Psyttalia cf. *concolor* (Hymenoptera: Braconidae) for Biological Control of Olive Fruit Fly (Diptera: Tephritidae) in California

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ABSTRACT The larval parasitoid, *Psyttalia* cf. *concolor* (Szépligeti), reared on Mediterranean fruit fly, *Ceratitis capitata* (Weidemann), by the USDA–APHIS–PPQ, Guatemala City, Guatemala, was imported into California for biological control of olive fruit fly, *Bactrocera oleae* (Gmelin), in olives, *Olea europaea* L. Mean percentage parasitism of olive fruit fly third instars infesting fruit in field cages ranged from 7.0 in Grapevine to 59.7 in Santa Barbara and in free releases ranged from 0 in Grapevine to 10.6 in Santa Barbara after 4- to 6-d exposures. In the laboratory, more parasitoids developed to adults in olive fruit fly larvae that were 11–13 d old than in larvae 8–10 d old. Adult parasitoids lived significantly longer when provided with water than adults without water in environmental chambers at 5°C, 85% RH; 15°C, 65% RH; 25°C, 25% RH; and 35°C, 25% RH. Adult parasitoids lived for 48 d with honey for food and water and 32 d with food and sugar solution at 15°C and 65% RH. Survival of adult parasitoids without food and water in greenhouse tests was ≈4 d in a simulated coastal climate and 1 d in a simulated inland valley climate and was significantly increased by providing food and water. The parasitoid did not develop in the beneficial seedhead fly, *Chaetorellia succinea* (Costa), in yellow star thistle. The rate of parasitism of walnut husk fly, *Rhagoletis completa* Cresson, larvae in green walnut husks was 28.4% in laboratory no-choice tests. In choice tests, the rate of parasitism of walnut husk fly versus olive fruit fly larvae in olives was 11.5 and 24.2%, respectively.

KEY WORDS larval parasitoid, *Bactrocera oleae* (Gmelin), *Olea europaea* L.

Olive fruit fly, *Bactrocera oleae* (Gmelin), was first detected in California in 1998 (Rice 2000). The introduction and distribution of the pest throughout the state has created a serious economic threat to the olive industry. High populations of olive fruit fly occur in the coastal areas, and low numbers of the pest are found in the San Joaquin Valley of California, where canning olives, *Olea europaea* L., are produced (Yokoyama et al. 2006). Quarantine strategies to mitigate pest populations in harvested fruit transported to processing plants were developed by Yokoyama and Miller (2004). Other methods to detect and control the pest were also studied (Yokoyama et al. 2006). However, biological control was found to have the greatest potential for reducing olive fruit fly populations in heavily infested areas (Yokoyama et al. 2004).

European countries have used parasitoids for biological control of olive fruit fly in commercial olives for many decades. A braconid, *Opius concolor* Szépligeti, from Tunisia was released in Greece (Kapatos et al. 1977, Neuenschwander et al. 1983) and was later found to be the most abundant parasitoid of olive fruit fly in southern Crete (Michelakis 1990).

The larval parasitoid used in our studies was collected from tephritids infesting coffee in Kenya (Wharton et al. 2000) and shipped to Guatemala for rearing in 1999, where it has been carefully maintained in a pure colony. This parasitoid was originally described as an *Opius* sp. and briefly considered a synonym of *Psyttalia humilis* (Silvestri) (Kimani-Njogu et al. 2001). Wharton et al. (2000) later described it as *Psyttalia* cf. *concolor* from Kenya. Based on DNA work, *P.* cf. *concolor* in our study differs by a single base pair from the *P. concolor* maintained in colonies in California and Hawaii that are labeled Kenya but originate from Italy (Wharton et al. 2006), including the *P. concolor* studied by Sime et al. (2006) for olive fruit fly control in California.

We reared *P.* cf. *concolor* on Mediterranean fruit fly, *Ceratitis capitata* Weidemann, at the Medfly Parasitoids Rearing Facility La Aurora, Programa La Mosca del Mediterráneo (MOSCAMED), Guatemala, and imported it into California to determine its potential for biological control of olive fruit fly (Yokoyama et al. 2004, 2008). We selected *P.* cf. *concolor* for further study because the parasitoid could be mass reared, was

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found to develop in olive fruit fly, and its origins could be determined.

Factors that would affect the effectiveness of *P.* cf. *concolor* to control olive fruit fly include the ability of the parasitoid to adapt to different climatic conditions in California, the availability of food for the adult stage, the capacity to parasitize different life stages of olive fruit fly, and the potential to attack nontarget fruit flies. The objectives of our study were to determine the ability of *P.* cf. *concolor* to survive on olive fruit fly under laboratory, greenhouse, and field conditions that represent the diverse climatic regions of California and elucidate the susceptibility of other tephritids as hosts.

Materials and Methods

Production and Shipment of Parasitoids. *Psyttalia* cf. *concolor* adults were reared from early third instars of the Antigua strain of Mediterranean fruit fly in the quarantine facility at MOSCAMED, San Miguel Petapa, Guatemala. Larval exposure cages were made from an inner plastic ring (10 cm inner diameter by 1.5 cm high by 0.5 cm wide) that was cut (0.2 cm wide) for flexibility, an outer plastic ring (10.8 cm inner diameter by 1.5 cm high by 0.3 cm wide), and nylon chiffon cloth (30 cm wide by 40 cm long). Naked Mediterranean fruit fly third instars were placed in the center of one side of the cloth. The cloth was folded in half to cover the larvae. The covered larvae were placed into the outer ring and secured in place with the inner ring. The larval exposure cage was moistened with water and placed on the screened top of a Plexiglas cage (30 cm wide by 30 cm long by 30 cm high) that contained *P.* cf. *concolor* females. Approximately 2,600 Mediterranean fruit fly larvae were exposed for 1.5 h to female parasitoids at a ratio of 1 female per 6.5–19.5 larvae. The parasitized larvae were placed on sawdust in Plexiglas cages for pupation, and the parasitoid adults that emerged were collected with glass aspirators.

