

# Biological Control of Olive Fruit Fly (Diptera: Tephritidae) by Releases of *Psytalia* cf. *concolor* (Hymenoptera: Braconidae) in California, Parasitoid Longevity in Presence of the Host, and Host Status of Walnut Husk Fly

Victoria Y. Yokoyama<sup>1</sup>, Pedro A. Rendón<sup>2</sup> & John Sivinski<sup>3</sup>

<sup>1</sup>USDA-ARS, SJVASC, 9611 South Riverbend Ave., Parlier, CA 93648, USA, email: vyokoyama@fresno.ars.usda.gov; <sup>2</sup>USDA-APHIS-PPQ, CPHST, 4a. Avenida 12-62, Zona 10, Guatemala City, Guatemala, email: prendon@aphisguate.com; <sup>3</sup>USDA-ARS, CMAVE, 1700 SW 23 Dr., Gainesville, FL 32604, USA, email: jsivinski@gainesville.usda.ufl.edu

**ABSTRACT:** The larval parasitoid, *Psytalia* cf. *concolor*, collected from tephritids infesting coffee in Kenya and reared on Mediterranean fruit fly, *Ceratitis capitata* Weidemann, in Guatemala by USDA-APHIS, PPQ, was imported into California for biological control of olive fruit fly, *Bactrocera oleae* (Gmelin), in olives, *Olea europaea*. Free releases of the parasitoids were made in olive trees infested with olive fruit fly at a coastal and inland valley location during the fall and early winter of 2005. The relative humidity during the releases was significantly higher at the coastal location. Mean percentage parasitism ranged from 0.5 to 4 and 1.5 to 30 at the coastal and inland valley locations respectively, based on same season recovery of the F<sub>1</sub> generation. One parasitoid was found in infested olives in the next crop of the following year in San Jose. Survival of the parasitoid in the greenhouse in the presence of olive fruit fly infested olives was not significantly different than in the presence of non-infested olives. The greatest number of progeny was produced from female parasitoids that were 12-16 d old. In laboratory tests, a few individuals of the parasitoid successfully completed one life cycle in walnut husk fly, *Rhagoletis completa* Cresson, infested English walnuts, *Juglans regia* L.

**Key Words:** *Bactrocera oleae*, *Olea europaea*, *Rhagoletis completa*

## INTRODUCTION

A larval parasitoid collected in Kenya from Mediterranean fruit fly, *Ceratitis capitata* Weidemann, in coffee was described by Wharton et al. (2000) as *Psytalia* cf. *concolor* (Szépligeti). This parasitoid was originally described as an *Opius* sp. and then briefly considered a synonym of *P. humilis* (Silvestri) (Kamani-Njogu 2001). *P. cf. concolor* differs from the *P. concolor* used in Europe for biological control of olive fruit fly, *Bactrocera oleae* (Gmelin) (Wharton et al. 2005). We reared this parasitoid on Mediterranean fruit fly, *Ceratitis capitata* Weidemann, at the Medfly Parasitoids Rearing Facility La Aurora, Programa La Mosca del Mediterráneo (MOSCAMED), Guatemala City, Guatemala, and imported it into California, U.S.A. to determine its potential for biological control of olive fruit fly (Yokoyama et al. 2004).

Olive fruit fly is a newly introduced species in California (Rice 2000). Large populations of

olive fruit fly occur in the coastal areas, and low numbers can be found in the inland valleys where canning olives, *Olea europaea* L., are produced (Yokoyama et al. 2006). Even though olive fruit fly damage has been mitigated by the warm, arid conditions in the inland valleys, the pest has potential to devastate the canned olive industry in the state which supplies olives to the nation. Quarantine strategies for harvested fruit, and other methods to detect and control the pest including biological control have been investigated by Yokoyama and Miller (2004, 2007), Yokoyama et al. (2004), and Sime et al. (2006).

