# Visual and Olfactory Stimuli and Fruit Maturity Affect Trap Captures of Oriental Fruit Flies (Diptera: Tephritidae)

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**ABSTRACT** An effective lure-and-kill trap is a potentially important instrument in monitoring and controlling oriental fruit flies, *Bactrocera dorsalis* (Hendel). A number of experiments were performed in an orchard of commercial guava, *Psydium guajava* L., to determine how fly captures are affected by combining visual and olfactory stimuli, and by the timing of trap deployment relative to host phenology. Baiting sticky Ladd traps with hydrolyzed liquid protein significantly increased the number of captured flies. Mostly male flies were caught in the absence of mature guava fruit, whereas mostly female flies were caught when ripe fruit was abundant. These results suggest that an effective oriental fruit fly trap should include both visual and olfactory lures, and that proper timing of trap deployment can be an important factor in monitoring female abundance in oriental fruit fly populations.

**KEY WORDS** *Bactrocera dorsalis*, trapping, behavioral control, population dynamics

ORIENTAL FRUIT FLY, *Bactrocera dorsalis* (Hendel), is a serious horticultural pest in many tropical and subtropical regions of the world, where it causes direct damage to >150 species of fruits and vegetables (Christenson and Foote 1960, Haramoto and Bess 1970). Developing lure-and-kill trap systems to detect, monitor, and control populations of this insect can provide immediate economic benefits to commercial growers. One of the most successful examples of such strategies for tephritid fruit fly management is control of apple maggot, *Rhagoletis pomonella* (Walsh), with fruit-mimicking red sphere traps baited with host fruit odor (butyl hexanoate) and food attractant (ammonium acetate) in North America (Prokopy 1975, 1991; Duan and Prokopy 1995). The baited red sphere traps represent "supernormal" fruit stimuli and are highly attractive to both sexes of apple maggot flies (Prokopy 1968). This trapping system alone not only eliminates three to four chemical sprays annually against apple maggot flies but also greatly facilitates biological control of secondary pests such as aphids and spider mites. It has become a promising component of apple orchard integrated pest management (IPM) programs in North America (Prokopy et al. 1990a).

No such system is yet available for managing oriental fruit fly populations. The existing methyleugenol-baited traps for oriental fruit fly control are highly effective in intercepting male flies. However, unless male density is decreased by at least 99%, these traps have virtually no impact on females (Steiner 1952, Steiner et al. 1965). High levels of male suppression within fairly large and diverse regions, such as the Hawaiian Islands, are hindered by the existence of large fly populations, often in hard-to-reach natural areas (Vargas et al. 1989, 1990). Because oriental fruit flies are highly polygamous, even very few surviving males have the potential to fertilize a substantial number of females (Cunningham 1989). Each mated female can produce >1,000 eggs (Vargas et al. 1984), almost certainly resulting in significant damage to horticultural crops. Therefore, successful behavioral control of oriental fruit flies is highly unlikely without a lure-and-kill trap effective against females.

Oriental fruit flies use both visual and olfactory cues to find essential resources (e.g., host fruit for oviposition and protein food sources for ovarian development). Prokopy et al. (1990b) showed that mature female oriental fruit flies respond positively to visual and olfactory stimuli from individual natural host fruit (kumquats, *Fortunella japonica* Swingle) or models of this fruit. In subsequent field tests, Vargas et al. (1991) demonstrated that yellow or white fruit-mimicking spheres were more attractive to both sexes than orange, red, light green, dark green, blue, and black spheres. The reflectance spectrum of yellow spheres in their experiments closely resembled that of ripe fruits of an important oriental fruit fly host, common guava, *Psidium guajava* L. Similarly, in a study by Cornelius et al. (1999), yellow spheres captured more female oriental fruit flies than spheres of other colors, or than yellow rectangular blocks. Jang and Light (1991) showed that headspace odor from ripe papaya, *Carica papaya* L., was attractive to sexually mature oriental fruit fly females in a wind tunnel. Also, Cornelius et al. (2000) demonstrated that the odor of orange, *Citrus sinensis* (L.), puree was attractive to both mature and immature female flies in field cage experiments, whereas proteinaceous food odor was attractive to young females in the field. More females were attracted to a combination of yellow sticky spheres with ammonia-based olfactory lures than to spheres or olfactory lures alone.

