aged lures among different lure/dispenser combinations. Traps with fresh and aged wafers caught nearly identical numbers of males, while traps with aged liquids captured more *B. cucurbitae* males than traps with fresh liquids, although this difference was not statistically significant. In contrast, trap catch was lower for aged vs. fresh CL and RKF plugs, and in the case of the CL plug, this decline was statistically significant.

Table 2. Average numbers of released *B. dorsalis* and *B. cucurbitae* males captured per replicate in traps baited with different lures and/or dispensers in Sarasota, Fl (as described in Table 1a). for each replicate, trap captures for a given lure/dispenser combination were added over the 4 cardinal directions to yield a single total capture. values within each column sharing a capital letter and values within each row sharing a lower case letter did not differ significantly (Tukey test).

Bactrocera dorsalis

Lures presented	Fresh lures Average (SE)	Aged lures Average (SE)
Liquid ME, CL	78.6 (7.8) A, d	121.5 (9.0) B, e
Plug ME, CL	73.5 (9.2) A, f	105.6 (17.5) B, f
Plug ME, RKF	99.6 (15.1) A, g	94.3 (17.0) B, g
Wafer ME/RK	79.8 (11.4) A, h	34.5 (8.1) C, j

Bactrocera cucurbitae

Lures presented	Fresh lures Average (SE)	Aged lures Average (SE)
Liquid ME, CL	25.9 (3.6) A, e	35.9 (7.7) C, e
Plug ME, CL	36.4 (5.4) AB, f	17.8 (3.0) D, g
Plug ME, RKF	28.5 (5.5) A, h	18.6 (3.5) D, h
Wafer ME/RK	43.5 (4.3) B, j	43.2 (5.3) C, j

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California Trials

Bactrocera dorsalis

Lure/dispenser ($F_{3, 64} = 2.9$, $P = 0.04$) had a significant effect on captures of *B. dorsalis* males, but lure age did not ($F_{1, 64} = 3.3$, $P = 0.07$; Table 3). The interaction term was significant ($F_{3, 64} = 5.6$, $P = 0.002$). For fresh lures, the Tukey test revealed that traps baited with the ME-only or combination wafers captured significantly more males than traps baited with liquid or plug formulations and that these latter types did not differ significantly. For aged lures, the Tukey test revealed no significant differences between traps of any of the different lure/dispenser combinations. While lure age did not have a significant effect overall, the interaction term was significant, which reflected pronounced differences in the relative performance of fresh vs. aged lures among different lure/dispenser combinations. In particular, trap catch was significantly greater for aged than fresh lures for liquid ME, whereas the converse was true for traps baited with ME-only wafers. No significant effect of lure age was observed for the ME plugs or the combination wafers.

Bactrocera cucurbitae

Both lure/dispenser ($F_{3, 64} = 8.5$, $P < 0.001$) and lure age ($F_{1, 64} = 17.7$, $P < 0.001$) had significant effects on captures of *B. cucurbitae* (Table 3). The interaction term was not significant ($F_{3, 64} = 0.2$, $P = 0.9$). Among both fresh and aged lures, traps baited with the combination wafer captured significantly more males than traps baited with CL liquid or plugs, while traps baited with CL-only wafers captured intermediate numbers. Male captures did not differ significantly between liquid CL and CL plugs for either fresh or aged lures. As indicated above, lure age had a significant overall effect on male captures, and while a significant difference between fresh and aged lures was noted only for combination wafers, large decreases in male captures with lure age were noted for all formulations.

Hawaii Trials

Bactrocera dorsalis

Across the 4 sites and over both age categories, there were few significant differences observed in trap captures of *B. dorsalis* males among the dispensers (Fig. 1). Traps baited with wafers caught significantly more males than traps baited with liquid or plugs among fresh lures at Kapolei, and the same result was observed among aged lures at Waimanalo. Captures for liquid- and plug-containing traps were similar in all cases.

Bactrocera cucurbitae

Captures of *B. cucurbitae* males were similar among the different dispenser/lure combinations at all 4 sites and for both age categories, with 2 exceptions (Fig. 2). At Kapolei, traps with fresh wafers caught significantly more males than traps with fresh liquid or plugs, and at Waialua, traps with aged wafers caught significantly more males than traps with aged liquid or plugs.

