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Response of Olive Fruit Fly (Diptera: Tephritidae) to an Attract-and-Kill Trap in Greenhouse Cage Tests

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ABSTRACT. A novel attract-and-kill trap for olive fruit fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae), was constructed with yellow corrugated plastic in an inverted cylindrical pan shape formed from a disk and collar. The trap components were tested under three greenhouse temperatures and humidities of warm, hot, and very hot for attractiveness to caged young or older adults. A greater proportion of adults regardless of age were found underneath the devices including disks, cylindrical pans, and pans with pheromone lures and test units of cylindrical pans sprayed with water, insecticidal bait spray, and with lures. The effect was related to lower temperatures on the underside compared with the top and the intolerance of the pest to heat. A circular collar added to the perimeter of the disk that formed the top of the inverted cylinder made the attract-and-kill trap more attractive to adults than the disk alone. Pheromone lures or bait sprays did not increase adult attraction, so were not needed for efficacy. The cylindrical pan was especially attractive to adults when temperatures were high by providing shelter from the heat. At very high temperatures, the pan became unattractive, possibly due to heating of the construction materials. Cylindrical pans sprayed with water on the underside attracted the highest number of adults especially at high temperatures. Greenhouse tests showed that the inverted cylindrical pan design has potential as an attract-and-kill device for olive fruit fly control.

Key Words: Bactrocera oleae, Olea europaea L., cultural control, bait station

Quarantine pest control techniques for olive fruit fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae), in California olives grown for canned fruit were developed shortly after the pest was first detected (Yokoyama and Miller 2004). Pest management programs were later implemented after the pest became widely distributed throughout the state, and the potential use of attract-and-kill traps was first studied (Yokoyama et al. 2004). The history of attract-and-kill traps, also called lure and kill traps, was described and reviewed by El-Sayed et al. (2009) for a diversity of insect pests including olive fruit fly. The function of such traps is to attract a pest with a semiochemical and cause mortality by exposure to an insecticide. The objective was to test different trap attributes for attractiveness to caged young or older adults. A greater proportion of adults regardless of age were found underneath the devices including disks, cylindrical pans, and pans with pheromone lures and test units of cylindrical pans sprayed with water, insecticidal bait spray, and with lures. The effect was related to lower temperatures on the underside compared with the top and the intolerance of the pest to heat. A circular collar added to the perimeter of the disk that formed the top of the inverted cylinder made the attract-and-kill trap more attractive to adults than the disk alone. Pheromone lures or bait sprays did not increase adult attraction, so were not needed for efficacy. The cylindrical pan was especially attractive to adults when temperatures were high by providing shelter from the heat. At very high temperatures, the pan became unattractive, possibly due to heating of the construction materials. Cylindrical pans sprayed with water on the underside attracted the highest number of adults especially at high temperatures. Greenhouse tests showed that the inverted cylindrical pan design has potential as an attract-and-kill device for olive fruit fly control.

A novel attract-and-kill trap for olive fruit fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae), was constructed with yellow corrugated plastic in an inverted cylindrical pan shape formed from a disk and collar. The trap components were tested under three greenhouse temperatures and humidities of warm, hot, and very hot for attractiveness to caged young or older adults. A greater proportion of adults regardless of age were found underneath the devices including disks, cylindrical pans, and pans with pheromone lures and test units of cylindrical pans sprayed with water, insecticidal bait spray, and with lures. The effect was related to lower temperatures on the underside compared with the top and the intolerance of the pest to heat. A circular collar added to the perimeter of the disk that formed the top of the inverted cylinder made the attract-and-kill trap more attractive to adults than the disk alone. Pheromone lures or bait sprays did not increase adult attraction, so were not needed for efficacy. The cylindrical pan was especially attractive to adults when temperatures were high by providing shelter from the heat. At very high temperatures, the pan became unattractive, possibly due to heating of the construction materials. Cylindrical pans sprayed with water on the underside attracted the highest number of adults especially at high temperatures. Greenhouse tests showed that the inverted cylindrical pan design has potential as an attract-and-kill device for olive fruit fly control.

