Weathering of Torula Yeast Borax Food Bait and Capture of Oriental, Mediterranean, and Melon Fruit Flies in Hawaii (Diptera: Tephritidae)

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Abstract. Detection of pestiferous tephritid fruit flies relies largely on traps baited with male-specific attractants, termed male lures. Although male lures are quite powerful, two factors limit their effectiveness: they do not target females, and males of many tephritid species are not attracted to these compounds. Consequently, food-baited traps are an important component of fruit fly monitoring programs, because, despite their relatively low attractancy, food baits are general attractants that are neither sex- nor species-specific. Enzymatic torula yeast in an aqueous solution is a standard food attractant used in tephritid trapping programs worldwide. Torula yeast bait is not particularly long-lasting, and replacement is recommended every 7–14 d. Few data exist regarding the attractiveness of this food bait over time, and the present study was undertaken to compare captures of wild *Bactrocera dorsalis* (Hendel), *Ceratitis capitata* (Wiedemann), and *Zeugodacus cucurbitae* (Coquillett) in Multilure traps baited with torula yeast slurry weathered for 3, 6, 9, or 12 d. No significant variation in trap catch was detected among these weathering intervals for any of the three species. In a second experiment, trap captures were compared between food bait weathered 3 vs. 21 d, and in this case significantly more *C. capitata* and *Z. cucurbitae* were captured in the 3-d-old bait, whereas catch of *B. dorsalis* was similar for traps containing food bait weathered for 3 or 21 d. Results are compared with those of previous studies.

Key words: Tephritidae, trapping, detection, food bait, torula yeast

Detection, monitoring, and control of pestiferous fruit flies often rely on the deployment of attract-and-kill devices baited with male-specific attractants, termed male lures (Vargas et al. 2010, Tan et al. 2014). Although male lures are both powerful and long-lasting, two factors limit their effectiveness: they do not target females, and males of many tephritid species are not attracted to these compounds (Drew and Hooper 1981, Metcalf and Metcalf 1992). Consequently, food-baited traps are an important component of fruit fly monitoring programs, because, despite their relatively low attractancy, food baits are general attractants that are neither sex- nor species-specific (Epsky et al. 2014). In addition, in certain instances, food-baited traps have been shown to detect fruit fly populations earlier in the flight season than male lure-baited traps (Papadopoulos et al. 2001).

A commonly used food bait, and one described as the standard food attractant in tephritid trapping programs worldwide (Heath et al. 1995), is torula yeast. Torula yeast is presented in an aqueous solution, often containing propylene glycol to reduce evaporation and decomposition of trapped insects (Thomas 2008), that contains dissolved pellets of enzymatic hydrolyzed yeast and borax added to reduce yeast and insect decomposition (López-D. et al. 1971). The torula yeast–borax slurry (TYB hereafter) acts both as the attractant and catch mechanism as attracted flies drown in the liquid (Thomas et al. 2001). Although synthetic food baits have replaced TYB in some locations, TYB-baited traps are still widely used (FAO/IAEA 2018). California, for example, currently deploys five TYB-baited traps per mi^2 ($2.59\ km^2$) as a component of a fruit fly detection network that covers approximately 25,000 mi^2 ($64{,}750\ km^2$) in the southern part of the state (Vargas et al. 2013).

The attractiveness of TYB-baited traps to tephritid fruit flies is thought to be relatively short-lasting. In a mark-release-recapture study on the Caribbean fruit fly, *Anastrepha suspensa* (Loew), Calkins et al. (1984) replaced TYB in traps on a weekly basis following the recommendation of an unpublished report produced by a state agency in Florida. Recent international guidelines (FAO/IAEA 2018) recommend a 2-week interval for re-baiting TYB traps in detection and monitoring programs. Similarly, for the state of California, Gilbert et al. (2013) recommended that TYB slurry should be replaced at every servicing, which is weekly in warmer months and biweekly in winter months. A short replacement interval clearly reduces the likelihood that the liquid bait evaporates before the next servicing event, but little data (see Discussion) exist regarding possible changes in the attractiveness of TYB slurry during a replacement cycle. Moreover, available data are inconsistent. On one hand, Malo (1992) found no significant difference in captures of *Anastrepha* spp. among traps baited with TYB weathered 2, 4, 6, 8, or 10 d. In contrast, the catch of *A. suspensa* showed consistent decline among traps baited with TYB weathered 2, 4, or 7 d (Epsky et al. 1993).