Discussion

The present study showed that, with relatively few exceptions, solid dispensers containing male lures were equally or even more effective in trapping *Bactrocera* fruit flies than the currently used liquid formulations applied to cotton wicks.

Table 3. Average numbers of released *B. dorsalis* and *B. cucurbitae* males captured per replicate in traps baited with different lures and/or dispensers in Anaheim, Ca (as described in Table 1b). for each replicate, trap captures for a given lure/dispenser combination were added over the 4 cardinal directions to yield a single total capture. Values within each column sharing a capital letter and values within each row sharing a lower case letter did not differ significantly (Tukey test).

Bactrocera dorsalis

Lures presented	Fresh lures Average (SE)	Aged lures Average (SE)
Liquid ME, CL	85.9 (9.3) B, d	135.9 (8.3) C, e
Plug ME, CL	77.4 (7.9) B, f	104.9 (11.3) C, f
Wafer ME, CL	132.6 (15.2) A, g	96.8 (12.7) C, h
Wafer ME/RK	115.1 (10.2) A, i	125.7 (8.4) C, i

Bactrocera cucurbitae

Lures presented	Fresh lures Average (SE)	Aged lures Average (SE)
Liquid ME, CL	25.2 (5.9) B, f	9.9 (1.1) E, f
Plug ME, CL	26.9 (6.8) B, g	14.9 (2.5) D, E, g
Wafer ME, CL	40.1 (6.7) AB, h	20.3 (2.5) C,D, h
Wafer ME/RK	57.1 (10.2) A, i	31.3 (5.3) C, j

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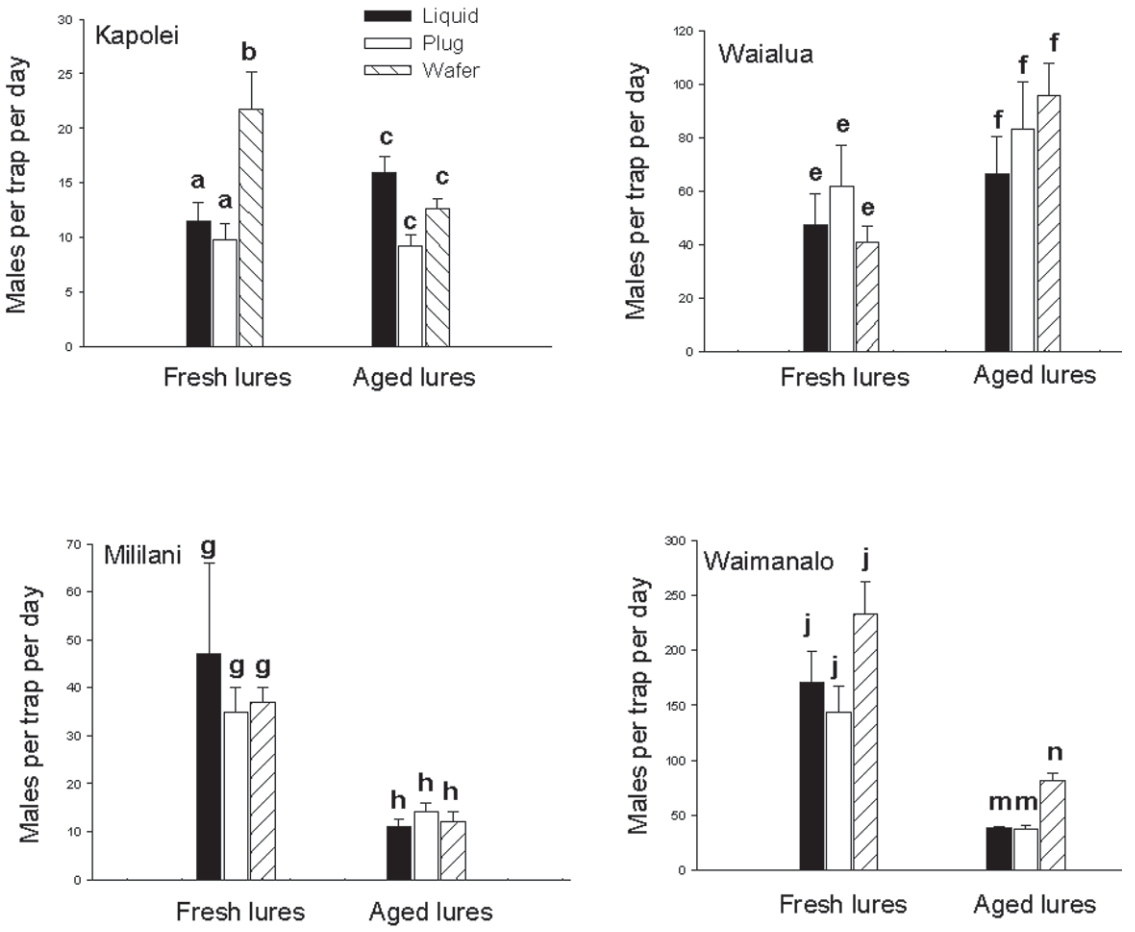