The attract-and-kill trap developed in this study is a modification of the oriental fruit fly bait station (Piñero et al. 2009). Construction material was selected to provide shade and insulation to reduce summer heat and to create a refuge for olive fruit fly adults. A yellow color was used for attractiveness to adults (Prokopy et al. 1975), and an application of insecticidal bait spray on the underside is designed to prevent weathering of the bait. Other considerations in development of the attract-and-kill trap were the necessity for a pheromone lure, the amount of insulation for adequate protection from heat, the need for a curved surface (Tsitispis 1977) to attract gravid females that normally lay eggs on rounded surfaces such as olive fruit, and the requirement for simple construction materials. The objective was to test different trap attributes for attractiveness to adults in greenhouse cage tests to construct an economical attract-and-kill trap to control olive fruit fly in variable weather conditions.
Materials and Methods

Insects. Mission or Manzanillo olives infested with olive fruit fly larvae were collected from olive trees in Davis, Lodi, and San Jose, CA, from October 2012 to January 2013. The infested fruit were placed in 7.8-liter plastic containers, covered with organdy cloth, and held at \( \approx 23^\circ C \) until the third instars emerged from the fruit and pupated. Pupae were placed in cages used in tests or placed in sleeve cages until adult emergence. Noninfested olive fruit were placed in the sleeve cages containing adults and exposed to oviposition for about 7 d. The fruit was then placed in plastic containers until mature larvae emerged and the process repeated to increase the number of insects in a laboratory colony as described by Yokoyama (2012a).

Attract-and-Kill Traps. Yellow corrugated plastic sheets (Corrugated Plastics, Hillsborough, NJ) were cut into disks 1.0-cm thickness by 40.6-cm diameter. The disks were used to form the top of the attract-and-kill trap. A circular collar was made from yellow corrugated plastic 0.2-cm thick by 7.6 wide by 56-cm long by riveting the ends together. Six equally placed 2-cm wide by 2.5-cm long tabs along one long edge of the collar were riveted to the top of the disk. The disk with collar formed a wide, short cylinder that was positioned with the disk at the top to create the pan-shaped attract-and-kill trap (Fig. 1). The disk alone and the cylindrical pan were evaluated for attractiveness to olive fruit fly adults.

Cage Tests. Temperature and relative humidity (RH) were maintained in a glass greenhouse 5.6-m wide by 4.5-m long by 4.1-m high and monitored and recorded with a hygrothermograph (model CT485, Omega Engineering, Stamford, CT). Temperatures and humidity were manually controlled during the day with shade cloths, swamp coolers, ground water misters, and propane, and electrical heaters. Olive fruit fly pupae were placed into polyethylene screen cages, 35-cm wide by 35-cm long by 56-cm high, supported by a polyvinyl chloride pipe frame that fit on greenhouse benches. Several hours after adults began to emerge, the pupae were removed to limit the number of adults in the cage. The adults were maintained for 1–5 d to obtain young adults and 6–13 d to obtain older adults. The insects were provided with honey for food smeared on the top of the cage and water in a plastic container with a sponge wick to enhance adult survival to the test age. Each cage was considered a replicate, and 3–4 replicate cages were used to test each device or unit with young or older adults.

A warm temperature of 22–24°C and \( \approx 65\% \) RH, a hot temperature of 31–32°C and \( \approx 35\% \) RH, and a very hot temperature of 36°C and \( \approx 26\% \) RH were used to simulate olive growing regions in the San Joaquin Valley and determine the attractiveness of different components of the attract-and-kill trap under varying weather conditions.

The devices tested in a cage for attractiveness to olive fruit fly adults were disk, inverted cylindrical pan (disk with collar), and cylindrical pan with pheromone (1,7-dioxaspiro [5,5] undecane) lure (Vioryl, Athens, Greece). Separate cages were used to evaluate units composed of inverted cylindrical pan and water (\( \approx 10 \) ml); pan and insecticidal bait spray (40% GF-120); and pan, bait spray, and pheromone lure. Before the start of each test, the number of live adults was counted in each cage. The attractiveness of the device or unit to young or older adults was determined by counting the number on the top and the underside every 15 min for 2 h after placement in the cage. After the 2-h observation period, the adults were dislodged and a different device in the order of disk, pan, and pan with pheromone lure or unit in the order of pan with water, bait spray, and bait spray with lure. Each cage with young or older adults was used no more than 6 h. In tests with pheromone lures, the air speed across the cage was maintained at \( \approx 0.1 \) m/s with fans and monitored with a thermo anemometer (model 8525, Alnor, Huntington Beach, CA).

At the end of the test, except for young adults with devices in warm temperature, the cage was held at 5°C until all insects died and the number of males and females counted. The sex ratio was reported as the percentage females of the total number of insects in each cage. The number of adults on the top and underside of the device or unit, and the number of all adults on the device or unit were reported as the percentage of the total number of live adults counted before the beginning of the test.