The present study presents the results of two field experiments designed to monitor captures of three economically important fruit fly species to TYB-baited traps weathered for varying intervals. The study species included *Bactrocera dorsalis* (Hendel), the oriental fruit fly, *Ceratitis capitata* (Wiedemann), the Mediterranean fruit fly (or medfly), and *Zeugodacus cucurbitae* (Coquillett), the melon fly. In Experiment 1, we compared captures among traps baited with TYB weathered for 3, 6, 9, or 12 d. In Experiment 2, we compared captures among traps baited with TYB weathered for 3 vs. 21 d.

Materials and Methods

Study sites. For both Experiments 1 and 2, trapping of *B. dorsalis* was conducted on Hawaii island (Big Island) at the edge of second-growth forest (170 m elevation) approximately 10 km south of Hilo, HI (19° 37′ 41.82″ N, 155° 04′ 18.29″ W). Strawberry guava (*Psidium cattleianum* L.), a preferred host of *B. dorsalis* (Vargas et al. 1990), was abundant in the forest. Experiment 1 was conducted in June–July, 2020, during which daily minimum and maximum air temperatures averaged 18.9°C and 26.4°C, respectively, with a rainfall total of 27 cm. Experiment 2 was conducted in February - March, 2021, during which daily minimum and maximum air temperatures averaged 16.5°C and 23.7°C, respectively (National Oceanographic and Atmospheric Administration Station, Keaau, HI; approximately 6 km from field site), with a rainfall total of 122 cm.

For both Experiments 1 and 2, trapping of *C. capitata* was performed in a commercial coffee field (*Coffea arabica* L., 65 ha, 100 m elevation) in central Oahu (21° 52´ 7.34´´ N, 158° 03´ 78.60´´ W) approximately 10 km southeast of Haleiwa, HI. Plant rows were spaced 3 m apart, and individual plants were maintained at a height of 2–3 m. Experiment 1 was carried out in September–October 2020, during which daily minimum and maximum air temperatures averaged 23.6°C and 30.3°C, respectively, with a rainfall total of 17 cm. Experiment 2 was conducted in December 2021–January 2022, during which daily minimum and maximum air temperatures averaged 20.8°C and 26.2°C, respectively (Wheeler Army Airfield Station, Wahiawa, HI; approximately 10 km from field site), with a rainfall total of 31 cm.

For Experiment 1, trapping of *Z. cucurbitae* was conducted in south central Oahu (21° 22´ 25.82´´N, 158° 02´ 44.40´´ W) in a small stand of citrus trees surrounded by wild bitter melon, *Momordica charantia* L. (MC), and cultivated cucurbit hosts (e.g., zucchini, *Cucurbita pepo* L.; melons, *Cucumis* spp.). Citrus trees were arranged in three long rows (≈ 100 m) separated by 10 m, and within each row trees were spaced about 5 m apart. Experiment 1 was carried out in March–May 2021, during which daily minimum and maximum air temperatures averaged 21.8°C and 27.9°C, respectively (National Weather Service Station, Kalaeloa Airport; approximately 8 km from field site), with a rainfall total of 9 cm. For Experiment 2, *Z. cucurbitae* were captured in the same traps and same time intervals used for *C. capitata* for Experiment 2 (i.e., coffee field during December 2021–January 2022).

