

Capture of Mediterranean Fruit Flies and Melon Flies (Diptera: Tephritidae) in Food-Baited Traps in Hawaii

Todd E. Shelly and Rick S. Kurashima

USDA-APHIS, 41-650 Ahiki Street, Waimanalo, HI 96795

Abstract. Food-based attractants are an important component of tephritid fruit fly detection programs, because they are general baits that are neither sex- nor species-specific. Two widely used food baits are enzymatic hydrolyzed torula yeast, which is presented as an aqueous solution that also serves to catch insects (wet trap), and a synthetic lure that combines ammonium acetate, putrescine, and trimethylamine and may be presented with or without a water-based catch system. Recently, the liquid attractant CeraTrap, which is an enzymatic hydrolyzed animal protein, has been shown to be equally or more effective than traditional protein baits in capturing species of *Anastrepha*. The present study compares capture of wild Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), and melon flies, *Zeugodacus cucurbitae* (Coquillett) in traps baited with torula yeast or CeraTrap. In addition, one sampling interval compared the catch of *C. capitata* in wet traps baited with torula yeast, a synthetic food lure, or CeraTrap. CeraTrap was generally more effective in capturing both sexes of *C. capitata* than the other food baits, while torula yeast resulted in higher captures of *Z. cucurbitae* than CeraTrap. Results are compared with other trapping studies of tephritids involving food-based attractants.

Introduction

Detection, monitoring, and control of pestiferous tephritid fruit flies often rely on the deployment of attract-and-kill devices baited with male-specific attractants, termed male lures (Vargas et al. 2010, 2014). Although male lures are both potent and long-lasting, two factors limit their effectiveness, i.e., they do not target females, and males of many tephritid species are not attracted to sex-specific lures (Drew and Hooper 1981). As a result, food-baited traps are also an important component of fruit fly management programs, because, even though they are less powerful than male lures, food baits are general attractants that are neither sex- nor species-specific (Epsky et al. 2014). In addition, in certain instances, food-baited traps may detect fruit fly populations earlier

in the season than male lure-baited traps (Papadopoulos et al. 2001).

Historically, many different food baits have been developed and tested in the field for trapping tephritid fruit flies (Epsky et al. 2014). At present, the most commonly used food baits are torula yeast and a synthetic blend of ammonium acetate and putrescine alone or in combination with trimethylamine. The former is presented in an aqueous solution, often containing propylene glycol to reduce evaporation and decomposition of trapped flies, that contains dissolved pellets of enzymatic hydrolyzed yeast and borax (added to reduce yeast and fly decomposition) (Burditt 1982, Cunningham 1989). Traps baited with torula yeast-borax slurry are wet traps, and the solution acts both as the attractant and catch mechanism as attracted

flies drown in the liquid (Thomas et al. 2001). However, in addition to attracting a large number of non-target insects, such traps are cumbersome to prepare and service, particularly in remote areas where water may have to be transported over considerable distances (Navarro-Llopis and Vacas 2014).

Largely to facilitate trap handling and monitoring, a dry synthetic blend was developed and intensively tested with wild populations of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), and various *Anastrepha* species, particularly the Mexican fruit fly, *A. ludens* (Loew) (e.g., Heath et al. 1995, 1997; Epsky et al. 1995, 1999, 2011; Katsoyannos et al. 1999; Martinez et al. 2007). Collectively, these studies revealed that (i) a 3-component lure of ammonium acetate, putrescine, and trimethylamine was most effective for *C. capitata*, while a 2-component lure of ammonium acetate and putrescine was more attractive for *Anastrepha* species, and (ii) traps containing dry synthetic food attractants captured equal or greater numbers of flies as traps baited with conventional liquid food lures, particularly in wetter environments where wet traps were not an important water source for the flies. The different food components may be presented in individual sachets or combined in a single device, and, although liquid may be used to capture insects, traps can be deployed with an insecticidal strip or a sticky surface, thus reducing handling time associated with trap servicing (Epsky et al. 2014).