Statistical Analysis. Test results are reported as the mean \( \pm \) SEM among the eight observations for each test duration in each of 3–4 replicate cages for each temperature and olive fruit fly adult age. A t-test (GraphPad Software 2012) was used to compare the temperature or percentage adults between the top and underside of each device or unit. Percentage adults was arcsine transformed and compared among each device or unit with a one-way analysis of variance and Tukey’s honestly significant difference (HSD) test (GraphPad Software 2012).

Results

Manipulation of the greenhouse environment resulted in simulated warm, hot, and very hot weather conditions at 22.5–23.6°C and 40–51% RH; 31.3–32.3°C and 28–36% RH; and 36.1°C and 29% RH, respectively (Tables 1–4). Only young adults with devices were tested at the highest temperature due to low olive fruit fly response under very hot conditions (Table 1). The mean number of adults tested in each cage was 125–329 young, and 140–240 older adults of which 43–50% were female.

Temperatures were consistently lower underneath than on top of disks and inverted cylindrical pans by \( \approx 1°C \) in the warm and by \( \approx 2–3°C \) in the hot and very hot greenhouse (Tables 1 and 2). In warm temperatures, \( \approx 1–2\% \) more of the young adults and \( \approx 12–14\% \) more of the older adults were underneath than on top of the devices. In hot temperatures, \( \approx 10–38\% \) more of the young and \( \approx 13–20\% \) more of the older adults were underneath the disk or cylindrical pan than on the top. When temperatures were very hot, fewer young adults were found underneath the disk or cylindrical pan than in warm and hot temperatures, and the difference between the underside and top was \( \approx 6–12\% \).

Temperatures were lower by \( \approx 2–4°C \) on the underside compared with the top of inverted cylindrical pans in all water and insecticidal bait spray treatments in the warm and by \( \approx 2–5°C \) in the hot greenhouse (Tables 3 and 4). The difference in percentage young olive fruit fly adults attracted to the underside compared with the top of cylindrical pans sprayed on the underside with water or insecticidal bait was 5.0 and 0.6% in warm, and 69.1 and 26.7% in hot temperatures, respectively. The difference in older adults on the underside compared with the top of cylindrical pans sprayed with water or insecticidal bait was 23.0 and 11.4% in warm and 61.3 and 19.2% in hot temperatures, respectively. Fewer young and older adults were attracted to cylindrical.
pans with bait spray and pheromone lures compared with pans with water or bait spray at both greenhouse temperatures.

The inverted cylindrical pans attracted more of the young olive fruit fly adults than the disk alone, and the addition of a pheromone lure did not increase attraction in all temperatures and RHs tested (Table 1). More of the young adults were found on all devices when the temperatures were hot compared with warm or very hot. Similarly, older adults were more attracted to the cylindrical pan than the disk, and response to the pan with pheromone lure was similar to the pan alone in all temperatures (Table 2). More of the older adults were attracted to the devices in hot compared with warm temperatures. HSDs ($F = 38.68$; df = 3, 28; $P < 0.01$) were found among the total mean percentages of young and older adults on the attract-and-kill, cylindrical pan trap in the warm and hot greenhouse.

The inverted cylindrical pan attracted more of the young and older olive fruit fly adults when water in comparison to insecticidal bait spray was added to the underside under warm and hot greenhouse temperatures (Tables 3 and 4). Attraction of both adult ages to the cylindrical pan with bait spray was similar with and without pheromone lures at warm temperatures, but at hot temperatures, fewer adults were found on the pans with pheromone lures. HSDs ($F = 217.7$; df = 3, 28; $P < 0.01$) were found among the total mean percentages of young and older adults on the attract-and-kill, cylindrical pan trap with bait spray in the warm and hot greenhouse.

### Discussion

Warm, hot, and very hot temperatures of $\approx 23$, 32, and $36^\circ$C were created in the greenhouse to simulate seasonal conditions in the
California San Joaquin Valley where table olives are grown (Tables 1–4). These temperatures would occur during fruit development from spring bloom, through summer maturation, and fall harvest. Olive fruit fly can be found in olives in low numbers during the spring and summer and abundantly during the fall when temperatures are mild. An effective attract-and-kill trap would reduce pest numbers throughout the olive growing season. Highest RHs were maintained among the temperatures to enhance olive fruit fly survival during the greenhouse tests, so responses were not caused by lack of moisture. The RHs achieved in the cages were above normal than expected for inland valleys but consistent with coastal areas. Numbers of adults in cage tests were based on availability in laboratory colonies and survival throughout young to older stages in greenhouse cages. The ages of young adults were based on the preovipositional period of 6 d (Yokoyama 2012a) prior to maturity. The sex of adults visiting the attract-and-kill traps could not be determined visually from the exterior of the screened cage, and therefore, the numbers were determined upon completion of the test. The smaller percentage of females than males in each cage was consistent with the sex ratio for olive fruit fly adults reared for similar tests (Yokoyama 2012b). Adults rarely fed on the honey and water in each cage during the test period, and insects not on test materials were on the cage screen.