The parasitoids were transported in paraffin-coated paper cups (11.0 cm diameter by 14.9 cm tall). A thin sponge (8.0 cm diameter by 2.5 cm high) was glued to the bottom of the cup and saturated with water. Newspaper (≈45 cm wide by 10 cm long) was pleated (8–10 folds) and glued to the inside of the cup. The top of the cup was covered with organdy cloth that was glued to the rim and fastened with a rubber band. Parasitoids (500 females and 500 males) that were 3 d old were placed inside the cup through a small opening in the cloth. The opening was sealed with cloth and glue. Honey was spread on the cloth for food. The cups were placed in a cardboard box with crushed paper for support, sealed with tape, and shipped by air freight.

On arrival at the USDA–ARS, Parlier, CA after 2 d in transit, the adults were placed into two wooden sleeve cages (72 cm wide by 42 cm deep by 50 cm tall) with Plexiglas tops. Mortality was calculated by counting the number of dead parasitoids in the bottom of the cups and reported as a percentage of the total number of parasitoids in the shipment. Honey was placed on the inside of the top of each cage for food, and deionized water was provided with a cellulose sponge (5 cm wide by 7 cm long by 2 cm high) placed through a slit in the lid of a plastic bowl (11.5 cm diameter by 4 cm high). The sleeve cages were maintained in an isolated holding room at a daily 24.0 ± 1.5°C and 63 ± 3% RH (±SEM), and a photoperiod of 12:12 (L:D) h with simulated dawn and dusk conditions (model HLT Dawn/Dusk Simulator; Hughes Lighting Technologies, Lake Hopatcong, NJ). The parasitoids were observed and allowed to mate for at least 2 d. Parasitoids (163–303) were retained in each of three sleeve cages to determine the maximum life span under these conditions, and reported as the mean ± SEM days parasitoids were alive after arrival in Parlier, CA.

Voucher specimens of *P.* cf. *concolor* were placed in the Entomology Laboratory, Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA, and the Systematic Entomology Laboratory, National Museum of Natural History, Smithsonian Institution, Washington DC.

Field Cages and Releases. Parasitoids were collected from the sleeve cages with cylindrical paper cartons (9.5 cm diameter by 10 cm high; model HD16; Solo Cup, Urbana, IL) by brushing the adults inside and closing the opening with organdy fabric held in place with the collar of the lid. Honey and a sponge (3 cm wide by 4 cm long by 2 cm high) saturated with deionized water were placed on top of the fabric and covered with plastic food wrap held in place with a rubber band. The cartons of parasitoids were transported in insulated coolers by automobile or airplane for field studies.

Cylindrical cages were sewn from polyethylene screen (48.3 cm diameter by 61.0 cm long; no. C32A, 12 openings per cm; Synthetic Industries, Gainesville, GA). The base of the cage was finished with a cotton muslin fabric sleeve (48.3 cm diameter by 53.3 cm long) that had a cord sewn into the hem. Boning was sewn into the seam around the diameter of the cage between the screen and muslin sleeve to help maintain the cylindrical shape of the cage.

Each cage was placed over olive branches that had a large amount of fruit (290–498) infested with olive fruit fly in the canopy. Parasitoids were introduced into the cage from the opened paper cartons. The cage was closed at the base by pulling the cord to tighten the fabric sleeve around the branches and by fastening the sleeve with nylon tie wraps. The cages were removed from the trees by cutting off the branch above the ties after each exposure period.

Parasitoids (450–2,000) were released in trees without cages by attaching open cartons with vinyl flagging tape (2.8 cm wide) to an olive branch near fruit infested with olive fruit fly. The number of parasitoids used in cage tests and releases were based primarily on quantities received in each shipment and the number allocated for other tests.

Fruit collected for pretest controls to determine the number of olive fruit fly larvae were sampled at random from the same trees used for cage tests and releases. Postrelease samples of fruit were collected at

random in the immediate vicinity of the same trees in which parasitoids were released. Each fruit sample was considered a replicate.

Temperature loggers (model XTI08–5 + 37; Intermountain Environmental, Logan, UT) with external thermistors (model TMC6–1T; Intermountain Environmental) on extension cables (1.8 m long) were used to determine the temperature, and humidity loggers (model SRHA08; Intermountain Environmental) were used to determine relative humidity. A temperature and a humidity logger was placed inside each of three cages and on branches outside of each of the three cages in the canopy of trees at each location and programmed to record 720 determinations/d.

Four yellow panel Pherocon AM traps (Trécé, Adair, OK) each with a clear plastic packet (10.5 cm wide by 10.5 cm high) of ammonium bicarbonate bait (15–20 g) and a plastic dispenser (1.7 cm wide by 4.8 cm long) containing pheromone (1,7-dioxaspiro[5,5]undecane, 80 mg) were used to trap olive fruit fly adults at each location. Traps were placed in trees with cages, and in general, one trap was used per tree spaced one to several trees apart. The traps were suspended at mid-canopy ≈2.4 m high in a shaded area near fruit (Yokoyama et al. 2006) that was close to the cage. Each trap was considered a replicate.

Test Locations. Field cage and release studies were conducted in California in the following locations from September to November 2002: Aborn Road and Ruby Avenue, San Jose; Mission Canyon Road and Las Canoas Road, Santa Barbara; Paseo del Verano Norte and Cumana Terrace, San Diego; and Interstate Highway 5 and Grapevine Road, Grapevine. Mature olive trees, primarily of the Mission type, with canopies topped for hand harvesting and bearing fruit infested with olive fruit fly were selected in the following arrangements at each site: San Jose, a single windrow of 9 trees; Santa Barbara, a staggered border row of 7 trees along a commercial orchard; San Diego, a windrow of 11 trees; and, Grapevine, 9 trees in a commercial, landscaped area.

On 18 October 2002, in San Jose, three replicates of 563–585 olive fruit infested with olive fruit fly were collected for controls. Six replicate cages were placed in each of two trees with 60 parasitoids in each cage. Approximately 1,950 parasitoids were released in a tree without cages. Two traps were placed in each of two trees with cages. After a 5-d exposure, all cages, loggers, traps, and five replicates of 431–543 postrelease fruit weighing 958–1,275 g were collected.