Fig. 1. Trap catch of wild *Bactrocera dorsalis* males at 4 sites on Oahu, Hawaii, in Jackson traps baited with ME applied to cotton wicks as a liquid or contained in solid plugs or wafers. Bar heights represent average capture of 12 traps (+ 1 SE). Separate 1-way ANOVAs were performed for the fresh and aged categories, respectively, and within these categories bars marked with different letters were significantly different ($P = 0.05$, Tukey test).

Regarding *B. dorsalis*, there was only 1 instance where traps baited with a solid formulation performed poorly relative to the standard liquid application (Table 4). In Florida, traps baited with aged wafers caught significantly fewer males than traps baited with aged liquid ME. This result appeared to arise from the lower loading of ME (3.1 g) in the wafers compared to the amount of liquid ME applied to wicks (6 mL or ~ 6 g). Subsequent tests in California and Hawaii used wafers with 6 g of ME (either alone or with RK), and in all of these cases traps baited with aged wafers captured equivalent or greater numbers of *B. dorsalis* males than the aged standard liquid ME-baited traps. As noted previously, the poor performance of the aged wafers in Florida was unexpected, since traps baited with this same type of wafer captured an equal or greater amount of *B. dorsalis* males as traps baited with 6 mL of liquid ME in 2 separate locations in Hawaii over 6 week sampling intervals (Shelly et al. 2011b). Likewise, in another study, slightly larger wafers (7.5 cm by 6.3 cm by 0.125 cm) were employed, but these contained only 3.54 g of ME (i.e., only 14% more ME than the wafers used in Florida) yet were as effective as 6 mL of liquid ME both when fresh and aged 6 weeks (Shelly et al. 2011a). Thus, the relatively low catch of *B. dorsalis* in traps baited with aged wafers in Florida was inconsistent with previous studies, the reason(s) for which remain unknown.

Another anomalous result involves the performance of fresh versus aged liquid ME. In both Florida and California, traps baited with aged liquid ME caught significantly more *B. dorsalis* males than traps with fresh liquid ME. This result was unexpected, particularly given the high volatility of methyl eugenol (Keiser et al. 1974). However, as this result was observed under different environmental conditions and for both