Recently, a food bait derived from enzymatic hydrolyzed animal protein, and commercially known as CeraTrap, has been tested intensively with *Anastrepha* species and found to be highly effective. De los Santos-Ramos et al. (2012), Lasa et al. (2013, 2015), and Herrera et al. (2015) all compared capture of *A. ludens*

in traps of a single type baited with different food lures and reported that traps with CeraTrap captured as many or more flies than traps with standard hydrolyzed yeast and/or synthetic food baits. Based on the same experimental design, similar results have been obtained for *A. obliqua* Macquart (Lasa and Cruz 2014; Herrera et al. 2015), *A. serpentina* (Wiedemann) (Herrera et al. 2015), and *A. fraterculus* (Wiedemann) (Bortoli et al. 2016). In other studies involving *A. ludens* that compared trap-lure combinations (and not lures per se), traps baited with CeraTrap performed equally or better than other trap-food bait pairings (Lasa et al. 2014, 2015). Fewer data are available for *Ceratitis* spp., and the results thus far have been mixed. Hafsi et al. (2015) compared captures of *C. capitata* in two trap-lure combinations and found traps baited with CeraTrap had similar catch to traps baited with 3-component synthetic protein baits. As a potential attractant in mass-trapping programs, Llorens et al. (2008) reported that traps baited with CeraTrap resulted in reduced fruit damage relative to protein bait spray or Malathion application. Thus, as a tool in mass trapping, CeraTrap may lead to significant reductions in pesticide use. In contrast, in comparing multiple trap-lure combinations, Peñarrubia-María et al. (2014) reported that traps baited with dry synthetic food baits captured significantly more *C. capitata* and *C. rosa* (Karsch) than traps baited with containing CeraTrap. To our knowledge, only one study (Royer et al. 2014) has examined the attraction of a *Bactrocera* species (*B. cucumis* (French)) to CeraTrap.

Here, we describe captures of wild medflies and melon flies, *Zeugodacus cucurbitae* (Coquillett) (the new generic classification proposed by Virgilio et al. 2015 is here adopted), in a Hawaiian coffee field in traps having different food baits. As described below, trapping was

conducted during three different intervals over 2014–2016. In the initial two periods, catch was compared between traps baited with torula yeast/borax and traps baited with CeraTrap. As part of their tephritid fruit fly detection programs, both California (Gilbert et al. 2010) and Mexico (Lasa et al. 2015) utilize the torula yeast/borax mixture as a food bait. Consequently, we considered it useful to gather data on the field performance of this standard bait relative to CeraTrap, a potential replacement. In the final sampling period, trap catch was compared among traps baited with torula yeast/borax, CeraTrap, and the 3-component synthetic food lure as the ease of handling the latter lure makes it an attractive alternative to the standard torula yeast/borax mixture.

Materials and Methods

Study site. Field work was conducted in a coffee field (*Coffea arabica* L., \approx 65 ha, 100 m elevation) 10 km southeast of Haleiwa, Oahu. The field is surrounded primarily by untended land overrun with koa haole (*Leucaena leucocephala* (Lam.) de Wit) and California grass (*Brachiaria mutica* (Forsk.) Stapf) and by pineapple fields (*Ananas comosus* (L.) Merr.), i.e., areas devoid of suitable medfly host plants. Coffee is a host plant of *C. capitata* and *Bactrocera dorsalis* (Hendel), but the latter species was rare over the entire study period and was therefore not included in this report. While coffee is not a host plant of *Z. cucurbitae*, several of its host plants, primarily bitter melon (*Momordica charantia* L. and ivy gourd (*Coccinia grandis* (L.)), occurred as feral populations in gullies adjacent to the coffee field.

Trapping was conducted during three different 6-week periods: January–February, 2015, March–April, 2015, and January–March, 2016. Average daily maximum and minimum air temperatures were 26.1°C and 19.5°C, respectively,

for the two sampling periods starting in January (data pooled over 2015 and 2016) and 27.2°C and 22.8°C, respectively, for the March–April interval (readings from Kaneohe Marine Corps Base (<http://wunderground.com>), approximately 10 km from the study area).