On 9 October 2002, in Santa Barbara, three replicates of 630–731 infested olive fruit were collected for controls. Six replicate cages were placed in each of four trees with 50 parasitoids in each cage. Approximately 450 parasitoids were released in a tree without cages. Four traps were placed in three trees with cages. After a 6-d exposure, all cages, loggers, traps, and three replicates of 432–614 postrelease fruit weighing 807–1,141 g were collected.

On 2 October 2002, in San Diego, three replicates of 293–311 infested olive fruit were collected for controls. Six replicate cages were placed in each of five trees with 50 parasitoids in each cage. Approximately 1,500 parasitoids were released in a tree without cages. Four traps were placed in each of four trees with cages. After a 5-d exposure, all cages, loggers, traps, and five replicates of 509–755 postrelease fruit weighing 761–1,146 g were collected.

On 30 September 2002, in Grapevine, three replicates of 157–237 infested olive fruit were collected for controls. Six replicate cages were placed in each of four trees with 30 parasitoids in each cage. Approximately 500 parasitoids were released in one tree. Four traps had been placed on 19 September in three trees adjacent to trees with cages and in one tree with a cage. After a 4-d exposure, all cages, loggers, traps, and three replicates of 383–452 postrelease fruit weighing 1,162–1,463 g were collected.

All materials were returned to the laboratory for evaluation. Fruit from controls, inside cages, or near parasitoid releases were removed from the branches, counted, and placed in plastic containers (22 cm wide by 32 cm long by 13 cm high) covered with organdy cloth and held in the laboratory at 23°C. The total number of fruit was reported as the mean ± SEM of the replicates. The weight of fruit in controls was reported as the mean ± SEM of the replicates. Olive fruit fly pupae and adults and parasitoid adults that emerged from fruit collected from cages and near releases were counted for ≥56 d from the beginning of the exposure period. Temperature and humidity data were reported as the daily mean ± SEM inside and outside of three replicate cages. Mean daily temperature and humidity were compared inside and outside of cages with a two-tailed paired t-test, α level at 0.05, and both inside and outside temperature and humidity were compared among locations using a one-way analysis of variance (ANOVA) and Tukey's test (GraphPad Software 2007). Percentage females and the total number of olive fruit fly adults captured per day were reported as the mean ± SEM of four replicate traps and compared among the locations using a one-way ANOVA and Tukey's test (GraphPad Software 2007).

Parasitoid-induced mortality of olive fruit fly in the field cage and release tests was calculated by 1 − the number of all life stages that emerged from fruit in each test divided by the expected number of all life stages to emerge. The latter value was based on the number of all life stages that emerged per fruit in the controls. Percentage parasitoid induced mortality was reported as the mean ± SEM of the replicates.

Calculation of Parasitism. The number of olive fruit fly larvae and pupae that emerged in controls for 4 d after the fruit was collected was reported as the mean ± SEM third instars and third instars per fruit. The number of third instars per fruit was multiplied times the number of fruit in each test replicate to estimate the number of third instars exposed to the parasitoids and reported as the mean ± SEM. Percentage parasitism was calculated by dividing the number of parasitoid adults that emerged in the exposed fruit by the estimated number of third instars and reported as the mean ± SEM of the replicates.

Larval Susceptibility to Parasitism. Olives were exposed to olive fruit fly adults for oviposition for 1 d to obtain 8- or 10-d-old larvae and for 2 d to obtain 11- to 13-d-old larvae. After exposure to oviposition, three replicates of 42, 36, and 39 olives were held at 23°C for 8, 10, and 11 d, respectively. Each replicate of postinfested fruit was placed in an aluminum frame cage (30.5 cm wide by 30.5 cm long by 30.5 cm high; model 1450B; BioQuip Products, Rancho Dominguez, CA) with polyethylene screening. Forty parasitoids were placed for 4 d in each cage. One cage each of 8- or 10-d postinfested fruit and one cage of 11- to 13-d postinfested fruit were used for nonexposed controls.

The number of olive fruit fly pupae and adults per fruit that emerged in the nonexposed controls was reported as the combined mean ± SEM of 8- and 10-d postinfested fruit and the mean of 11- to 13-d postinfested fruit. This value was multiplied times the number of fruit in each replicate to estimate the number of larvae that were exposed to the parasitoids and reported as the mean ± SEM of the replicates. Percentage parasitism was calculated by dividing the number of parasitoid adults that emerged from the exposed fruit by the estimated number of larvae, reported as the mean ± SEM of the replicates. The results for the exposed 8- and 10-d postinfested fruit and respective nonexposed controls were combined, and reported as values for 8–10 d. Percentage parasitism was arcsine transformed and compared between 8- to 10- and 11- to 13-d postinfested fruit with a two-tailed unpaired *t*-test (GraphPad Software 2007).

Parasitoid Survival in Laboratory Tests. Cages were constructed with cylindrical paper cartons (9.5 cm diameter by 10 cm high; model HD16; Solo Cup, Urbana, IL). A plastic cup lid (6.5 cm diameter) was glued to the side of the carton and used as a platform when the cage was placed on its side. A hole (1.3 cm diameter) was cut into the top of the cage near the opening. The neck of an inverted glass vial (1.6 diameter by 6 cm tall) was filled with deionized water or 5% sucrose in deionized water, plugged with cotton, and placed through the hole in tests in which liquid was provided. Parasitoids (22–60) were placed in each cage, and the opening was covered with organdy fabric held in place with the lid collar. Replicate cages (4–15) were prepared with parasitoids that were provided with honey for food on the fabric cover and no water, water, or 5% sucrose in water. The cages were placed in laboratory environmental chambers (model E32560; Lab-Line, Melrose Park, IL), and parasitoid survival was evaluated every 1–5 d for the following constant temperature and relative humidity combinations, 5°C, 85%; 15°C, 65%; 25°C, 25%; and 35°C, 25%, until all adults had died. Results were reported as the mean ± SEM number of days adults were observed alive in each replicate cage. Survival was compared with or without water or 5% sucrose in water using a one-way ANOVA and Tukey's test (GraphPad Software 2007).