In all tests, the underside of disks and inverted cylindrical pans were lower in temperature than the top (Tables 1–4), and the reduction in heat was attributed to the insulating properties of the corrugated plastic. The temperature difference was especially evident in hot compared with warm greenhouse tests. The adults would require shelter from heat during hot temperatures, so the inverted cylindrical pan would be more effective when conditions in the orchard were the warmest during the summer. However, when the greenhouse temperature was very hot (Table 1), the temperature difference was evident between the top and underside of the devices, but the entire structure heated to a very high temperature, thus limiting the attractiveness of corrugated plastic as a substrate for heat sensitive olive fruit flies. Bait sprays rapidly dried in a thin layer after application to the underside of the inverted cylindrical pans, so the presence of insecticidal bait or small plastic pheromone lure dispensers would not be expected to cause major temperature differences between the top and underside of the pans as found in Table 4. These differences were primarily caused by the random position of each cage in the greenhouse and the amount of exposure to sunlight on the top of the inverted cylindrical pan during the test.

Space for cages was limited in the greenhouse, and no-choice tests rather than choice tests were selected to determine the attractiveness of the structure of the attract-and-kill trap with different components. Choice tests would have required larger cages to accommodate the size of the cylindrical pan. Olive fruit fly adults that alighted on the disk, untreated cylindrical pan, or pans treated with water, insecticidal bait spray, or pheromone lures tended to remain throughout the test period because the numbers counted and the position of individuals on test surfaces remained rather constant. Therefore, the number of insects observed on each device was reported as a mean of each 15-min interval for 3 h test. Of the number of adults observed on disks and inverted cylindrical pans in all tests, the highest percentage was found on the underside (Tables 1–4). Placing the disk horizontally rather than vertically on its edge was attractive to adults, and the addition of a collar to form the cylindrical pan increased the number of both young and older adult visits at all temperatures tested. The inverted cylindrical shape is a necessary attribute of the attract-and-kill trap because a simple disk alone will not attract as many insects. The curvature of the collar suggests the curvature of cones, which was found by Tsitsipis (1977) to attract adults and stimulate oviposition. The percentage of young and older adults beneath the inverted cylindrical pan was greater at hot than warm temperatures (Tables 1 and 2). At very hot temperatures, the percentage of young adults decreased on the inverted cylindrical pan and was probably due to the high surface temperature of 36 °C beneath the pan. Avidov (1954) reported that olive fruit fly adult activity ceases at 35 °C, which would greatly reduce the number of adults capable of moving to the pan. Furthermore, adults may not visit the cylindrical pan at 35 °C because Fletcher (1987) found that adults move to the low undergrowth of the tree at this temperature. These observations suggest that the cylindrical pan would become ineffective if not protected within the canopy of the tree during very hot periods of the day on the hottest summer days in the inland valleys. Under such conditions, the cylindrical pan would be most attractive when orchard temperatures were moderate in the morning and especially in late afternoon and when adults were sexually active (Fletcher 1987).

When the attractiveness of the inverted cylindrical pan to young and older olive fruit fly adults with and without insecticidal bait spray at hot temperatures was compared, the pan was somewhat more attractive to younger adults than older adults (Tables 3 and 4). This is an advantage that would help control olive fruit fly before females began to oviposit in fruit. The most attractive additive to the cylindrical pan was water as the adults require sufficient water to survive, especially under warm conditions (Yokoyama 2012a). When the underside of the cylindrical pan was sprayed with water under warm greenhouse conditions, about 91% of the adults in the cage were on the pan. The strong attraction of olive fruit fly adults to water on the underside of the pan was predicted, and the response was used for comparative purposes. Water alone was more attractive when compared with bait spray as a food source suggesting a need for improved bait spray formulations. Solutions of water and yeast are used as attractants in plastic bottles in hot and arid regions for mass trapping of olive fruit fly (Tabic et al. 2011) with varying results. The use of water in any trap device is nonselective, and

Table 4. Mean (± SEM) number and sex ratio of older (≥6-d old) olive fruit fly adults in greenhouse cage tests, greenhouse conditions, and temperatures and percentage total adults on the top and underneath attract-and-kill traps with water, insecticidal bait spray, and pheromone lures