Baits and trapping protocol. The two study periods in 2015 involved comparison between Multilure traps (FAO/IAEA 2013; Better World Manufacturing, Fresno, CA) baited with a solution containing torula yeast/borax pellets (Scentry Biologicals Inc., Billings, MT) or CeraTrap (CT hereafter, Bioibérica, Barcelona, Spain). The torula yeast bait (TY hereafter) was prepared by placing one torula yeast/borax pellet (45% torula yeast, 55% borax by weight) per 100 ml of a water/antifreeze solution (95% and 5% by volume, respectively, using SPLASH RV & Marine Antifreeze [14% propylene glycol]; SPLASH Products Inc. St. Paul, MN). The antifreeze was added to reduce evaporation and decay of captured insects (FAO/IAEA 2013). Leblanc et al. (2010) found that the addition of propylene glycol to TY bait reduced captures of medfly and *Bactrocera* species. However, these authors used an unusually high concentration (20%) of antifreeze, and an auxiliary field test (Shelly, unpublished data) showed no difference in captures of medfly or melon fly between TY traps containing or lacking 5% antifreeze. CT is sold ready for use and hence was used directly from the bottle. Sampling in 2016 included the TY and CT baits described above plus 3-component food cones that contained putrescine, ammonium acetate, and trimethylamine (hereafter CN; Scentry Biologicals Inc). Jang et al. (2007) found that these food cones were equally or more attractive to medflies than torula yeast/borax solution. The cones were placed in a well in the upper half of the Multilure trap, and the same water/

antifreeze solution described for TY traps was placed in the lower part of the trap. All traps contained 300 ml of liquid.

In all sampling periods, traps were placed in Norfolk pine trees (*Araucaria heterophylla* (Salisb.) Franco) planted as windbreaks along the perimeter of a rectangular coffee field. Traps containing different baits were alternated around the field, and adjacent traps were separated by a minimum of 30 m. For all sampling intervals, 15 traps per bait type were deployed, i.e., 30 total traps were used in the three intervals comparing TY and CT, and 45 total traps were used in the final interval comparing TY, CT, and CN baits. Traps were placed 2.0–2.5 m above ground in shaded locations. Traps were serviced weekly. In the field, the trap's liquid was poured through a sieve to retain captured insects, which were returned to the laboratory for counting. TY bait was replaced weekly, whereas CT bait was recycled and replenished as needed. CN bait was not replaced and remained in the field for the six-week duration of the sampling period, and the water/antifreeze solution was recycled and replenished as required.

Data analysis. Sufficiently large numbers of medflies were captured to permit statistical analyses for all sampling intervals, whereas analysis for the melon fly was restricted to two intervals both of which used torula yeast/borax and CeraTrap baits only. For both species, data were analyzed separately for individual sampling intervals using 3-way ANOVA, with sex, bait, and week as main effects followed by the Holm-Šidák method (test statistic t) for all pairwise multiple comparisons. Raw data, computed as flies per trap per day (FTD), were transformed as $\log_{10}(x + 1)$ to increase normality. The transformed data did not meet the parametric assumptions of normality and equal variances in all cases. To assess the robustness of our analyses, we also

performed a non-parametric equivalent of ANOVA using ranked data (following Conover and Iman 1981). This procedure generated results identical to those obtained using the raw data, indicating that the parametric analyses of raw data were sufficiently robust to accommodate the levels of non-normality and heteroscedasticity present in the data set.

Results

Mediterranean fruit fly. January–February 2015. Each of the main factors had a significant effect on medfly captures (Table 1, Fig. 1). With one exception (week \times sex, $F = 3.28$, $P = 0.007$), none of the interaction terms were significant ($P > 0.05$ in all cases). Both sexes were captured in significantly higher numbers in CT than TY traps (females: $t = 2.9$, $P = 0.004$; males: $t = 4.2$, $P < 0.001$). Over the 6-week sampling period, average weekly FTDs for females ranged from 8.6–29.6 for CT traps (overall average = 14.8) compared to 3.8–16.3 (overall average = 9.4) for TY traps. For males, the corresponding ranges were 3.1–28.1 for CT traps (overall average = 11.5) and 1.9–16.5 for TY traps (overall average = 6.2). As the FTD values suggest, females were captured in significantly greater numbers than males in both TY ($t = 4.5$, $P < 0.001$) and CT ($t = 3.2$, $P = 0.002$) traps. The significant week \times sex term reflected the large intersexual difference in the proportional increase in total captures (over both TY and CT traps) over the sampling period, i.e., the total number of males captured increased roughly 7-fold from the initial two weeks to the final two weeks compared to only a two-fold increase for females from the initial to the final samples.