Preparation and weathering of food baits. Following standard protocol, TYB slurry was prepared by dissolving one TYB pellet (5 g; Scentry Biologicals, Inc., Billings, MT) per 100 mL of a water/antifreeze solution (90:10 v:v; SPLASH RV and Marine Antifreeze [14% propylene glycol], SPLASH Products Inc., St. Paul, MN). The TYB solution was prepared and thoroughly stirred in large buckets and then placed in Multilure traps (300 mL per trap) for weathering. Multilure traps are two-piece, plastic McPhail-like traps (FAO/IAEA 2018). The top portion is clear plastic, while the bottom is bright yellow and holds the liquid food bait. The bottom of the trap has a central, open invagination through which insects enter; the liquid reservoir acts as the killing mechanism. A wire hanger at the top of the trap is used to suspend the trap from tree branches.

Capture effectiveness was compared among four different weathering intervals of the TYB slurry (namely, 3, 6, 9, or 12 d) in Experiment 1 and 2 different weathering intervals (3 or 21 d) in Experiment 2. The different ageing durations are hereafter referred to as treatments. A new batch of TYB solution was prepared at the start of the weathering interval for each treatment. These intervals were scheduled to end on the same day, which also marked the day of trap deployment in the field. For example, in Experiment 1, the 12-d treatment was started first followed by the 9-d treatment started 3 d later and so on. As noted below, 10 Multilure traps were deployed in the field per treatment, but 12–15 traps were set up per treatment to ensure that, after evaporative loss, a sufficient amount of TYB bait would remain for the field sampling. The traps were weathered outdoors on covered outdoor porches at temperatures similar to those listed above and were either suspended above ground or placed on slatted tables, such that the traps were open to the air. When weathering was complete, the TYB solution was poured from the traps into separate buckets for the different treatments, and the buckets were transported to the field. It should be noted that, during the weathering procedure, very few insects were attracted to the traps, consequently any potential impact of decomposing insects on field-collected samples was assumed to be negligible.

Trap deployment: Experiment 1. In the field, 300 mL of TYB slurry were placed in 10 traps per treatment, with a total of 40 traps deployed (10 traps for each of the four treatments). Newly aged batches of food bait were used for each replicate, and traps operated for 2 d for all replicates at all sites. Upon trap collection, the liquid was poured through a sieve to retain the catch, and samples were transported to the laboratory to identify and count the flies.

At the Big Island site (oriental fruit fly), the traps were placed 30 m apart along the edge of the forest in shaded locations 1.5–2 m above ground. The different treatments were alternated in repeating sequences (e.g., 3-6-9-12-3-6-9-12 and so on), with the starting treatment in the sequence rotated among replicates (e.g., 3-6-9-12 then 6-9-12-3 in the subsequent replicate and so on) to minimize possible position effects. Eight replicates were conducted at weekly intervals.

In the Oahu coffee field (medfly), traps were arranged in 4 x 10 grids, with four traps (one per treatment) placed in 10 different rows of coffee. Traps were placed in shaded locations 1–1.5 m above ground and were separated by 15–20 m both within and between rows. The sequence of treatments was rotated among rows (e.g., 3-6-9-12 then 6-9-12-3 and so on) to reduce potential position effects. Five replicates were conducted at weekly intervals.

In the Oahu citrus stand (melon fly), 20 traps (four traps per treatment; five repeating sequences per row) were placed in each of two adjacent rows (traps were 10 m apart within a row). Within each row, traps were placed in every other tree (i.e., traps were separated by 10 m). As above, starting treatments within a row were alternated among replicates to minimize possible position effects. Four replicates were conducted at weekly intervals.