Parasitoid Survival in Greenhouse Tests. Temperature and relative humidity were maintained in a partitioned glass greenhouse (5.6 m wide by 9.3 m long) with swamp coolers and heaters and monitored with a hygrothermograph (model CT485; Omega Engineering, Stamford, CT).

Cages were sewn from polyethylene screen (35 cm wide by 35 cm long by 56 cm high). An opening (12 cm wide by 12 cm high) was cut into one side of the cage for access and covered with a flap (30 cm wide by 30 cm high). The cage was suspended inside a polyvinyl chloride pipe frame (2.7 cm diameter; 42 cm wide by 42 cm long by 65 cm high).

The greenhouse was maintained to simulate a coastal climate on the cooler side and an inland valley climate on the warmer side and reported as the daily mean ± SEM of the diurnal and nocturnal temperatures and relative humidities. Newly eclosed parasitoid adults (35–58) were placed in a cage, and their survival tested with either no food and no water or honey for food and water. Deionized water was provided through a cellulose sponge (5 cm wide by 7 cm long by 2 cm deep) placed through the lid of a plastic bowl (11.5 cm diameter by 4 cm deep). Six replicate cages were used on each side of the greenhouse. Survival in each cage was determined every 1–4 d until all adults had died. Results were reported as the mean ± SEM number of days adults were observed alive in each replicate cage. Parasitoid survival with and without food and water and between the cool and warm side of the greenhouse was compared with a two-tailed paired *t*-test (GraphPad Software 2007).

Seedhead Fly as a Host. Whole plants of yellow star thistle, *Centaurea solstitialis* L., with floral buds (fruit) infested with the larval stage of a tephritid seedhead fly, *Chaetorellia succinea* (Costa), were collected from fields in Hercules, CA, on 10 October 2004. Infested bouquets of floral buds with 25-cm stem lengths were cut at random from different plants. The bouquets were placed in 250-ml Erlenmeyer flasks filled with deionized water in aluminum frame cages (30.5 cm wide by 30.5 cm long by 30.5 cm high; model 1450B; BioQuip Products) with polyethylene screening.

Fifty females and one to three male parasitoids were placed in each cage and held in a room at a daily 24.0 ± 1.5°C and 63 ± 3% RH (mean ± SEM) and a photoperiod of 12:12 (L:D) h. The infested yellow star thistle was exposed to the parasitoids for 2 d in three replicate cages and for 7 d in six replicate cages in no-choice tests. At the end of the exposure period, the bouquets were removed from each cage, and all parasitoids were removed. The bouquet was placed in a new cage and observed every 1–3 d for 67–75 and 56–67 d in tests with 2- and 7-d exposures, respectively. The number of seedhead fly adults and parasitoids that emerged from the bouquets was counted. The floral buds were removed from the stems, counted, and dissected, and all life stages of the seedhead fly and parasitoids were counted.

A control to estimate the number of seedhead fly larvae in the yellow star thistle buds at the time of exposure to the parasitoids in no-choice tests was made by collecting five replicates of a random sample of yellow star thistle floral buds (50–51) from all har-

Table 1. Mean ± SEM trap captures of olive fruit fly adults, temperatures, and relative humidities over 4–6 d with parasitoid cages in olive trees with fruit infested with olive fruit fly in 2002

Location	Trap captures[a]		Cage daily temperature (°C)		Cage daily %RH	
	Percent females	No. per day	Interior	Exterior	Interior	Exterior
San Jose[b]	44.1 ± 3.0ab	27.2 ± 3.4a	15.2 ± 0.1a	15.0 ± 0.2a	78.1 ± 2.6	73.8 ± 0.8a
Santa Barbara[c]	42.5 ± 5.5ab	7.6 ± 0.6b	15.3 ± 0.5a	14.8 ± 0.3a	84.2 ± 1.7	85.4 ± 0.8b
San Diego[d]	47.1 ± 1.3a	36.6 ± 2.7a	16.6 ± 0.3ab	17.0 ± 0.2b	70.0 ± 0.3	66.3 ± 0.4c
Grapevine[e]	30.9 ± 3.4b	10.0 ± 2.0b	16.9 ± 0.2b	17.0 ± 0.1b	38.8 ± 2.0	35.8 ± 2.1d

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's test; GraphPad Software 2007).
[a] Four replicates.
[b] 18–23 Oct.
[c] 9–15 Oct.
[d] 2–7 Oct.
[e] 30 Sept. to 4 Oct.

vested plants. The floral buds were dissected and inspected for the larval stages of the seedhead fly. The length of each larva was measured and determined as first, second, and third instars according to lengths that ranged from <2.5, 2.5–3.5, and 4.0–5.0 mm, respectively. Each instar was reported as the percentage of all larvae that were dissected from the floral buds in the control. The total number of larvae were counted and reported as the mean ± SEM per floral bud. The number of seedhead fly larvae exposed to the parasitoids was calculated by multiplying the number of larvae per floral bud in the control times the number of fruit in each test replicate and reported as the mean ± SEM.

Female parasitoid behavior in the presence of seedhead fly larvae in the host was studied by dissecting five green, open floral buds with yellow or dried petals, which indicated larval feeding, and counting the number of third instars in each bud. Based on the number of third instars per bud, four bouquets of infested floral buds (61–74) were each placed in a separate cage with mated female parasitoids (46–56) at the ratio of 15 per 20 floral buds. The females were observed for searching or probing behavior on the infested floral buds for ≈1 h two to four times each day between 0700 and 1500 hours for 2.5 d. The percentage of live females per cage (4) that showed searching or probing behavior was reported as the mean ± SEM for all observation periods (9).