March–April 2015. Each of the main factors had a significant effect on medfly captures (Table 1, Fig. 2), and two of the interactions were significant (week \times sex, $F = 3.36$, $P = 0.01$; week \times bait, $F = 3.80$,

Table 1. Results of 3-way ANOVA (week, bait, and sex as main factors; significant interaction terms are noted in the text) for captures of the Mediterranean fruit fly, *C. capitata*, in Multilure traps baited with torula yeast/borax pellets or CeraTrap during the 2015 sampling periods and with torula yeast/borax pellets, CeraTrap, or 3-component food cones in 2016. Trapping was conducted for 6 weeks in all sampling periods. In all cases, 15 traps were run per bait type.

Sampling period	Source of variation	DF	F	P
January–February 2015	Week	5	21.93	< 0.001
	Bait	1	25.15	< 0.001
	Sex	1	29.25	< 0.001
	Error	336		
March–April 2015	Week	5	7.23	< 0.001
	Bait	1	65.41	< 0.001
	Sex	1	27.68	< 0.001
	Error	336		
January–March 2016	Week	5	82.66	< 0.001
	Bait	2	7.88	< 0.001
	Sex	1	102.01	< 0.001
	Error	504		

$P = 0.002$). Both sexes were captured in significantly higher numbers in CT than TY traps (females: $t = 4.8$, $P < 0.001$; males: $t = 6.6$, $P < 0.001$). Over the 6-week sampling period, average weekly FTDs for females ranged from 3.4–8.4 for CT traps (overall average = 5.5) compared to 0.9–6.7 (overall average = 2.9) for TY traps. For males, the corresponding ranges were 3.6–6.1 for CT traps (overall average = 4.4) and 0.7–2.2 for TY traps (overall average = 1.3). Females were captured in significantly greater numbers than males in both TY ($t = 4.6$, $P < 0.001$) and CT ($t = 2.8$, $P = 0.006$) traps. The significant week x sex interaction reflected the opposite trends in trap catch of the sexes over time: over both TY and CT traps, female captures declined, while male captures were relatively constant over the 6-week sampling period. The significant week x bait term appeared to derive from the

temporal difference in relative catch in TY vs. CT traps observed for both sexes, i.e., the CT traps captured a greater proportion of flies in the final weeks of the sampling period.

January–March 2016. Each of the main factors had a significant effect on medfly captures (Table 1, Fig. 3), and two of the interactions were significant (week x sex, $F = 2.89$, $P = 0.01$; week x bait, $F = 3.23$, $P < 0.001$). Both sexes were captured in significantly higher numbers in CT than TY traps (females: $t = 2.4$, $P = 0.02$; males: $t = 3.1$, $P = 0.002$). For females, CT traps also had significantly higher captures than CN traps ($t = 2.2$, $P = 0.03$), while catch did not differ significantly between TY and CN traps ($t = 0.23$, $P = 0.82$). For males, captures did not differ significantly between CT and CN traps ($t = 1.5$, $P = 0.13$) or between TY and CN traps ($t = 1.5$, $P = 0.13$). Although captures of both