<table>
<thead>
<tr>
<th>Greenhouse Adults Device type</th>
<th>Device temp. (°C)</th>
<th>% Total adults</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Top</td>
<td>Underside</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp. (°C) % RH n % Female</td>
<td></td>
<td></td>
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<tr>
<td>22.5 ± 0.4 40.0 ± 2.7 156.7 ± 17.6 43.6 ± 1.6</td>
<td>Pan-Water 24.8 ± 0.2 21.2 ± 0.1</td>
<td>6.2 ± 0.9 29.2 ± 8.2* 36.1 ± 1.1a</td>
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<tr>
<td>32.3 ± 0.4 35.8 ± 2.0 239.7 ± 8.4 49.8 ± 2.5</td>
<td>Pan-Bait 23.2 ± 0.3 20.9 ± 0.7*</td>
<td>2.6 ± 0.8 14.2 ± 5.0 17.1 ± 0.4b</td>
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<tr>
<td></td>
<td>Pan-Bait-Lure 22.7 ± 0.1 21.1 ± 0.8</td>
<td>2.3 ± 1.0 13.4 ± 4.4 15.8 ± 0.9b</td>
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<tr>
<td></td>
<td>Pan-Water 35.8 ± 0.4 32.4 ± 0.7*</td>
<td>8.4 ± 1.7 69.7 ± 2.4** 78.0 ± 1.6a</td>
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<tr>
<td></td>
<td>Pan-Bait 33.3 ± 0.1 31.7 ± 0.0**</td>
<td>5.7 ± 1.0 24.9 ± 4.3* 30.6 ± 2.2b</td>
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<tr>
<td></td>
<td>Pan-Bait-Lure 32.5 ± 0.4 31.8 ± 0.4</td>
<td>4.7 ± 0.2 12.1 ± 1.9* 16.8 ± 1.4c</td>
</tr>
</tbody>
</table>

Temperature or percentage adults on underside significantly different than top (* P < 0.05; ** P < 0.01; t-test; GraphPad Software 2012). Column means for total percentage adults within each greenhouse temperature followed by different letters are significantly different (P < 0.05, one-way analysis of variance, Tukey’s HSD test, GraphPad Software 2012).
precautions must be implemented to prevent adverse effects on beneficial insects, especially in attract-and-kill traps that utilize toxicants.

The least attractive component tested with the cylindrical pan was the pheromone lure in most tests and may have been related to the abnormal environment caused by presenting the pheromone to adults confined to small cages in a greenhouse. Use of a pheromone lure for detection trapping was recommended for yellow sticky traps (Yokoyama et al. 2006) but would not be needed for the inverted cylindrical pan, attract-and-kill trap based on greenhouse cage test results. The trapping efficacy of the inverted cylindrical pan is not comparable with detection traps, because the adults are not captured in the former device. However, greenhouse tests suggest that detection traps may be more attractive during high orchard temperatures if placed in a horizontal instead of a vertical position. Olive fruit fly adults attracted to either type of trap would be transiting the canopy of the olive tree in the vicinity of the traps.

Greenhouse cage tests were used to determine olive fruit fly adult response to the cylindrical pan, attract-and-kill trap for short durations under diurnal conditions, which would not be sustained for very long in olive orchards. Many other variables would affect attractiveness throughout the day such as potential dew formation on the pans in the evening and morning, which would greatly increase attraction. Trap placement will greatly affect pan temperatures and installation in the shaded canopy of the trees would reduce exposure to sunlight, heating, and high ambient temperatures. During daily fluctuations in temperature in orchard environments favorable conditions will prevail for trap visitations by olive fruit fly adults. Visitations must coincide with the application of insecticidal bait sprays to control the adults and future pest numbers. The capacity of the cylindrical traps to reduce weathering of bait sprays also needs consideration.

Olive fruit fly infestation sites were described in table olive growing regions in previous biological control studies in California (Yokoyama et al. 2012). Field testing of the attract-and-kill trap would be preferable in these or similar sites as their infestation histories are known. The yellow, inverted cylindrical pan, attract-and-kill trap may have potential for economical olive fruit fly control in commercial olive orchards used for canned fruit. Insecticidal bait spray would be applied as a spot spray to the underside of the cylindrical pan rather than on foliage, reducing insecticide costs, and orchard contamination. The inverted cylindrical pan can be easily constructed from simple materials and would be a readily available pest management tool for growers.

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