Walnut Husk Fly Compared with Olive Fruit Fly as a Host. The susceptibility of walnut husk fly to parasitism was determined in a laboratory no-choice test and a comparative choice test between walnut husk fly and olive fruit fly. English walnuts, *Juglans regia* L., infested with walnut husk fly larvae were collected from trees in Fresno and Hanford, CA, and placed in aluminum frame cages. In the no-choice test, 20 infested walnuts were placed in each of three replicate cages with 30 female and 15 male parasitoids per cage for 7 d. Twenty walnuts were used for nonexposed controls and placed over sand in a plastic container to collect emerging third instars as described by Yokoyama et al. (1992). To evaluate mortality caused by exposure to parasitoids, the total number of walnut husk fly pupae that emerged in the control and the total number of walnut husk fly pupae and parasitoid adults that emerged in each no-choice test was compared for significance by a two-tailed paired *t*-test (GraphPad Software 2007) and reported as the mean (±SEM) of the replicates.

In choice tests, 16 walnuts infested with walnut husk fly larvae and 32 olive fruit infested with olive fruit fly second to third instars in the laboratory were placed in each of three replicate cages with 11–12 females and 6 male parasitoids for 7 d. Sixteen infested walnuts and 32 infested olive fruit were used for nonexposed controls and placed in plastic containers to collect the emerging larvae. These larvae were used to determine the relative density of the host in the fruit as described below.

The no-choice and choice tests were held at 24.0 ± 1.5°C and 63 ± 3% RH (mean ± SEM) and a photoperiod of 12:12 (L:D) h. The total number of pupae that emerged in controls 26–27 d after the start of each test was counted in walnuts in no-choice tests and in walnuts and olive fruit in choice tests. This number was divided by the number of fruit in each control to calculate the number of larvae per fruit and multiplied by the number of fruit in each no-choice and choice test replicate to estimate the number of larvae that were exposed to the parasitoid and reported as the mean ± SEM of the replicates.

The number of adult parasitoids that emerged from fruit in no-choice and choice tests was counted 43–44 d after the beginning of the test when parasitoids ceased to emerge. The number of parasitoids was divided by the estimated number of larvae in each test to calculate percentage parasitism and reported as the mean ± SEM of the replicates. Percentage parasitism between walnut husk fly larvae in walnuts and olive fruit fly larvae in olive fruit was compared with a two-tailed unpaired *t*-test (GraphPad Software 2007).

Results

Shipment of Parasitoids. Mortality of *P.* cf. *concolor* adults imported from MOSCAMED, Guatemala City, Guatemala, was ≈1% on arrival in Fresno, CA, after 2 d of transport by air and ground. The parasitoids were 3 d old when collected and packaged in Guatemala, 5 d old when they were received, and ≥7 d old when used in laboratory and field tests. The maximum life span of

Table 2. Mean ± SEM no. of fruit, estimated olive fruit fly third instars exposed 4–6 d to parasitoids, and percentage parasitism of olive fruit fly third instars in four locations in California in 2002

Location	Test	No. parasitoids released	No. fruit	No. third instars	Percent parasitism
San Jose	Control	0	572.7 ± 6.5	129.0 ± 16.6	
	Cage	60	370.8 ± 54.1	83.4 ± 12.2	24.1 ± 5.2
	Release	2,000	489.4 ± 22.5	110.1 ± 5.0	1.6 ± 0.5
Santa Barbara	Control	0	689.0 ± 30.4	90.7 ± 0.9	
	Cage	50	497.8 ± 75.0	65.7 ± 9.9	59.7 ± 9.4
	Release	450	548.0 ± 58.2	72.3 ± 7.7	10.6 ± 0.3
San Diego	Control	0	301.3 ± 5.2	267.7 ± 43.4	
	Cage	50	397.8 ± 67.1	352.9 ± 59.6	13.1 ± 2.1
	Release	1,500	630.4 ± 40.0	559.2 ± 35.52	4.1 ± 0.8
Grapevine	Control	0	196.0 ± 23.1	3.3 ± 0.9	
	Cage	30	289.5 ± 25.2	5.2 ± 0.5	7.0 ± 4.5
	Release	500	410.7 ± 21.0	7.4 ± 0.4	0

the parasitoids that were maintained in sleeve cages was 69.3 ± 6.7 (mean ± SEM) from the day the shipment was received.

Evaluation of Parasitism in Test Locations. The mean percentage of females of olive fruit fly adults collected in yellow panel traps was <50% for all locations and significantly different ($F = 3.77$; df = 3,12; $P = 0.041$) among the locations (Table 1). Trap captures ranged from a mean of 7.6 in Santa Barbara to 36.6 adults per day in San Diego and were significantly different ($F = 33.5$; df = 3,12; $P < 0.0001$) among the locations. Mean ± SEM fruit size measured by weight in controls was 2.02 ± 0.01, 1.26 ± 0.04, 1.77 ± 0.02, and 2.94 ± 0.18 g in San Jose, Santa Barbara, San Diego, and Grapevine, respectively. Mean temperatures inside cages ranged from 15.2°C in San Jose to 16.9°C in Grapevine and outside of cages ranged from 14.8°C in Santa Barbara to 17.0°C in San Diego and Grapevine. Mean relative humidity inside cages ranged from 38.8% in Grapevine to 84.2% in Santa Barbara and outside of cages ranged from 35.8% in Grapevine to 85.4% in Santa Barbara. Temperatures inside and outside of cages were not significantly different in each location, but the relative humidity was significantly higher inside ($t = 8.38$, df = 2, $P = 0.014$) than outside the cage in San Diego. Temperatures on the outside of cages were significantly different ($F = 36.35$; df = 3,8; $P < 0.0001$) among locations. The San Diego and Grapevine location temperatures were significantly higher ($P < 0.001$) than the Santa Barbara and San Jose locations. The mean relative humidity on the outside of the cage was significantly different ($F = 426.4$; df = 3,7; $P < 0.0001$) among the locations.