Trap deployment: Experiment 2. For *B. dorsalis,* 300 mL of TYB slurry were placed in 12 traps for each of two treatments (food bait aged 3 or 21 d, 24 total traps deployed). For both *C. capitata* and *Z. cucurbitae*, which were captured at the same site and time (as noted above), 300 mL of TYB slurry were placed in each of the two treatments (20 traps total). As above, newly aged batches of food bait were used for each replicate, and traps operated for 3 d for all six replicates involving *B. dorsalis* and 2–3 d for all five replicates involving *C. capitata* and *Z. cucurbitae*. Trap placement followed the general procedure given above for the different sites and was designed to minimize both potential interference between neighboring traps and position effects on trap catch.

Statistical analysis. For Experiment 1 and for all three species, a Kruskal-Wallis test was used to compare, separately for each replicate, the percentage of females occurring among traps of the four treatments. Results showed no significant variation among treatments in the proportion of females captured for any replicate for any of the three species. Analyses were therefore based on total captures per trap (i.e., sexes combined). These total counts were analyzed using generalized linear models (GLM) with food bait age and week (replicate) as independent variables. Data were non-normal for all three species, and a Poisson distribution was chosen with the log link. In all tests, the significance of the independent variables was tested using a likelihood ratio chi-square, with df = 3 for food bait age and df = no. weeks –1 for week.

Data for Experiment 2 were analyzed in the same manner. Sex ratios did not vary between 3- and 21-d treatments for

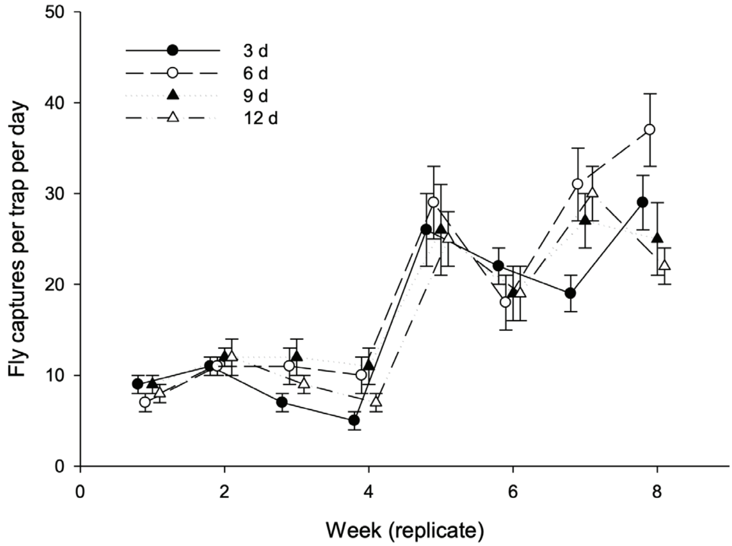

Figure 1. Captures of oriental fruit flies (males and females combined) in Multilure traps baited with torula yeast borax weathered 3, 6, 9, or 12 d. Traps operated for 2 d at weekly intervals over 8 consecutive weeks. Symbols represent means ± 1 SE (N = 10 traps/treatment/sampling period).

any replicate for any species, and numbers were combined over the sexes for analysis. Data for *C. capitata* and *Z. cucurbitae* were normal, consequently the identity link function was used in GLM. Data for *B. dorsalis* were not normally distributed, and a Poisson distribution was chosen with the log link. Significance testing was conducted as above, with df = 1 for food bait age and df = no. weeks –1 for week. Analyses were performed with JMP 14 software (SAS Institute, Carey, North Carolina, USA).

Results

Experiment 1

Sex ratios. As noted above, preliminary analyses revealed that sex ratios did not vary among treatments for any replicate for any of the three species. Trap catch was female-biased for *B. dorsalis* and *C. capitata* but slightly male-biased for *Z. cucurbitae*. Based on data pooled over all traps and replicates, the average proportions of trapped *B. dorsalis* that were females ranged between 92.1% (6-d-old TYB) and 93.6% (12-d-old TYB) among treatments, while female proportions for *C. capitata* varied between 68.2% (9-d-old TYB) and 72.4% (12-d-old TYB). In contrast, male *Z. cucurbitae* comprised a minimum of 54.5% (12-d-old TYB) and a maximum of 62.0% (9-d-old) of the total catch among the treatments.