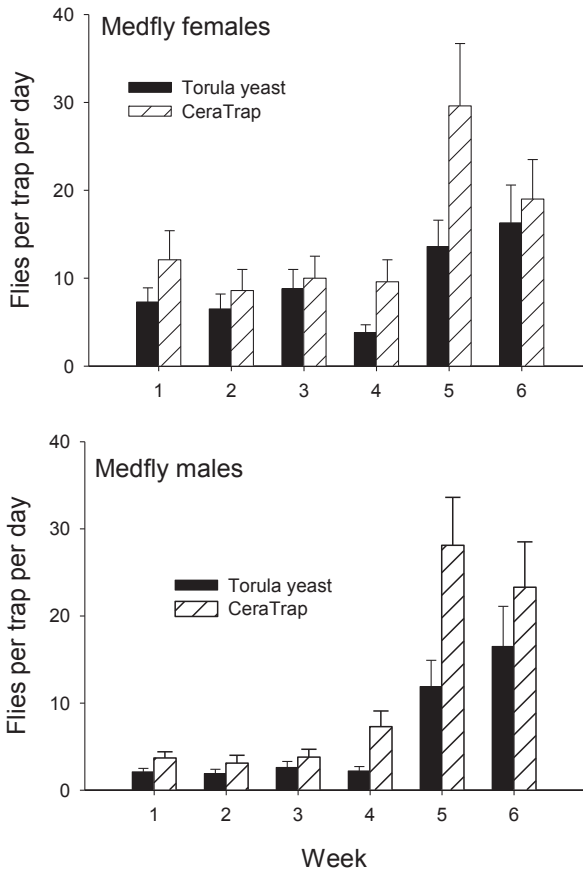


Figure 1. Numbers of female and male *C. capitata* captured in Multilure traps baited with torula yeast/borax pellets (TY) or Ceratrap (CT) over the 6-week sampling period in January–February 2015. Bar heights represent averages of 15 traps per bait type; whiskers represent ± 1 SE.

sexes declined greatly over the sampling interval, FTD values for females generally exceeded those of males for each bait for any given week (Fig. 4), and statistical analyses confirmed significantly higher numbers of female than male captures for TY ($t = 6.5$, $P < 0.001$), CT ($t = 5.8$, $P < 0.001$), and CN ($t = 5.2$, $P < 0.001$) traps. The significant week \times sex interaction appeared to reflect the slightly greater decline in male captures over time, and the significant week \times bait interaction may

have reflected the high captures of both females and males in (i) CT traps in week 1 and (ii) CN traps in week 3 relative to other weeks.

Melon fly. January–February 2015.

Each of the main factors had a significant effect on melon fly captures (Table 2; Fig. 4), and with one exception (week \times sex, $F = 22.6$, $P < 0.001$) none of the interaction terms were significant ($P > 0.05$ in all cases). Significantly more females were captured in TY traps than CT traps ($t =$

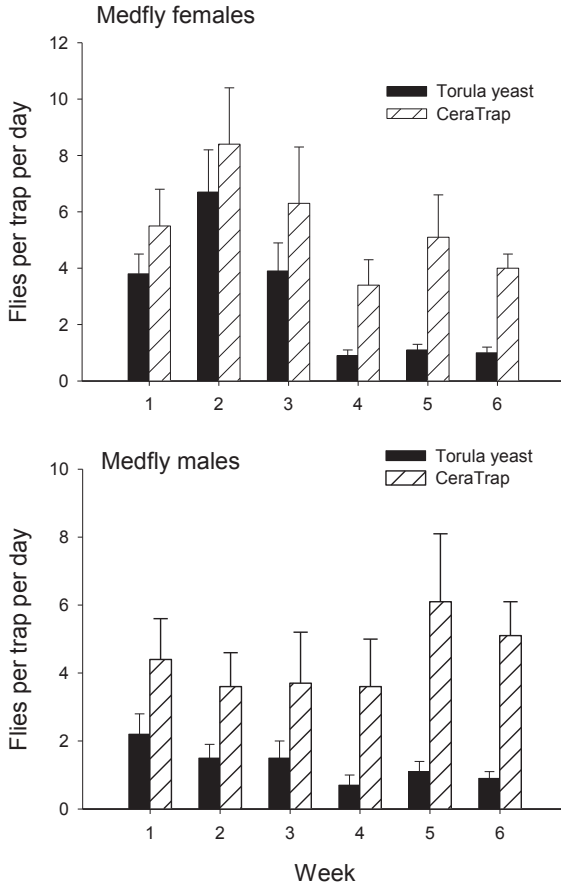


Figure 2. Numbers of female and male *C. capitata* captured in Multilure traps baited with torula yeast/borax pellets (TY) or Ceratrap (CT) over the 6-week sampling period in March–April 2015. Bar heights represent averages of 15 traps per bait type; whiskers represent ± 1 SE.