Biological control of olive fruit fly by *P. cf. concolor* might be accomplished through "classical" release and establishment of the parasitoid. However, factors that affect the effectiveness of the parasitoid need further consideration. To determine levels of parasitism in different climates, we selected a coastal and an inland valley location as representative of the two regions where olives are grown and olive fruit fly is present. We considered parasitoid longevity in the presence or absence of the host, which may af-

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Corresponding author:  
Victoria Y. Yokoyama

fect the ability of the parasitoid to become established on olive fruit fly. We investigated the potential of *P. cf. concolor* to attack the walnut husk fly, *Rhagoletis completa* Cresson, in English walnuts, *Juglans regia* L.

## MATERIALS AND METHODS

**Source of Parasitoids.** Parasitoids, *P. cf. concolor*, were reared from third instars of Mediterranean fruit fly at MOSCAMED, San Miguel Petapa, Guatemala. Parasitoid adults that were 1-5 d-old and of a 50:50 sex ratio were shipped from Guatemala City by air freight in paraffin-coated paper cups as described by Yokoyama et al. (2007). The parasitoids arrived at the USDA-ARS, Parlier, CA, after 3 d enroute. Live adults were placed into wooden sleeve cages and provided with honey for food, and separate vials of water. Dead adults in the cups were counted and reported as the mean  $\pm$  SEM percentage mortality in seven shipments from Oct. through Dec. 2005. The parasitoids were held for 2-d at  $24.0 \pm 1.5^\circ\text{C}$  for observation and mating, then transferred in the laboratory to  $0.03 \text{ m}^3$  (30 liter) screened cages for field releases.

**Study Locations, Traps, Temperature and Relative Humidity Loggers.** Parasitoid release studies were conducted in California from Oct. 2005 to Jan. 2006 at Villages Parkway and San Filipe Road in San Jose (Lat.  $37^\circ 17' \text{ N}$ , Long.  $121^\circ 45' \text{ W}$ ), and Interstate 99 at Grapevine Road (Lat.  $34^\circ 56' \text{ N}$ , Long.  $118^\circ 55' \text{ W}$ ). The San Jose location is near the coast and had extensive plantings of ornamental olive trees. The Grapevine Road site is an isolated location in the inland valley and had a few ornamental olive trees.

Four replicate yellow panel Pherocon® AM traps (Trécé, Adair, OK) each with a clear plastic packet (10.5 cm wide by 10.5 cm high) of 15-20 g of ammonium bicarbonate bait, and a plastic dispenser (1.7 cm wide by 4.8 cm long) containing 80 mg of pheromone

(1, 7-dioxaspiro[5,5]undecane) were used to trap olive fruit fly adults at each location. Two temperature and humidity loggers (Onset Computer Corp., Bourne, Massachusetts) were placed on branches at each location near parasitoid release sites and programmed to record 720 determinations per day, except for 26 Oct. through 14 Nov. 2005 when National Oceanic and Atmospheric Administration Weather Service data for Bakersfield, California (Lat.  $35^\circ 25' \text{ N}$ , Long.  $119^\circ 03' \text{ W}$ ) were reported for Grapevine 55.1 km away.

**Parasitoid Releases.** Parasitoids were released from the screened cages near trees with olive fruit infested with olive fruit fly. The number of parasitoids released in San Jose on 19 Oct.; 2, 7, and 21 Nov.; 5 and 9 Dec. were 2,587; 3,600; 4,630; 3,655; 5,089; 4,683; and, in Grapevine on 26 Oct.; 14 and 28 Nov. were 2,661; 1,482; and 3,450, respectively.

After the first parasitoid release, loggers and traps were collected and replaced, and a sample of olive fruit was collected on each following release date. The samples were collected at random from the canopy of trees in the vicinity where parasitoids were released and consisted of 473-2,195 and 282-478 fruit in San Jose and Grapevine, respectively. The last fruit samples were collected on 23 Jan 2006 in San Jose, and on 14 Dec. 2005 in Grapevine. Each sample of fruit was considered a replicate and three and 1-3 replicates were collected in San Jose and Grapevine, respectively. All materials were returned to the laboratory for evaluation. Percentage females and the total number of olive fruit fly adults captured per day were reported as the mean  $\pm$  SEM of 1-3 traps every 14-35 d for San Jose, and 14-19 d for Grapevine. The total number of adults per day per trap from Oct. through Dec. was compared between San Jose and Grapevine by a two-tailed paired t-test (GraphPad Software 2004). Temperature and humidity data were reported as the daily mean  $\pm$  SEM during 14-35 d and 14-19 d intervals in San Jose and Grapevine, respectively.