Week and treatment effects. Plots of raw data for each of the three species revealed that, while there was considerable weekly variation, trap captures were similar among the four treatments within any one week (Figs. 1–3). Results of the GLMs reflect this trend (Table 1). For *B. dorsalis* and *Z. cucurbitae*, week had a significant effect on trap captures, but food bait age did not (Table 1). For *C. capitata*, neither week nor food bait age had a significant effect. The interaction

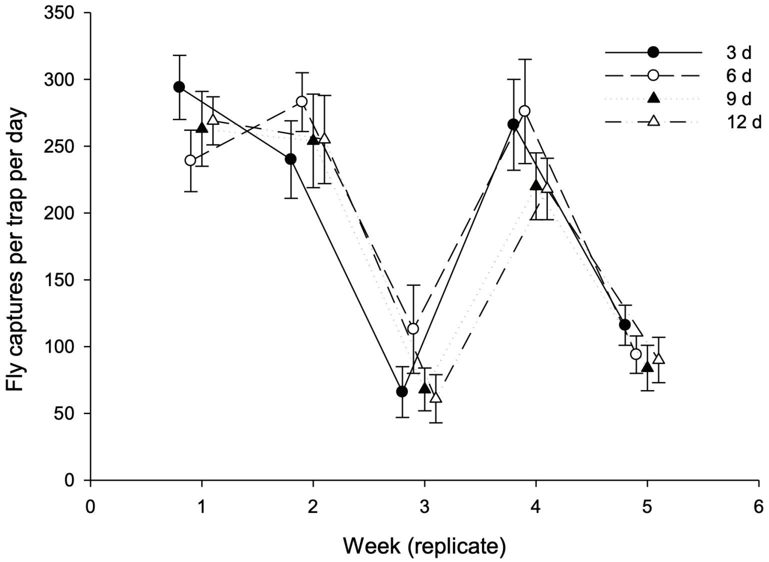

Figure 2. Captures of Mediterranean fruit flies (males and females combined) in Multilure traps baited with torula yeast borax weathered 3, 6, 9, or 12 d. Traps operated for 2 d at weekly intervals over 5 consecutive weeks. Symbols represent means ± 1 SE (N = 10 traps/treatment/sampling period).

Table 1. Results of GLMs for Experiments 1 and 2.

	B. dorsalis			***C. capitata***			***Z. cucurbitae***		
Source	**df**	χ^2	**P**	**df**	χ^2	**P**	**df**	χ^2	**P**
Experiment 1									
Week	7	15.8	0.03	4	8.6	0.07	3	14.3	0.002
Food bait	3	0.7	0.88	3	0.1	0.99	3	0.5	0.92
Week*Food bait	21	1.9	1.00	12	0.2	1.00	9	0.9	1.00
Experiment 2									
Week	5	28.3	<0.001	4	12.0	0.02	4	10.4	0.03
Food bait	1	3.2	0.07	1	17.2	<0.001	1	12.4	<0.001
Week*Food bait	5	9.6	0.09	4	0.2	1.00	4	4.1	0.39

term was not significant for any of the species. The factors responsible for temporal variation in trap catch were not studied, but this variation presumably reflected both natural population dynamics as well as possible differences in environmental conditions during sampling (e.g., wind speed and direction).

Experiment 2.