3.3, $P < 0.001$). Over the 6-week sampling period, average weekly FTDs for females ranged from 2.8–32.7 for TY traps (overall average = 10.5) compared to 1.2–16.4 (overall average = 4.7) for CT traps. Male captures did not differ significantly between trap types ($t = 1.4$, $P = 0.16$). Over the 6-week sampling period, average weekly FTDs for males ranged from 0.4 to 3.2 for TY traps (overall average = 2.0) and from 0.5 to 2.3 (overall average = 1.5)

for CT traps. As the FTD values suggest, females were captured in greater numbers than males in both TY ($t = 7.6$, $P < 0.001$) and CT ($t = 5.7$, $P < 0.001$) traps. The significant interaction term (week \times sex) reflected the fact that the sexes showed opposite temporal trends in catch, with female numbers decreasing, and male numbers increasing, through time.

March–April 2015. Bait and sex had significant effects on melon fly captures,

Table 2. Results of 3-way ANOVA (week, bait, and sex as main factors; significant interaction terms are noted in the text) for captures of the melon fly, *Z. cucurbitae*, in Multilure traps baited with torula yeast/borax pellets or CeraTrap for two sampling periods in 2015. Fifteen traps were run per bait type for 6 weeks during each sampling period.

Sampling period	Source of variation	DF	F	P
January–February 2015	Week	5	4.25	< 0.001
	Bait	1	11.26	< 0.001
	Sex	1	88.43	< 0.001
	Error	336		
March–April 2015	Week	5	0.71	0.614
	Bait	1	5.81	0.016
	Sex	1	7.74	0.006
	Error	336		

but week did not (Table 2; Fig. 5), and none of the interaction terms were significant ($P > 0.05$ in all cases). Both females ($t = 2.2$, $P = 0.03$) and males ($t = 2.7$, $P = 0.01$) were captured in significantly higher numbers in TY than CT traps. Over the 6-week sampling period, average weekly FTDs for females ranged from 1.4 to 5.2 for TY traps (overall average = 3.5) compared to 0.4–2.7 (overall average = 1.7) for CT traps. For males, average weekly FTDs ranged from 1.1 to 2.2 for TY traps (overall average = 1.7) and from 0.5 to 1.0 (overall average = 0.8) for CT traps. Females were captured in significantly greater numbers than males in both TY ($t = 2.2$, $P = 0.03$) and CT ($t = 3.1$, $P = 0.002$) traps.

Discussion

For the Mediterranean fruit fly, comparisons between TY and CT traps generated consistent results across two sampling intervals. Both sexes were captured in significantly higher numbers in CT traps than TY traps. In addition, and as typically found for TY or other liquid protein traps (Katsoyannos et al. 1999, Epsky et al. 1999, Alemany et al. 2004; present study),

CT traps in the present study consistently captured significantly more females than males of *C. capitata*. Over the two intervals in which TY and CT traps were compared, females comprised 56%–64% of the total catch. Hafsi et al. (2015) reported a much more pronounced female bias for CT traps deployed in Tunisian citrus orchards, as females comprised over 90% of all *C. capitata* captured in CT traps in each of three study sites. Reasons for this large difference are unknown but presumably reflect differences in availability of alternate protein sources between the Hawaiian and Tunisian study sites.

Deployment of TY, CT, and CN traps in the final sampling interval yielded slightly different results for females and males of *C. capitata*. For females, the CT traps caught significantly more individuals than either TY or CN traps, which, in turn, did not differ significantly from one another. For males, however, catch in CT traps was significantly greater than that in TY traps but not different from catch in CN traps. As with females, male captures were not statistically different between CN and TY traps. These results contrast with the find-