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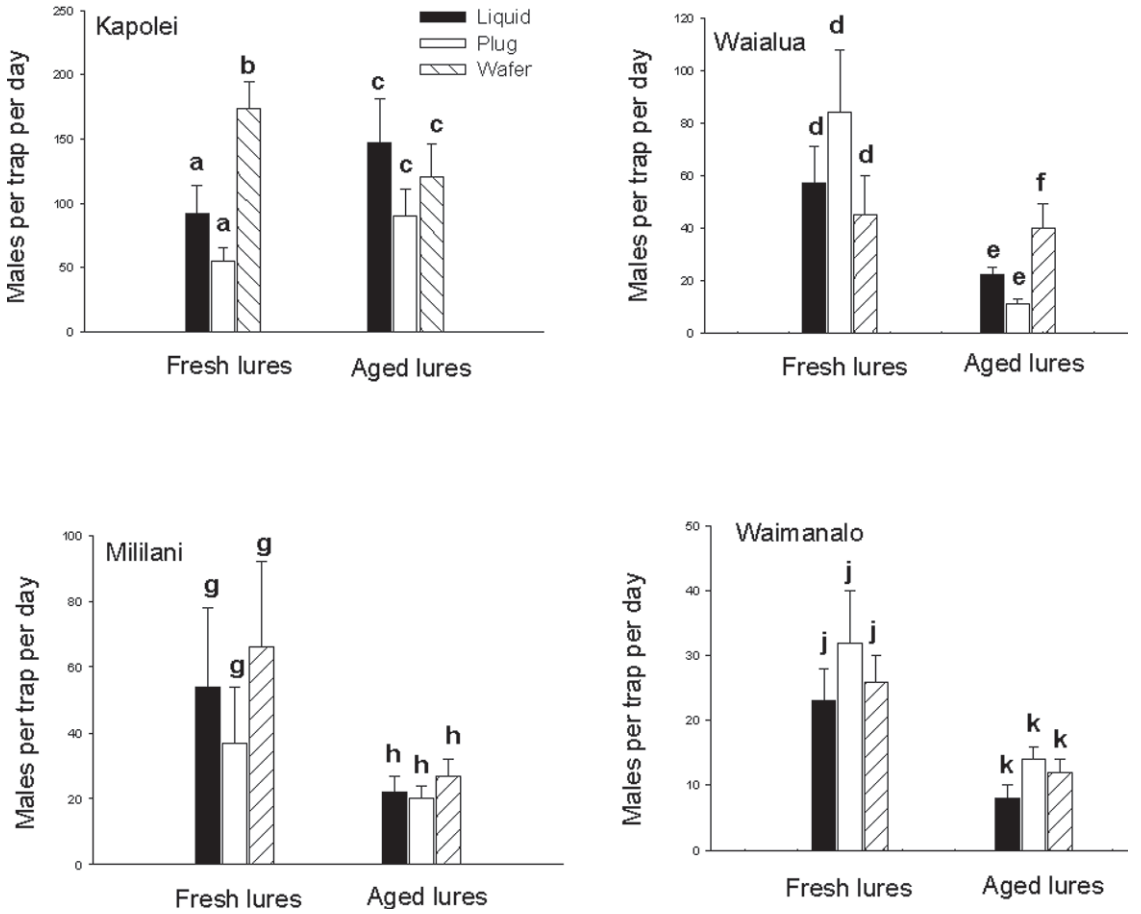


Fig. 2. Trap catch of wild *Bactrocera cucurbitae* males at 4 sites on Oahu, Hawaii, in Jackson traps baited with CL applied to cotton wicks as a liquid or contained in solid plugs or with RK contained in solid wafers. Bar heights represent average capture of 12 traps (+ 1 SE). Separate 1-way ANOVAs were performed for the fresh and aged categories, respectively, and within these categories bars marked with different letters were significantly different ($P = 0.05$, Tukey test).

wild-like (Florida) and mass-reared (California) flies, it does not appear to be an artifact of the experimental design or methodology.

Regarding *B. cucurbitae*, there were only 2 cases over all tests where solid formulations of CL, RKF, or RK resulted in lower trap catch than liquid CL (Table 5). In Florida, traps baited with aged plugs containing CL or RKF captured significantly fewer flies than traps baited with aged liquid CL. This result was unexpected, because these compounds have relatively low volatility (Keiser et al. 1974), and the plugs contained an equal amount of attractant (6 g of CL and RKF, respectively) as the liquid CL baits (6 mL or ~ 6 g) and a higher amount compared to the wafers (2.1 g of RK), which, in Florida, performed as well as the liquid CL both when fresh and aged. Moreover, in all subsequent tests in California and Hawaii, traps containing aged CL plugs captured as many or more *B. cucurbitae* males as traps baited with aged CL liquid. The reason for the poor performance of aged CL plugs in Florida is unknown. RKF plugs were tested only in Florida, and consequently their efficacy is less easily evaluated. Prior results with RKF plugs were inconsistent. In Hawaii, Jang et al. (2007) compared RKF plugs and liquid CL over a 32-week interval and found the plugs resulted in significantly higher trap catch of *B. cucurbitae* males than the liquid CL. In contrast, however, a more recent study (Shelly et al. 2012a) found that traps baited with RKF plugs generally captured fewer *B. cucurbitae* males than traps baited with liquid CL.