Each sample of fruit from parasitoid release sites was 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  $\pm$  SEM of the replicate samples. Parasitoid adults that emerged from olive fruit fly pupae from the fruit were counted for  $\geq 50$  d from the beginning of the exposure period.

Every 1-4 wks from 17 Jul. through 23 Oct. 2006 in San Jose, one to two samples of about 250 to 750 olive fruit per tree were collected at random from four trees where parasitoids had been released in 2005. The fruit that was infested with olive fruit fly was held in cloth covered plastic containers and observed for the emergence of parasitoids.

**Calculation of Parasitism.** Olive trees infested with olive fruit fly in adjacent to each release site were selected for controls, and a sample ranging in number from 1,230-1,837 fruit in San Jose, and 90-299 fruit in Grapevine were collected from these trees after each release date. The fruit were returned to the laboratory, placed in plastic containers, and the number of olive fruit fly larvae and pupae that emerged for 4 d after collection was reported as the number of 3<sup>rd</sup> instars per fruit. The observational period was based on 4 d duration of the 3<sup>rd</sup> instar. This value was multiplied times the total number of fruit collected from each release site to estimate the total number of 3<sup>rd</sup> instars that were exposed to the parasitoids. Percentage parasitism was calculated by dividing the total number of parasitoid adults that emerged by the estimated total number of 3<sup>rd</sup> instars for each release date.

**Parasitoid Survival in the Presence of the Host.** To obtain larvae, olive fruit were exposed to approximately 500 olive fruit adults in a 0.1 m<sup>3</sup> screened cage for 1-3 d, removed from the cage, and held in the laboratory for 6-11 d at 23°C. Thirty-five to 55 fruit infested with olive fruit fly larvae were

placed into each of three 0.1 m<sup>3</sup> screened cages. Approximately 196-214 adult parasitoids that were of an equal sex ratio and 4-5 d-old were placed into each cage. Thirty-five to 55 non-infested fruit were placed into each of three cages with 184-201 parasitoids per cage. Each cage was considered a replicate, and all cages were placed in a greenhouse with a daily mean ( $\pm$  SEM) diurnal temperature and humidity of 26.5  $\pm$  0.2°C and 62.7  $\pm$  0.9%, and a daily mean nocturnal temperature and humidity of 24.7  $\pm$  0.2°C and 58.5  $\pm$  1.5%. Fruit infested with olive fruit fly larvae or non-infested fruit were replaced every 1-3 d until parasitoid mortality was about 85% or 26 d after the start of the test, when all fruit were removed from the cages. Throughout the test, whether infested or non-infested fruit was present, the parasitoids were fed honey and water and dead parasitoids were counted every 1-4 d until all adults had died. The mean number of parasitoids that were dead 1-26 d from the beginning of the test was compared between three replicate cages with fruit infested with olive fruit fly larvae and three replicate cages with non-infested fruit by a two-tailed paired t-test (GraphPad Software 2004). The results were reported as the mean  $\pm$  SEM percentage parasitoid survival in three replicates cages provided with infested versus non-infested fruit 68 d from the beginning of the test.

**Progeny per Female.** Fruit infested with olive fruit fly larvae that had been exposed to parasitoids and removed from cages every 1-3 d in survival tests were placed in plastic containers that were covered with fabric until all larvae had emerged and pupated. The olive fruit fly pupae were placed in petri dishes and held in the laboratory until all parasitoid adults emerged. The number of F1 adult parasitoids that emerged after  $\geq 31$  d was reported as the mean  $\pm$  SEM progeny per female. Progeny per female was based on equal mortality rates between the sexes.

**Host Status of Walnut Husk Fly.** The susceptibility of olive fruit fly and walnut husk fly to parasitism was compared in laboratory no-choice tests. Sixteen olives infested with 12 d-old olive fruit fly larvae were exposed for 7 d to 30 mated females and 7 male parasitoids in a 0.03 m<sup>3</sup> screened cage, and 16 identically infested olives were used for a non-exposed control. Olive fruit was unavailable to replicate the test. Parasitoid sex ratio was based on the addition of a small number of males to ensure that all females were mated.