The mean ± SEM number of third instars per fruit in controls for cage and release tests were 0.2 ± 0.0 in San Jose, 0.1 ± 0.0 in Santa Barbara, 0.9 ± 0.1 in San Diego, and 0.02 ± 0.01 in Grapevine. Based on controls, the mean number of third instars in cage tests ranged from 5.2 in Grapevine to 352.9 in San Diego and in release tests ranged from 7.4 in Grapevine to 559.2 in San Diego (Table 2). Mean percentage parasitism in cages ranged from 7.0 in Grapevine to 59.7 in Santa Barbara and in releases ranged from 0 in Grapevine to 10.6 in Santa Barbara. Mean percentage parasitism was highest in cages versus releases in all sites.

Mean percentage of parasitoid-induced mortality of olive fruit fly larvae after exposure to parasitoids in cages ranged from 20.2 in Santa Barbara to 55.6 in San Diego and in releases ranged from 0 in Santa Barbara and Grapevine to 42.0 in San Diego (Table 3).

Larval Susceptibility to Parasitism. The mean ± SEM number of 8- to 10-d-old and 11- to 13-d-old olive fruit fly pupae and adults per fruit that emerged in controls was 0.9 ± 0.4 (two cages of 36–42 fruit) and 1.1 (one cage of 39 fruit), respectively. Percentage parasitism was significantly higher ($t = 2.946$, df = 7, $P = 0.0215$) in olive fruit fly larvae that were 11–13 d old than in larvae 8–10 d old (Table 4).

Parasitoid Survival in Laboratory Tests. Survival of adult parasitoids at different temperatures and humidities in laboratory environmental chambers with food and no water, with water, or with a 5% sugar water solution are shown in Table 5. Adults lived significantly longer when provided with water than adults

Table 3. Mean ± SEM percentage parasitoid induced mortality of all olive fruit fly larvae after 4- to 6-d exposure to *P.* cf. *concolor* in four locations in California

Location	Test	Expected no. adults[a]	Percent mortality
San Jose	Cage	404.2 ± 59.0	38.9 ± 9.6
	Release	533.4 ± 24.5	1.1 ± 0.8
Santa Barbara	Cage	348.5 ± 52.5	20.2 ± 9.2
	Release	383.6 ± 40.7	0
San Diego	Cage	823.5 ± 139.0	55.6 ± 6.6
	Release	1,304.9 ± 82.9	42.0 ± 7.7
Grapevine	Cage	37.6 ± 3.3	22.2 ± 13.4
	Release	53.4 ± 2.7	0

[a] Number of fruit collected in each test multiplied by the no. of all stages of olive fruit fly per fruit collected in controls.

Table 4. Mean ± SEM percentage parasitism of olive fruit fly larvae exposed 4 d to *P.* cf. *concolor*

Larval age (d)	No. parasitoids	No. fruit	No. larvae	Percent parasitism
8–10	40	39 ± 1.3	35.1 ± 1.2	24.4 ± 7.2a
11–13	40	39.0 ± 0.0	42.9 ± 0.0	73.7 ± 13.4b

Means within a column followed by the same letter are not significantly different ($P > 0.05$, *t*-test; GraphPad Software 2007).

Table 5. Mean ± SEM survival of *P.* cf. *concolor* adults at constant temperature and humidity in laboratory environmental chambers with or without water or a sugar water solution and with food (honey)

Temperature (°C)	Percent relative humidity	Survival (d)		
		No water + food[a]	Water + food[b]	5% sugar water + food[c]
5	85	7.2 ± 0.2a	15.1 ± 1.3b	9.8 ± 0.2ab
15	65	4.0 ± 0.0a	47.6 ± 5.0b	32.5 ± 3.8ab
25	25	1.0 ± 0.0a	22.6 ± 2.1b	13.8 ± 1.5b
35	25	0.0 ± 0.0a	11.7 ± 1.4b	3.0 ± 0.0a

Means within a row followed by the same letter are not significantly different ($P > 0.05$, Tukey's test; GraphPad Software 2007).

[a] Four replicates of 22–34 adults.

[b] Fifteen replicates of 22–40 adults.

[c] Four replicates of 32–60 adults.

without water at 5°C and 85% RH ($F = 6.98$; df = 2,20; $P = 0.0050$, Tukey's test, $P < 0.01$); 15°C and 65% RH ($F = 11.41$; df = 2, 20; $P = 0.0005$, Tukey's test, $P < 0.001$); 25°C and 25% RH ($F = 16.53$; df = 2, 20; $P = 0.0001$, Tukey's test, $P < 0.001$); and 35°C and 25% RH ($F = 12.86$; df = 2, 20; $P = 0.0003$, Tukey's test, $P < 0.001$). Survival of adults provided with a sugar solution was longer than those without water but not significantly so at 5 and 15°C. At 25°C, survival of adults provisioned with either water or a sugar solution was similar, but at 35°C, adults receiving the sugar solution lived only 3 d longer that adults without water. Adults that received no water lived for 7 d at 5°C and 85% RH, and the length of survival decreased with an increase in temperature and corresponding decrease in humidity. Adult parasitoids lived the longest at 15°C and 65% RH when provided with food and water (48 d) or food and sugar solution (32 d).

Parasitoid Survival in Greenhouse Tests. In the cooler side of the greenhouse, the daily mean ± SEM diurnal temperature and humidity were 26.5 ± 0.2°C and 62.7 ± 0.9%, and the daily mean nocturnal temperature and humidity were 24.7 ± 0.2°C and 58.5 ± 1.5%. In the warmer side, the daily mean diurnal temperature and humidity were 36.2 ± 0.3°C and 31.4 ± 1.4%, and the daily mean nocturnal temperature and humidity were 25.6 ± 0.3°C and 47.5 ± 2.8%. Survival of adult parasitoids was ≈4 d on the cool side and 1 d on the warm side of the greenhouse without water and food and was significantly increased ($t = 5.71$, df = 5, $P = 0.0023$) on the cool side and on the warm side ($t = 10.25$, df = 5, $P = 0.0002$) when provided with water and food (Table 6). Survival was significantly higher with ($t = 9.50$, df = 5, $P = 0.0002$) and without ($t = 3.25$, df = 5, $P = 0.0227$) water and food on the cool side versus the warm side of the greenhouse. Survival with food and water at mean daily temperatures of 26 and 32°C in greenhouse tests was similar to survival at constant temperatures of 25 and 35°C in environmental chambers.