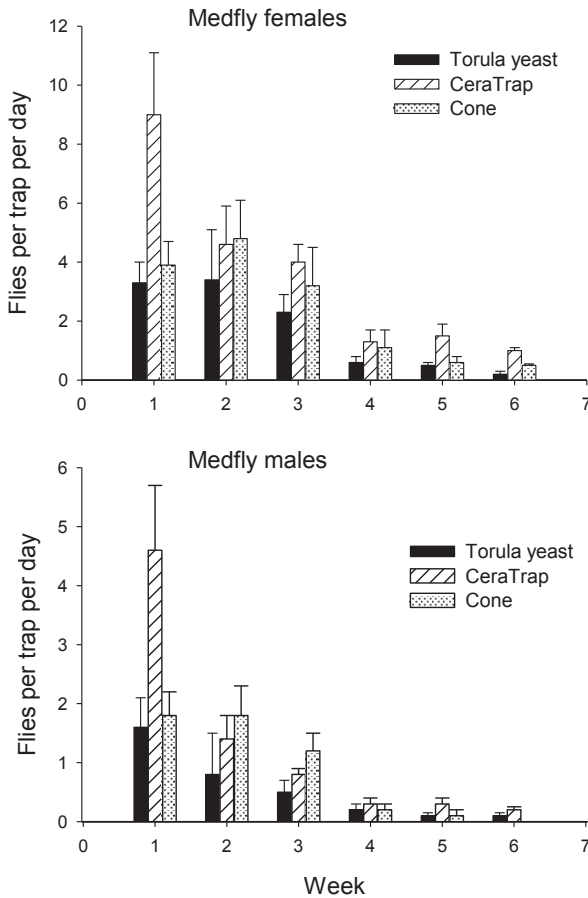


Figure 3. Numbers of female and male *C. capitata* captured in Multilure traps baited with torula yeast/borax pellets (TY), Ceratrap (CT), or 3-component synthetic food cones (CN) over the 6-week sampling period in January–March 2016. Bar heights represent averages of 15 traps per bait type; whiskers represent ± 1 SE.

ings of Hafsi et al. (2015), who reported that similar numbers of medflies were caught in traps baited with dry synthetic food lures or with CT, as well as those of Peñarrubia-María et al. (2014), who found that dry synthetic food baits were more effective than CT in trapping *C. capitata* and *C. rosa* (Karsch). In addition to being more effective than the standard TY lure in the present study, CT had the advantage of greater field longevity. Whereas the at-

tractiveness of the TY slurry may decline markedly after as few as 4–6 d in the field (Epsky et al. 1993), CT was apparently attractive to *C. capitata* for at least six weeks. Replenishment of evaporated CT was necessary, but the amounts required were minimal.

In contrast to the medfly, females of *Z. cucurbitae* were captured in significantly higher numbers in TY than CT traps. For males, the same trend held for one

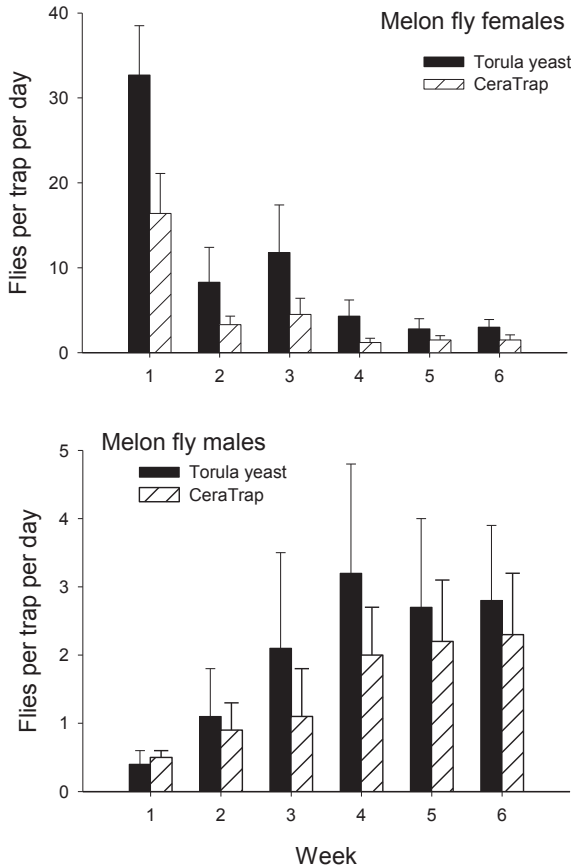


Figure 4. Numbers of female and male *C. cucurbitae* captured in Multilure traps baited with torula yeast/borax pellets (TY) or Ceratrap (CT) over the 6-week sampling period in January–February 2015. Bar heights represent averages of 15 traps per bait type; whiskers represent ± 1 SE.