English walnuts, *Juglans regia* L., with intact green husks were infested according to methods described by Yokoyama and Miller (1992). Four replicates of 6-7 walnuts infested with 6-13 d-old walnut husk fly larvae were exposed for 8 d to 30 mated females and 7-20 male parasitoids in 0.03 m<sup>3</sup> screened cages, and two replicates of 3-4 infested walnuts were used for a non-exposed control. The number of fruit used in the exposure test was reported as the mean  $\pm$  SEM of the replicates.

The cages were held at  $24.0 \pm 1.5^\circ\text{C}$  and  $63 \pm 3\%$  relative humidity ( $\pm$  SEM), and a photoperiod of 12:12 (L:D) h. The number of pupae and adults that emerged per fruit in the controls was reported as the mean  $\pm$  SEM. This value was multiplied by the total number of fruit in each replicate to estimate the number of larvae that were exposed to the parasitoids. Percentage parasitism was calculated by dividing the total number of parasitoid adults that emerged from exposed fruit by the estimated number of larvae in each test and reported as the mean  $\pm$  SEM.

## RESULTS AND DISCUSSION

The parasitoid, *P. cf. concolor*, was shipped 7 times from Guatemala to Parlier, California with mortality during shipment that ranged from 3.2-16.0%, and a mean  $\pm$  SEM of  $7.1 \pm 1.6\%$ . The parasitoids were 4-8 d-old when re-

ceived, 6-10 d-old when released, and 4-10 d-old when used in laboratory or greenhouse tests. *P. cf. concolor* survived well under extended shipping and handling conditions during importation, which is a valuable attribute for its successful use in a biological control program.

During the olive growing season, the weather is cool and humid in San Jose, and hot and arid in Grapevine. The percentage of olive fruit fly females captured with yellow sticky panel traps ranged from 44 to 58 during the parasitoid release period in both locations (Table 1). Olive fruit fly adults were captured daily in San Jose and Grapevine throughout the study period, but the total number of olive fruit fly adults did not significantly differ between the two locations from Oct. through Dec. During the period of parasitoid releases, the highest mean temperature,  $15^\circ\text{C}$ , was recorded in Grapevine, and the highest mean humidities,  $> 82\%$ , were recorded in San Jose. Mean relative humidity in San Jose was significantly higher than in Grapevine throughout the study period. The highest number of adults was collected in San Jose in Oct. which is consistent with the observation that olive fruit fly populations are highest in coastal locations and when fruit is abundant (Yokoyama et al. 2006). Both locations were ideal study locations to test the capacity of *P. cf. concolor* to parasitize olive fruit fly in field releases under different environmental conditions.

The estimated number of olive fruit fly 3<sup>rd</sup> instars in control fruit ranged from 0.12 to 0.21 per fruit in San Jose, and 0.03 to 0.21 per fruit in Grapevine. Based on the controls, the estimated mean number of olive fruit fly 3<sup>rd</sup> instars in fruit samples collected after parasitoids were released in San Jose and Grapevine are shown in Table 2. Mean percentage parasitism determined from the number of *P. cf. concolor* adults that emerged from olive fruit fly 3<sup>rd</sup> instars in the fruit samples ranged from 0.5 to 4% in San Jose and 1.5 to 30% in

**Table 1.** Mean ( $\pm$  SEM) olive fruit fly trap captures, daily temperatures, and humidities in two locations where parasitoids were released in 2005 in California.