In a series of recent experiments, Cornelius et al. (1999) identified standard Ladd traps as a more efficient trap for capturing oriental fruit fly females than several other trap types. The Ladd trap is a combination of a flat, yellow foliage-mimicking panel with a red fruit-mimicking sphere attached in the middle of the panel so that there is a hemisphere on each side of the panel. It is thought that female flies perceive dark spherical objects contrasted against a light yellow background as a host habitat comprising both fruit and foliage (Cornelius et al. 1999). Cornelius et al. 2000 also determined that liquid hydrolyzed proteinaceous bait (NuLure) attracted more female flies in a guava orchard than several ammonia-based olfactory lures. At the same time, few flies were attracted to the odor of orange puree under field conditions, probably because of the competition between the bait odor and the odors of fruit naturally occurring in the orchard. Simultaneous use of fruit and protein odors did not increase overall numbers of captured flies in their study.

In the current study, we attempted to determine whether combining the most attractive visual trap known at present (Ladd trap) with the most attractive protein odor has a synergistic effect on oriental fruit fly captures. Another objective was to determine whether the timing of trap deployment relative to host phenology affects the number of flies captured. Oriental fruit fly is a multivoltine species, and there is no obligatory diapause in its life cycle (Fletcher 1989). Thus, adults are present in the environment throughout the year. However, their populations undergo significant temporal and spatial fluctuations, with fly abundance in Hawaii closely related to availability of guava fruit (Vargas et al. 1983, 1989, 1990). A good understanding of the relationship between trap capture and host phenology may be important for optimization of trap efficiency within future IPM systems.

## Materials and Methods

**Experimental Site.** The study was conducted between 7 March and 27 June 1999 in a 194-ha unsprayed commercial guava ('Beaumont') orchard located in Kilauea, island of Kauai, HI. Guava trees were planted in 1977. At the time of this study, they were ≈4 m tall and had a canopy circumference of ≈25 m. The orchard is subdivided into several sections by windbreaks and dirt roads. The pruning schedules are coordinated among the sections, allowing continuous harvesting of guava fruit throughout the year. Previous experiments conducted in the same orchard (Cornelius et al. 1999) revealed substantial Oriental fruit fly populations.

**Experiment 1.** The major objective of the first experiment was to determine whether combining visual and olfactory stimuli increases overall attractiveness of lure-and-kill traps to oriental fruit flies. Fly captures by unbaited Ladd traps (visual cue only), McPhail-type traps baited with NuLure (olfactory cue only), and Ladd traps baited with NuLure (a combination of visual and olfactory cues) were compared. McPhail-type traps (AgriSense, Columbia, MD) used in our experiments were composed of clear plastic dome-shaped covers on invaginated clear plastic bases. Unlike the original McPhail traps, which are baited with torula yeast pellets and water, our traps were filled with 200 ml of a commercially available formulation of NuLure (Miller Chemical and Fertilizer, Hanover, PA). Protein odor bait for the Ladd traps (Ladd Research Industries, Burlington, VT) was prepared by filling 250-ml plastic containers with 200 ml of NuLure. Fourteen holes (8 mm diameter)were drilled in the upper part of each container to allow the odor to escape into the environment. The total area of the holes was approximately equal to the area of an aperture in a McPhail-type trap (≈700 $mm^2$). Similar empty containers were used with the unbaited traps. Containers were attached with wire ≈1 cm below each Ladd trap. Both baited and unbaited Ladd traps were covered with a layer of Tangletrap (Tanglefoot, Grand Rapids, MI), a clear, odorless, nondrying adhesive.

The experiment was conducted in six blocks (two blocks per treatment). All the blocks were located within a single orchard section, which contained mature fruit at the time of the experiment. Each block had an area of ≈625 $m^2$ (25 m by 25 m, or 5 by 4 trees). The distance between neighboring blocks was 50 m. Four traps of the same kind were hung with wire 10–12 cm below the tree canopy (≈1.7 m above the ground) on four randomly selected trees within each block for ≈48 h. The number of male and female flies captured by each trap was recorded. The experiment was repeated six times at weekly intervals. Reissig (1975) observed that visual traps were more effective in catching apple maggot flies when placed inside the canopy. However, further investigations by Drummond et al. (1984) and Owens and Prokopy (1984) determined that such an increase in fly captures is explained by a better trap visibility against the foliage background rather than by trap position itself. Furthermore, trimming away the foliage around traps improved their efficiency, probably because of their increased visibility to flies (Drummond et al. 1984). Because Ladd traps by themselves provide a contrast between dark spherical objects (fruit mimic) and a light yellow background (foliage mimic) (Cornelius et al. 1999), we decided that placing the traps slightly below tree canopy would increase their visibility to flies.