Sex ratios. As in the previous experiment, trap catch was heavily female-biased for *B. dorsalis* and *C. capitata* but male-biased for *Z. cucurbitae*. Based on data pooled over all traps and replicates, the average proportions of trapped *B. dorsalis* that were females were 84.3%

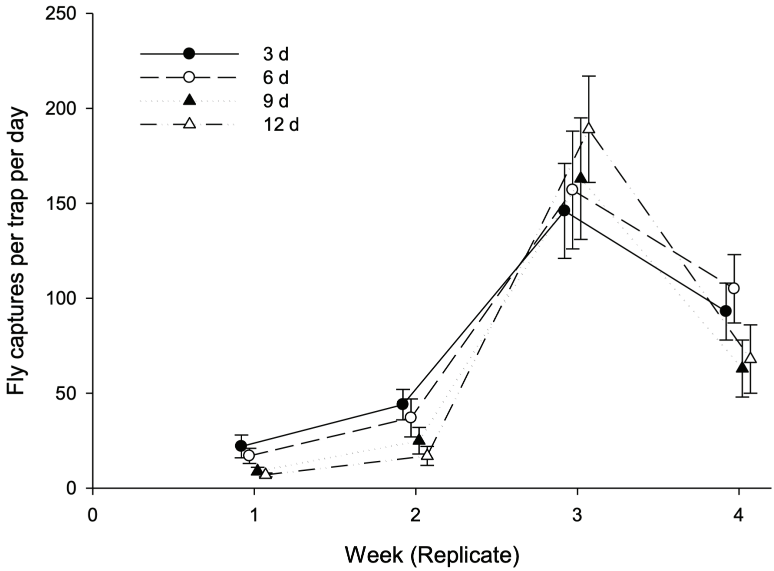

Figure 3. Captures of melon flies (males and females combined) in Multilure traps baited with torula yeast borax weathered 3, 6, 9, or 12 d. Traps operated for 2 d at weekly intervals over 4 consecutive weeks. Symbols represent means ± 1 SE (N = 10 traps/treatment/sampling period).

and 85.4% for the 3- and 21-d treatments, respectively, while female proportions for *C. capitata* were 60.2% and 60.4% for the 3- and 21-d treatments, respectively. In contrast, male *Z. cucurbitae* comprised 62.1% and 65.8% of the total catch for the 3- and 21-d treatments, respectively.

Week and treatment effects. For *B. dorsalis*, week had a significant effect on trap captures, but food bait age did not (Table 1). For *C. capitata* and *Z. cucurbitae*, both the effects of week and food bait age had significant effects. For both of these species, trap catch was significantly greater in 3-d-old bait than in 21-d-old bait. The interaction terms were not significant for any of the species. Average captures (± 1 SE; flies [sexes pooled] per trap per day) across all replicates for food baits weathered 3- vs. 21-d were: *B. dorsalis* –4.99 ± 0.23 vs. 4.59 ± 0.20 (N = 72); *C. capitata* –2.35 ± 0.3 vs. 1.05 ± 0.2 (N = 50); *Z. cucurbitae* –2.33 ± 0.24 vs. 1.36 ± 0.16 (N = 50).

Discussion

The present results show that the attractiveness of TYB slurry to *B. dorsalis*, *C. capitata*, or *Z. cucurbitae* did not change markedly between 3 and 12 d of weathering. Thus, under the ambient conditions of this study, TYB traps were effective for at least 12 d, suggesting the recommended interval (7–14 d) is appropriate (FAO/IAEA 2018). However, extending the replacement interval beyond 14 d seems problematic, as trap captures with TYB weathered for 21 d were significantly lower than those with TYB weathered for 3 d for both *C. capitata* and *Z. cucurbitae*. *B. dorsalis* was attracted equally to TYB weathered 3 or 21 d.