Location	Month	Trap captures		Daily temp., °C	Daily % relative humidity
		% Females	No. per day per trap		
San Jose	Oct.	44.3 $\pm$ 4.4	17.2 $\pm$ 8.1	14.2 $\pm$ 0.1	83.5 $\pm$ 0.4
	Nov.	45.8 $\pm$ 5.2	6.2 $\pm$ 3.6	12.7 $\pm$ 0.1	82.1 $\pm$ 0.4
	Dec.	54.8 $\pm$ 22.6	0.9 $\pm$ 0.7	9.3 $\pm$ 0.1	86.0 $\pm$ 0.4
	Jan.	45.7 $\pm$ 6.9	0.5 $\pm$ 0.4	11.5 $\pm$ 0.1	85.3 $\pm$ 0.2
Grapevine	Oct.	50.5 $\pm$ 6.6	1.6 $\pm$ 0.6	15.0 $\pm$ 0.4	60.8 $\pm$ 1.9
	Nov.	49.8 $\pm$ 3.8	6.6 $\pm$ 0.4	15.2 $\pm$ 0.1	41.4 $\pm$ 0.5
	Dec.	58.1	1.9	9.5 $\pm$ 0.1	64.2 $\pm$ 0.6

Grapevine. Rates of parasitism were similar to earlier studies (Yokoyama et al. 2007) and in general relatively low, especially in San Jose. Recapture of the progeny from parasitoid adults that were originally released in the San Jose olive trees may have been affected by the small sample of fruit in proportion to the large olive crop available at the location, a low population of < 1 olive fruit fly 3<sup>rd</sup> instar per fruit, and the increased likelihood of adult dispersal when released in a very large area with many olive trees. The highest rate of parasitism observed in Grapevine is attributed to

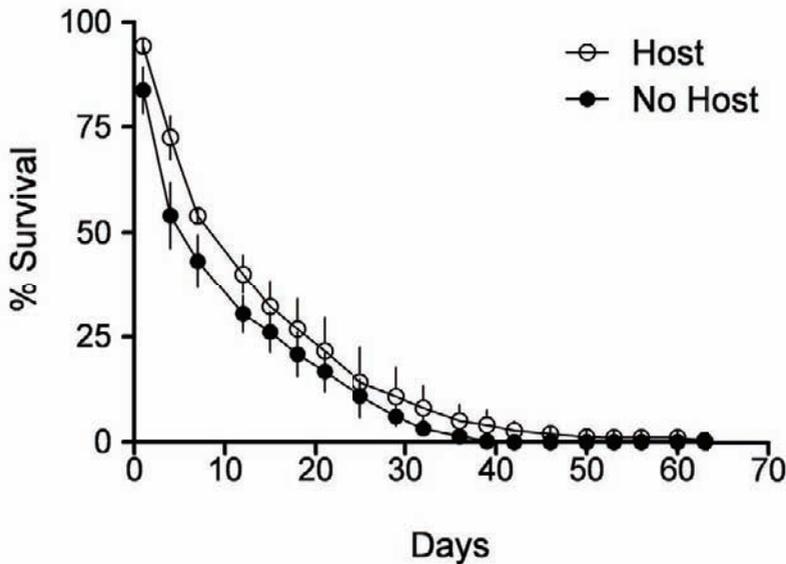
the small amount of fruit on a few trees in the isolated location. The limited number of olive fruit fly 3<sup>rd</sup> instars would be highly susceptible to parasitism because the low number of trees and small crop of fruit would have been inundated with the released parasitoids. One parasitoid adult was found in olives collected on 16 Oct. 2006 in San Jose indicating that a small number of parasitoids had become established on olive fruit fly from releases in the previous year. These results suggest that *P. cf. concolor* may have potential for biological control of olive fruit fly.

**Table 2.** Mean ( $\pm$  SEM) number of fruit, estimated number of olive fruit fly 3<sup>rd</sup> instars, and percentage parasitism by *P. cf. concolor* in 2005 in two locations in California.

Location	Collection date	No. fruit	No. 3 <sup>rd</sup> instars	% Parasitism
San Jose	Nov. 2	1,632.7 $\pm$ 258.1	195.9 $\pm$ 31.0	0.64 $\pm$ 0.42
	Nov. 21	767.0 $\pm$ 96.4	161.1 $\pm$ 20.2	0.54 $\pm$ 0.54
	Dec. 5	1,795.3 $\pm$ 342.8	323.2 $\pm$ 61.7	0.70 $\pm$ 0.33
	Dec. 9	1,336.0 $\pm$ 446.3	187.0 $\pm$ 62.5	3.57 $\pm$ 2.7
Grapevine	Nov. 14	324.0 $\pm$ 0.0	68.04 $\pm$ 0.0	1.47 $\pm$ 1.47
	Nov. 28	283.5 $\pm$ 1.5	8.50 $\pm$ 0.04	29.6 $\pm$ 29.6
	Dec. 14	371	11.13	9.0