Table 6. Mean ± SEM survival of *P.* cf. *concolor* adults in a greenhouse with or without water and food (honey)

Daily temperature (°C)	Daily percent relative humidity	Survival (d)	
		No water, no food[a]	Water + food[a]
25.9 ± 0.2	61.1 ± 1.1	4.3 ± 1.3	21.3 ± 2.2
32.2 ± 0.2	37.4 ± 2.2	≤1.0 ± 0.0	3.5 ± 0.3

[a] Six replicates of 35–58 adults.

Seedhead Fly, Walnut Husk Fly, and Olive Fruit Fly as Hosts. The mean ± SEM number of seedhead fly larvae per floral bud in no-choice test controls was 0.15 ± 0.06. The percentage of first, second, and third instars in the control was 8.1, 62.2, and 29.7%, respectively. The parasitoid did not develop in seedhead fly larvae in yellow star thistle, which was confirmed by dissections (Table 7). Yellow star thistle floral buds that were used to observe female parasitoid searching or probing behavior had a mean of 1.6 ± 0.4 (SEM), and a range of one to three seedhead fly third instars per floral bud. A mean of 1.6 ± 0.6% (SEM) of the females showed searching behavior on the infested floral buds per observation period. The mean percentage of females that searched was highest (5.65%) when the parasitoids were first introduced into the cages with the bouquets. No females were observed to probe or insert the ovipositor into the infested floral buds.

The mean number of walnut husk fly larvae per fruit was 12.5 (20 infested walnut fruit) in the no-choice test control and 23.5 (16 infested walnut fruit) in the choice test control. The mean number of olive fruit fly second and third instars per fruit was 3.7 (32 infested olive fruit) in the choice test control. The parasitoid reproduced in walnut husk fly larvae in walnuts and olive fruit fly larvae in olives (Table 7). Parasitism of walnut husk fly was higher in no-choice tests than in choice tests with olive fruit fly. Parasitism of olive fruit fly larvae in choice tests was not significantly different ($t = 1.36$, df = 4, $P = 0.2450$) than walnut husk fly larvae. The total number of walnut husk fly pupae that emerged in the control was not significantly different ($t = 3.69$, df = 2, $P = 0.0662$) than the mean (±SEM) total number of walnut husk fly pupae and parasitoid adults (286.7 ± 77.7) that emerged in no-choice tests, indicating that no additional host mortality occurred from exposure to parasitoids

Discussion

The parasitoid, *P.* cf. *concolor*, was successfully imported into California from Guatemala City, Guatemala, with a high rate of survival after shipment. On arrival, the adults were long lived under laboratory conditions and easily maintained until used in experiments or released into olive trees infested with olive fruit fly. Four locations in California (Fig. 1) were selected to study field interactions between parasitoids and olive fruit fly. The study areas included San Jose, in the central coast; Santa Barbara, in the southern coast; San Diego, in the southern coast just north of Mexico; and Grapevine, at the southern end of the San Joaquin Valley where olives are grown primarily for canning. These locations represented regional habitats where olive fruit fly occurred as a newly intro-

Table 7. Mean ± SEM percentage parasitism in no-choice tests with seedhead fly in yellow star thistle, walnut husk fly in green walnut husks, and in a choice test with walnut husk fly in walnuts versus olive fruit fly in olives

Cage test	Species	No. fruit	No. larvae	Exposure (d)	Percent parasitism
No-choice	Seedhead fly	141.0 ± 13.0	21.2 ± 2.0	2	0
		270.8 ± 18.7	40.6 ± 2.8	7	0
No-choice	Walnut husk fly	20.0 ± 0.0	250.0 ± 0.0	7	28.4 ± 9.3
Choice	Walnut husk fly	16.0 ± 0.0	376.0 ± 0.0	7	11.5 ± 2.2
	Olive fruit fly	32.0 ± 0.0	118.4 ± 0.0	7	24.2 ± 9.1

duced pest on olives and the effectiveness of the parasitoid could be evaluated.

Numbers of olive fruit fly adults were monitored in the study locations with yellow panel traps that had been used in previous studies (Yokoyama et al. 1992, Yokoyama and Miller 2007) and were found to be more effective in capturing adults than ChamP traps (Yokoyama et al. 2006). Two traps per 2–4 ha are recommended for monitoring olive fruit fly adults, and use of more than one trap in our small test locations provided greater accuracy in determining adult numbers (Johnson et al. 2006). More males than females were captured in these traps in all locations (Table 1), which is similar to previous findings (Yokoyama et al. 2006). The highest number of adults per day were captured in San Diego, and the lowest number was in Santa Barbara, which represents the adult population of olive fruit fly in these locations.

The physical attributes of the cage designed for this study was acceptable to evaluate parasitism of olive fruit fly and did not cause detrimental internal conditions. Temperature and humidity were found to be similar inside and outside of cages in all locations except San Diego, where the humidity was higher inside the cage. The humidity in Grapevine was lower than the other locations, reflecting the arid conditions of the lower San Joaquin Valley.

The method we used to calculate percentage parasitism was similar to those used by Neuenschwander et al. (1983). In our study, olive fruit fly third instars were used to determine the rate of parasitism because development of the parasitoid to the adult stage was more successful in thirds than in earlier instars. Fruit samples were collected for laboratory controls at each test location to determine the number of olive fruit fly larvae in each instar during the period of exposure to parasitoids. The laboratory controls were used instead of field cage controls to eliminate monitoring at distant locations and to allow daily observations of olive fruit fly pupal emergence. Variability in the number of parasitoids in field cage tests and releases resulted from the number of parasitoids shipped and divided among laboratory and greenhouse tests, whereas variability in exposure to olive fruit fly in the field resulted from travel schedules to distant test locations.