As noted above, few studies have assessed temporal variation in the attractancy of TYB-baited traps. Moreover, to our knowledge, only one study (Malo 1992) deployed variably weathered TYB traps simultaneously, an important procedure as it ensures the treatments were presented to the same size of the target population. In this case, Malo (1992) found no significant difference in captures of *Anastrepha* spp. among traps baited with TYB weathered 2, 4, 6, 8, or 10 d. Other studies (López-D. et al. 1971, Epsky et al. 1993) deployed TYB-baited traps and compared captures over time. While captures may have reflected age-related attractiveness of the bait, they may have also reflected changing sizes of fly populations. Data interpretation is therefore somewhat compromised in these studies. Nonetheless, Epsky et al. (1993) found a significant decrease in the catch of females (but not males) of *A. suspensa* among traps baited with TYB weathered 2, 4, or 7 d. Working with the same species, López-D. et al. (1971) reported results from two different 14-d periods of trapping. In the first test, captures of *A. suspensa* were uniform over the 14-d period. In the second test, however, trap catch declined significantly in the final days of the test. The authors attributed the variable results to differing temperature regimes: maximum daytime temperatures during the second test were, on average, 4°C warmer than during the first test (32°C vs. 28°C, respectively). Considering all these studies as a whole, we conclude that replacing TYB at 14-d intervals probably assures uniform attraction (see Iglesias et al. 2014 for a similar conclusion regarding yeast-based traps for *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) except in hot locations, where replacement should occur every 7–10 d.

Tephritid fruit flies are presumably attracted to volatiles derived from the decomposition of proteinaceous substances in the food bait (Epsky et al. 2014). To our knowledge, the identification and quantification of volatiles released by TYB solution have not yet been determined, but the aggregate aroma is likely a complex mixture of compounds. For example, Buttery et al. (1983) identified 43 volatile chemicals derived from PIB-7, a corn-based protein bait. Attraction is likely modulated by multiple volatiles (McPhail 1939, Mazor et al. 1987, Lasa and Williams 2021), but several studies indicate that ammonia has a key role. For example, Mazor et al. (1987) found a positive correlation between the pH of liquid protein baits, ammonia release rate, and catch of *C. capitata*, and Bateman and Morton (1981) observed the same general relationships for the Queensland fruit fly, *B. tryoni* (Froggatt) (but see Lasa and Williams [2021] for contrasting results for the West Indian fruit fly, *Anastrepha obliqua* (Macquart)).

Production of ammonia appears to increase with increasing pH in liquid protein food baits (Bateman and Morton 1981, Mazor et al. 1987), and increased pH has been associated with higher fruit fly captures (Epsky et al. 1993, Heath et al. 2009). In this regard, it is interesting that, over 7 d of field deployment, TYB solution maintained a relatively high and constant pH of 9.03 (Heath et al. 1994). This constancy may explain the uniform attractiveness of TYB observed here over 3–12 d of weathering. Also, the positive relationships observed between pH, ammonia release, and fly catch were not monotonic (Mazor et al. 1987, Bateman and Morton 1981), and captures declined at high values of pH and ammonia release. Whether the low attractiveness noted here for TYB weathered 21 d resulted from increases in pH and ammonia production is unknown. Alternatively, reduced attractiveness of long-weathered food baits may reflect, not ammonia levels, but increased production of certain bacterial metabolites that repel fruit flies (Epsky et al. 1993).

Finally, a female bias in captures of food-baited traps has been reported for several tephritid species, including *A. suspensa* (Hall et al. 2005), *A. ludens* (Heath et al. 1994), *C. capitata* (López-D. et al. 1971), and *B. dorsalis* (Steiner 1952). This result is believed to reflect females' greater need for dietary protein for egg production (Hagen and Finney 1950, Tsitsipis 1989). In contrast, and consistent with several previous studies, the present data revealed a male bias for *Z. cucurbitae* in TYB-baited traps. Working in Hawaii, Leblanc et al. (2010) observed that males comprised 70% of the total catch of *Z. cucurbitae* in TYB traps (see also Shelly et al. 2022). Similarly, a field cage study (Duyck et al. 2004) conducted on Reunion Island showed that *Z. cucurbitae* males were twice as likely to be captured in TYB-baited traps than females. The factors responsible for this male-bias in *Z. cucurbitae* are unknown but presumably reflect lower protein requirements for egg production by females and/or higher protein requirements for sexual signaling and mating by males.

Acknowledgments

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