The longevity of *P. cf. concolor* adults after importation and exposure to olives infested with 6-11 d olive fruit fly larvae or non-infested olives is shown in Fig. 1. Survival was not significantly different between adults in the presence of infested or non-infested olives ( $t = 0.1114$ ;  $df = 16$ ;  $P = 0.91$ ). Adults survived as long as 67 d in the greenhouse in cages

with infested fruit and as long as 43 d in cages with non-infested fruit. Longevity in the presence of the host would be beneficial for successful parasitism. In contrast, Sime et al. (2006) reported that the life span of *P. concolor* significantly declined when hosts were available, suggesting an energetic cost for reproduction.



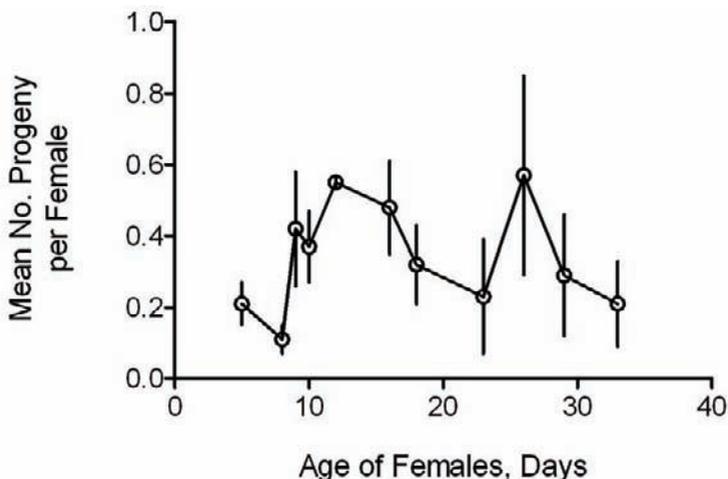
**Fig. 1.** Longevity of *P. cf. concolor* adults exposed to either non-infested or fruit infested with olive fruit fly larvae in greenhouse cage tests.

The mean number of F1 adult parasitoids produced for one month after importation on olive fruit fly larvae in olives is shown in Fig. 2. The greatest number of progeny was produced from parasitoids that were about 12-16 d-old. These observations are similar to Sime et al. (2006) who found that for *P. concolor* progeny production rates were highest during the first 12-14 d.

The number of olive fruit fly and walnut husk fly pupae and adults that emerged per fruit in controls was  $3.3$  and  $5.9 \pm 5.9$  (mean  $\pm$  SEM), respectively. In laboratory tests, a few individuals of *P. cf. concolor* successfully completed one life cycle in walnut husk fly (Table

3). Walnut husk fly is a pest of walnuts and can be an alternative host for the parasitoid in the absence of olive fruit fly. However, walnut husk fly produces only one generation per year (Yokoyama and Miller 1992) and overwinters in the pupal stage in the soil. Therefore, walnut husk fly would not help sustain the parasitoid when olive fruit and olive fruit fly infestations were not available during the winter months.

We conclude that *P. cf. concolor* has potential for use for biological control of olive fruit fly in California based on its ability to adapt to different environmental conditions and reproduce in the host. In this investigation,



**Fig. 2.** Mean number of F1 *P. cf. concolor* adults produced for one month after importation on olive fruit fly larvae in olive fruit.

we found that parasitoid survival is not adversely affected by shipping conditions or interactions with the host, and the parasitoid may also have potential for biological control of walnut husk fly, a pest of walnuts.

**Table 3.** Mean ( $\pm$  SEM) percentage parasitism of walnut husk fly in green walnut husks, and olive fruit fly in olive fruit by *P. cf. concolor* in laboratory cage tests.

Host	No. fruit	No. larvae	% Parasitism
Olive fruit fly	16	52.8	7.6
Walnut husk fly	6.8 $\pm$ 0.2	39.8 $\pm$ 1.5	5.6 $\pm$ 5.6

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