In addition to reducing handling time and health risks, solid dispensers that contain both ME and CL (or RK) would obviously reduce by half the number of *Bactrocera* detection traps placed in the environment and consequently the time required for their servicing as well as the financial cost of purchasing trapping supplies. In

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Table 4. Summary of trap catch data of *B. dorsalis* males from Florida, California, and Hawaii. Symbols represent outcome of statistical comparison with the standard liquid lures, where relative to the liquid lure ≈ indicates no significant difference in trap catch, > indicates significantly greater trap catch, and < indicates significantly lower trap catch.

Location	Lures presented	Fresh	Aged
Florida	Plug ME, CL	≈	≈
	Plug ME, RKF	≈	≈
	Wafer ME/RK	≈	<
California	Plug ME, CL	≈	≈
	Wafer ME, CL	>	≈
	Wafer ME/RK	>	≈
Hawaii-Kapolei	Plug ME, CL	≈	≈
	Wafer ME/RK	>	≈
Hawaii-Waialua	Plug ME, CL	≈	≈
	Wafer ME/RK	≈	≈
Hawaii-Mililani	Plug ME, CL	≈	≈
	Wafer ME/RK	≈	≈
Hawaii-Waimanalo	Plug ME, CL	≈	≈
	Wafer ME/RK	≈	>

this context, recent work (Shelly et al. 2012b; Vargas et al. 2012) has demonstrated that traps containing a wafer loaded with the 2 *Bactrocera* lures plus trimedlure (attractive to male *C. capitata*) capture similar numbers of *B. dorsalis*, *B. cucurbitae*, and *C. capitata* males as separate traps bearing the respective male lures. If these "trilure" dispensers prove satisfactory in additional testing, then only a third as many traps would be required in the field as presently used. The use of solid formulations would also reduce the chance of inadvertent lure contamination of objects in the environment that might attract flies away from traps, thus reducing their efficiency.

Table 5. Summary of trap catch data of *B. cucurbitae* males from Florida, California, and Hawaii. Symbols represent outcome of statistical comparison with the standard liquid lures, where relative to the liquid lure ≈ indicates no significant difference in trap catch, > indicates significantly greater trap catch, and < indicates significantly lower trap catch.

Location	Lures presented	Fresh	Aged
Florida	Plug ME, CL	≈	<
	Plug ME, RKF	≈	<
	Wafer ME/RK	>	≈
California			
	Plug ME, CL	≈	≈
	Wafer ME, CL	≈	>
	Wafer ME/RK	>	>
Hawaii-Kapolei	Plug ME, CL	≈	≈
	Wafer ME/RK	>	≈
Hawaii-Waialua	Plug ME, CL	≈	≈
	Wafer ME/RK	≈	>
Hawaii-Mililani	Plug ME, CL	≈	≈
	Wafer ME/RK	≈	≈
Hawaii-Waimanalo	Plug ME, CL	≈	≈
	Wafer ME/RK	≈	≈

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In conclusion, there is substantial benefit to be obtained by using solid dispensers loaded with male lures for trapping *Bactrocera* fruit flies. However, the plugs and wafers tested here will require registration prior to use in field detection programs. This process constitutes a major obstacle to implementation, because financial costs may be high, and considerable time may be required. Prior to registration, however, detection traps containing solid dispensers with male lures only, with a separate insecticidal strip positioned nearby, could be used. Ongoing work (Shelly, unpublished) in Hawaii has found that traps containing a wafer having only ME and CL plus a separate DDVP-strip capture similar numbers of *B. dorsalis* and *B. cucurbitae* males as traps baited with standard ME and CL liquids (with naled). Additional testing is required to identify the minimum dose of DDVP required to ensure captures comparable to those achieved using the standard mixture of liquid lures plus naled.

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