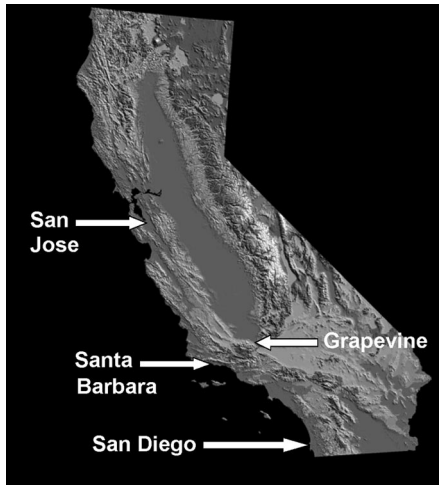

Fig. 1. Parasitoid and olive fruit fly study locations in California.

Tests in Santa Barbara resulted in the highest rate of parasitism of olive fruit fly in cages and releases (Table 2) compared with other locations, although higher numbers of olive fruit fly adults were captured (Table 1), and older larvae were more abundant in San Diego than in Santa Barbara. The lower temperature and higher humidity in Santa Barbara versus San Diego may have enhanced parasitism. These conditions were found by Yokoyama and Miller (2007) and Yokoyama et al. (2006) to support olive fruit fly growth and development. Olive fruit fly larvae were least abundant and rates of parasitism were lowest in Grapevine, where temperatures tended to be higher and the humidity lower than in the other locations. Weather conditions or low host density alone may be related to the small number of parasitoid progeny recovered from fruit infested with olive fruit fly. Based on the results of these tests, biological control of olive fruit fly by the parasitoid would be more effective in cool, coastal climates where the host is abundant.

Olive fruit fly larvae exposed to parasitoids in cage tests in all locations and in the San Diego release test showed high levels of parasitoid induced mortality (Table 3). Mortality was based on the number of all life stages that emerged in the control fruit and in most cases was higher than expected from parasitism of third instars alone (Tables 2 and 4). Yokoyama et al. (2004) attributed this effect to mortality among first and second olive fruit fly instars caused by the parasitoid. This observation was also reported by Calvitti et al. (2002) in olive fruit fly by the egg parasitoid, *Fopius arisanus* (Sonan). He reported that a proportion of dead eggs are always associated with parasitism even if percentage parasitism is low.

A greater number of *P.* cf. *concolor* completed development to the adult stage in olive fruit fly third instars that were 11–13 d old than in younger larvae (Table 4). In a similar study with two cultures of *P. concolor*, Sime et al. (2006) also found the parasitoids to reproduce most successfully in third instars. Canale and Loni (2006) found that *P. concolor* was more successful in locating third instars than second instars of Mediterranean fruit fly in laboratory dishes. Development of *P.* cf. *concolor* in olive fruit fly second instars (Yokoyama et al. 2004) would result in higher larval mortality. Based on our findings, *P.* cf. *concolor* developed more successfully in 11- to 13-d-old olive fruit fly larvae than in 8- to 10-d-old larvae.

In laboratory tests, parasitoid longevity was ≈7 wk at 15°C and 65% RH when food and water were provided (Table 5). These conditions were similar to the test environments of the coastal locations of San Jose and Santa Barbara (Table 1), where parasitism was highest in cage tests and in release tests in Santa Barbara (Table 2).

Parasitoid longevity when provided with food and water was ≈2, 3, and 1.5 wk at 5 (85%), 25 (25%), and 35°C (25% RH), respectively (Table 5). The dry environment of Grapevine (Table 1) may help explain the low level of parasitism in this location (Table 2). Sime et al. (2006) showed *P. concolor* also had higher survival at a constant temperature of 15°C than at higher temperatures when provided with food and water. To ensure accuracy in our study in relation to the different regional climates in California, more replicates were used to determine optimal parasitoid survival in the presence of water and food than without water or with sugar water in the four combinations of temperature and humidity.

The controlled environment in greenhouse tests was more similar to natural conditions than laboratory tests at constant temperature and humidity because the diurnal and noctural temperatures and humidities fluctuated during the day. Parasitoids lived longer in the cool and humid side of the greenhouse than in the warm and dry side (Table 6). The presence of food and water enhanced survival in both greenhouse climates and would help the parasitoid to survive under less than optimum temperature conditions in the field. The maximum length of survival in both laboratory and greenhouse tests was based on the days in which live individuals were last seen in each test because observations were not done on a daily basis. Therefore, actual survival may have been longer than we reported. These studies have shown that biological control of olive fruit fly by *P.* cf. *concolor* would be most effective in regions with mild climates and a source of food and water for adults.

Host specificity is a primary consideration for potential release of parasitoids for olive fruit fly control (Nadel et al. 2005). Before a large-scale release program for *P.* cf. *concolor* could be implemented, the status of a beneficial tephritid, a seedhead fly, in yellow star thistle and a walnut pest, walnut husk fly, were evaluated (Table 7). The results of laboratory no-choice tests showed that the parasitoid did not attack the seedhead fly. This observation is important because yellow star thistle is commonly found near olive groves and orchards. Testing the acceptability of seedhead fly larvae as a host for *P.* cf. *concolor* was needed because Daane et al. (2006) reported that another opiine braconid, *Diachasmimorpha kraussii* (Fullaway), reproduced in *C. succinea.* Our parasitoid developed in walnut husk fly in no-choice tests indicating that the pest could serve as an alternative host. However, in choice tests between walnut husk fly and olive fruit fly, the parasitoid showed a higher rate of parasitism of olive fruit fly, suggesting that olive fruit fly may be a more susceptible host.

Distribution of *P.* cf. *concolor* in California may be limited by climatic conditions, but temperatures and humidities that are suitable for the development of the host (Yokoyama and Miller 2007) are also suitable for the parasitoid. The parasitoid has potential to reduce coastal populations of olive fruit fly that serve as perpetual reservoirs of the pest and where no other method of control is practical or economical. The use of *P.* cf. *concolor* for augmentative or classical biological control may be limited because of the potential impact on endemic tephritids, lack of mass rearing procedures for olive fruit fly for parasitoid production, and establishment has yet to be achieved.

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