**Experiment 2.** Our second experiment was designed to investigate how timing of trap deployment affects the number of flies captured. The experimental protocol was very similar to that in experiment 1, but this time we also compared the numbers of flies captured before and after the appearance of ripe guava fruit on trees. To do so, we replicated the study at intervals of 4–6 d three times before fruit ripened within the orchard section where the experiment was conducted (7–21 March 1999), and three more times after the fruit had ripened (10–27 June 1999). Fruit

**Table 1. Number (mean ± SE) of oriental fruit flies captured by unbaited Ladd traps, baited Ladd traps, and McPhail-type traps in a guava orchard**

| Trap | No. ♂♂ | No. ♀♀ |
|---|---|---|
| Ladd | 6.39 ± 2.03 | 5.06 ± 0.69 |
| Baited Ladd | 9.42 ± 1.91 | 14.08 ± 1.36 |
| McPhail-type | 0.12 ± 0.05 | 0.42 ± 0.11 |

was not harvested during the entire duration of the experiment; therefore, ripe fruit was abundant on the trees and on the ground. The number of male and female flies captured by each trap was recorded.

**Experiment 3.** Because the protocol for experiment 2 did not allow us to discriminate between the effects of host plant phenology and the effects of other seasonal factors, we performed an additional experiment in an attempt to clarify this issue. Two adjacent orchard sections were selected. One section had abundant ripe fruit but the fruit in the other section was immature. Sections were located ≈7 m from each other, separated by a dirt road. Ten yellow plastic spheres (7 cm diameter) (Euro-matic, Ashland, OH) were hung by wire on 10 trees within each section, 10–12 cm below tree canopy (≈1.7 m above the ground). The trees were selected at random, but all were located at least 25 m away from the border between the sections. After ≈48 h, the number of male and female flies captured by each sphere was recorded. The experiment was repeated four times at intervals of 2–3 d between replications. We used fruit-mimicking yellow spheres instead of Ladd traps because Vargas et al. (1991) provided background information on their relative attractiveness to male and female flies. No such information was available for the Ladd traps.

**Statistical Analysis.** Analysis of variance (ANOVA) (Analytical Software 1996) was used to analyze data on the numbers of male and female flies captured by the traps. The Tukey honestly significant difference (HSD) tests were used for mean separation. Rank transformations were applied to the data before the analysis to equalize variances among the treatments and normalize the data distribution (Conover and Iman 1981). Means and standard errors were calculated from the nontransformed data only. Chi-square goodness-of-fit tests (Analytical Software 1996) were used to test the null hypotheses that the sex ratio of captured flies was not different from 1:1.

## Results

**Experiment 1.** McPhail-type traps were least efficient in capturing Oriental fruit flies, while baited Ladd traps were most efficient (Table 1). The difference among trap types was statistically significant both for males (ANOVA, $F = 150.93$; df = 1, 108; $P < 0.0001$) and females (ANOVA, $F = 218.27$; df = 1, 108; $P < 0.0001$). For both sexes, the number of flies captured by each trap type was significantly different from the number of flies captured by the other two types (Tukey HSD test, $P < 0.01$). There were no significant differences in fly captures among the blocks (ANOVA, $F = 0.17$; df = 1, 108; $P = 0.6845$). Ladd traps captured more males than females, whereas baited Ladd traps and McPhail-type traps captured more females than males. For Ladd traps, the difference between the sexes was only marginally significant (chi-square test, $\chi^2 = 3.74$, df = 1, $P = 0.0532$), whereas for the other two trap types the difference was significant (chi-square tests, $\chi^2 = 22.46$, df = 1, $P = 0.0001$ and $\chi^2 = 4.06$, $P = 0.0438$, respectively).