sampling period (March–April 2015), but no difference in captures was detected between TY and CT traps in another period (January–February 2015). In an earlier study conducted in Hawaii, Leblanc et al. (2010) found that significantly greater numbers of *Z. cucurbitae* and *B. dorsalis* were caught in TY traps than in traps containing individual sachets of the synthetic dry baits. In evaluating a female attractant based on host odor, Siderhurst and Jang (2010) reported that traps containing plugs

emanating cucumber volatiles captured significantly more *Z. cucurbitae* females than traps baited with TY slurry. Similarly, Royer et al. (2014) found that these plugs captured more *B. cucumis* than either orange ammonia or CT. Contrary to these findings, however, Shelly et al. (2016) observed that the standard TY slurry outperformed the cucumber volatile plugs in trapping melon flies. Use of different trap-lure combinations in different habitats confounds interpretation of these

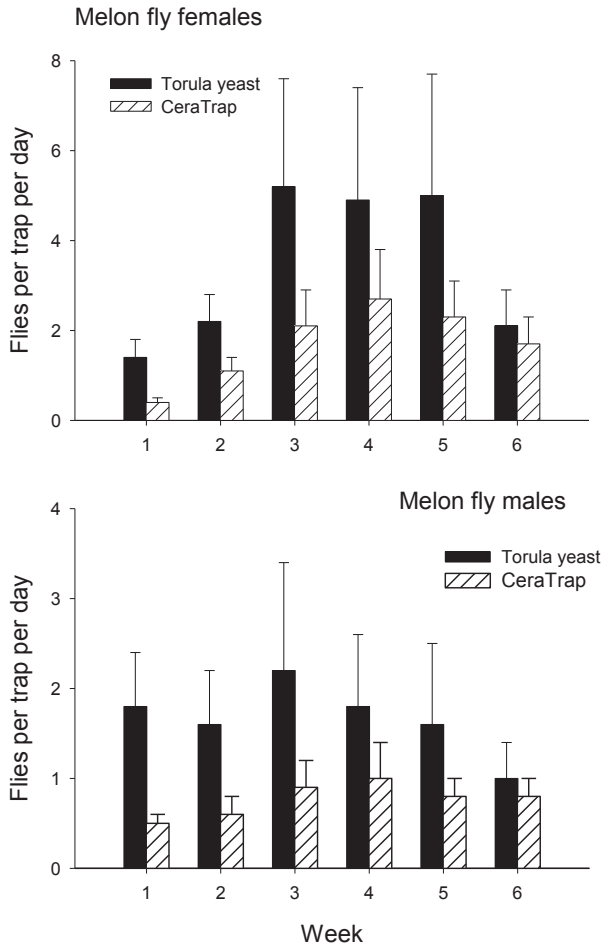


Figure 5. Numbers of female and male *C. cucurbitae* captured in Multilure traps baited with torula yeast/borax pellets (TY) or CeraTrap (CT) over the 6-week sampling period in March–April 2015. Bar heights represent averages of 15 traps per bait type; whiskers represent ± 1 SE.

results, and more work is clearly needed on the relative attractiveness of different food- and host-based lures to *Dacina* fruit flies.

The present study highlights two of the major challenges to implementing an effective fruit fly trapping program for detecting pestiferous tephritids. First, as shown for *Anastrepha* species (Diaz-

Fleischer et al. 2009), the notion that one trap-lure combination works equally well for all tephritid species, or even for all individuals within a species, is unrealistic. In the present study, CT traps were more effective than TY traps for the Mediterranean fruit fly, while the reverse was true for the melon fly. Thus, choice of any one food-based attractant likely represents a

compromise, which has variable effectiveness for different tephritid pests. Second, selecting the most economically effective trap-lure option is a complex problem that necessarily includes consideration of the costs associated with trapping materials, trap servicing, and environmental and safety issues. Such analysis is beyond the aims of this study, but inclusion of different food-based attractants hints at the problem. For example, the TY slurry is probably the least expensive bait, yet captures of medfly were significantly lower than observed for CT. Moreover, the TY slurry must be replaced weekly, whereas CT is longer-lasting. Similarly, although not so deployed in this study, synthetic lures can be used in dry traps, which can be serviced far more quickly than wet TY or CT traps. Independent of their capture effectiveness, use of dry synthetic food baits might dramatically reduce labor costs and result in considerable cost saving. Thus, just as Díaz-Fleischer et al. (2009) rightfully questioned the idea of a “magic” trap that effectively captures all fruit flies, it seems evident as well that there exists no “magic” trapping system that optimizes capture efficiency while simultaneously minimizing monetary and environmental costs.

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