**Experiment 2.** Overall, there was a highly significant difference in the number of oriental fruit fly males (ANOVA, $F = 323.02$; df = 1, 108; $P < 0.0001$) and females (ANOVA, $F = 79.16$; df = 1, 108; $P < 0.0001$) captured by different trap types (Table 2). The timing of trap deployment also had a significant effect on captures of both male (ANOVA, $F = 221.92$; df = 1, 108; $P < 0.0001$) and female (ANOVA, $F = 315.71$; df = 1, 108; $P < 0.0001$) flies. As fruit ripened, the number of male flies caught decreased dramati-

**Table 2. Effect of fruit ripening on the number of oriental fruit flies captured by unbaited Ladd traps, baited Ladd traps, and McPhail-type traps**

| | Ripe fruit absent | | | | Ripe fruit present | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean no. ♂♂ (SE) | Mean no. ♀♀ (SE) | Chi-square tests[a] $\chi^2$ | Chi-square tests[a] $P$ | Mean no. ♂♂ (SE) | Mean no. ♀♀ (SE) | Chi-square tests[a] $\chi^2$ | Chi-square tests[a] $P$ |
| Ladd traps | 89.79a (7.41) | 0.25a (0.11) | 1419.4 | <0.0001 | 1.42a (0.40) | 7.08a (1.14) | 51.0 | <0.0001 |
| Baited Ladd traps | 119.0a (10.71) | 0.36a (0.19) | 1878.1 | <0.0001 | 6.0b (0.83) | 17.21b (1.99) | 68.92 | <0.0001 |
| McPhail-type traps | 0.08b (0.06) | 0.08b (0.06) | NA[b] | NA[b] | 0.21c (0.08) | 0.50c (0.19) | 1.54 | 0.2140 |
| ANOVA[c] | | | | | | | | |
| $F$ | 611.36 | 1.11 | | | 139.75 | 47.58 | | |
| df | 2, 54 | 2, 54 | | | 2, 54 | 2, 54 | | |
| $P$ | <0.0001 | 0.3374 | | | <0.0001 | <0.0001 | | |

Means followed by the same letter within each column are not significantly different from each other (Tukey HSD test, $P > 0.05$).
[a] Chi-square goodness-of-fit tests testing the null hypotheses that sex ratio of captured flies was not different from 1:1.
[b] Exactly the same number of males and females per trap per replication were captured.
[c] Because there was a statistically significant interaction between the effects of trap type and timing of deployment (see *Results*), we analyzed trap effects on number of captured flies separately for the periods when the fruit was present and when it was absent.

cally, whereas there was a substantial increase in the number of captured females (Table 2). As a result, the sex ratio of flies captured by both unbaited and baited Ladd traps was significantly skewed toward a predominance of males during the first 3 wk of the experiment, and became significantly skewed toward a predominance of females during the last 3 wk. Interaction between the timing of trap deployment and trap type was highly significant (ANOVA, $F = 78.20$; df = 2, 108; $P < 0.0001$ for males and $F = 54.51$, $P < 0.0001$ for females). Regardless of deployment timing, McPhail-type traps captured very few oriental fruit flies. At the same time, baiting Ladd traps with NuLure increased their attractiveness to flies. The increase was statistically significant for both sexes during the last 3 wk of the experiment. During the first 3 wk, when the overall number of captured females was very low, their captures by Ladd traps were not affected by the presence of proteinaceous odor. The number of males captured by baited Ladd traps during the same period was slightly higher than the number of males captured by the unbaited Ladd traps. This difference was not statistically significant according to the Tukey HSD test, but it was statistically significant at $\alpha = 0.05$ when we used a less conservative least significant difference (LSD) test. There were no significant differences in fly captures among the blocks (ANOVA, $F = 0.18$; df = 1, 108; $P = 0.6689$).

**Experiment 3.** On average, 4.28 ± 1.27 male and 1.50 ± 1.29 female oriental fruit flies were captured in the nonfruiting orchard section, and 2.10 ± 0.43 male and 5.13 ± 0.78 female flies were captured in the fruiting orchard section. The difference between the sections was statistically significant for the number of females (ANOVA, $F = 21.48$; df = 1, 72; $P < 0.0001$), but not for the number of males (ANOVA, $F = 0.10$; df = 1, 72; $P = 0.7579$). Sex ratio of the flies captured by sticky spheres was significantly skewed toward predominance of males in the nonfruiting section (chi-square test, $\chi^2 = 28.25$, df = 1, $P < 0.0001$), and toward predominance of females in the fruiting section (chi-square test, $\chi^2 = 26.53$, df = 1, $P < 0.0001$).

## Discussion

Protein odor significantly increased the number of oriental fruit flies captured by Ladd traps in the current study. Our observations are consistent with findings by a number of other authors, who tested responses of several tephritid species to various combinations of visual and olfactory stimuli. Integrating food odor with visual traps increased captures of *Rhagoletis pomonella* (Walsh) (Neilson et al. 1976), *R. completa* (Osten-Sacken) (Riedl and Hoying 1981), *R. mendax* Curran (Prokopy and Coli 1978), and *Bactrocera oleae* (Gmelin) (Prokopy and Economopoulos 1975). Baiting visual traps with host fruit odors had a similar effect because both Ladd traps (AliNiazee et al. 1987) and red fruit-mimicking spheres (Reissig et al. 1982, 1985) captured more apple maggot flies when they were baited with synthetic apple volatiles. More females of oriental fruit fly were attracted to a combination of yellow sticky spheres and ammonia-based food baits than to either of these lures alone (Cornelius et al. 2000), and more males were captured in methyleugenol-baited bucket traps when those traps were painted white or yellow (Stark and Vargas 1992).

From a pest management perspective, our findings lend additional support to the idea that female oriental fruit fly traps should be based on a combination of visual and olfactory lures. Such traps can provide a valuable tool in detection and monitoring of oriental fruit flies, similar to the traps currently in use for detection and monitoring of the apple maggot fly (Prokopy et al. 1990a). Potentially, they also can be used to suppress oriental fruit fly populations on individual farms. However, much additional research is required before lure-and-kill traps can be used in commercial settings. The majority of female flies attracted to protein odors are sexually immature (Cornelius et al. 2000). Therefore, protein-baited traps may not be efficient in intercepting gravid females that migrate into orchards from surrounding vegetation. Such females may be targeted by incorporating host odors into olfactory lures (Cornelius et al. 2000). Unfortunately, no long-lasting synthetic fruit volatiles attractive to oriental fruit flies and capable of competing with the odors of naturally occurring ripening fruit are yet available. Developing such compounds will be an important step in advancing monitoring techniques and behavioral control of oriental fruit flies.

Using Tangletrap to capture and kill the flies lured into traps is unlikely to be adopted on a wide scale by commercial growers, especially for control purposes. This substance is difficult to handle, and costs of servicing traps are likely to surpass potential benefits of reducing fruit fly populations (Prokopy et al. 1990a). A mixture of pesticides, phagostimulants, and residue-extending agents might be a feasible alternative to Tangletrap as a killing agent (Duan and Prokopy 1995 and references therein), but no such system is yet available for the oriental fruit fly.

The number of oriental fruit flies caught by our lure-and-kill traps changed significantly both in space and in time. Female abundance appeared to follow the availability of mature guava fruit within the area of trap deployment. This is not surprising because guava is a very important larval host of *B. dorsalis* on Kauai (Vargas et al. 1983, 1989, 1990), and areas where ripe fruit is plentiful probably attract females searching for oviposition sites. Our observations are similar to the results obtained by Stark et al. (1991), who reported that fogging of guava trees with pyrethrins started to yield significantly more female than male oriental fruit flies as the season progressed and guava ripened. Conversely (and unexpectedly), male captures in our experiments decreased dramatically with the increase in abundance of ripe guava. It is hard to provide a tangible explanation for this observed phenomenon because of a lack of information on oriental fruit fly biology. It is possible that the flies undergo a dispersal phase, similar to that described by Fletcher (1974) for the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), and that dispersal patterns and habitat colonization behavior are different in males and females. Also, if males stake out fruit, awaiting the arrival of females seeking oviposition sites, reduction in male captures when ripe fruit is present can be caused by competition between such fruit and the traps. However, additional work is required before we can draw more definite conclusions because the current study provided only a "snapshot" of complex processes taking place within oriental fruit fly populations. Nevertheless, our results indicate that there are significant variations in the abundance of male and female oriental fruit flies on a relatively small temporal and spatial scale, and that these variations are probably connected to the phenology of host plants. Further investigations of this issue are essential for successful incorporation of different control methods, such as male annihilation, sterile insect releases, behavioral, cultural, and biological control, into an integrated system for managing this pest.

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