Crop domestication relaxes both top-down and bottom-up effects on a specialist herbivore

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Abstract

Domestication of crop plants selects for numerous traits that often distinguish them dramatically from their wild progenitors. In some cases, these modifications lead to increased herbivory, by enhancing their attractiveness to herbivorous insects or reducing the efficiency of natural enemies, or both. This study investigated the effects of fruit enlargement on the olive (\textit{Olea europaea} L.), the specialist olive fruit fly, \textit{Bactrocera oleae} (Rossi), and its specialized larval parasitoids. Wild olive fruit are small (∼2 mm pulp thickness) and the larval parasitoids associated with \textit{B. oleae} have short ovipositors (∼3 mm), while cultivated fruit are larger (4–8 mm pulp thickness). Female flies allocate more offspring to large than to small fruit within or across different-sized commercial cultivars, without reducing the fitness of their offspring. Fly larvae move deeper into the olive pulp with their increasing age and fruit size. In contrast, the specialist larval parasitoid, \textit{Psyttalia lounsburyi} (Silvestri), more effectively parasitizes hosts in smaller than larger fruit. The inverse relationship between the performance of the fly and its co-evolved parasitoids on fruit of increasing sizes indicates that olive cultivation favors the success of the fly by providing a better food resource and more enemy-free space. These findings offer some explanation for the failure of the decades-old classical biological efforts to manage \textit{B. oleae} using specialized larval parasitoids in the Mediterranean Basin and provide further evidence that crop domestication can alter host–parasitoid interactions.

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### Introduction

In natural ecosystems, a high degree of co-adaptation exists between plants, specialized herbivores, and their natural enemies (Gauld, Gaston, & Janzen 1992; Price et al. 1980). In agroecosystems, however, crop domestication may alter plant morphological, nutritional, and chemical traits (Evans 1993). An inadvertent consequence of plant breeding is that insect herbivore populations may be at higher densities on domesticated crops in agroecosystems than on their wild counterparts in natural ecosystems (Andow 1991). Such changes in insect herbivore densities may be caused by disruption of the bottom-up (plant resources) or top-down (natural enemies) forces that normally regulate insect populations (reviewed by Hunter, Varley, & Gradwell 1997).

Morphological changes are the most visible and dramatic results of plant domestication. In general, the increased amount of food promotes herbivore performance (Price 1991; Stiling & Moon 2005). Cultivation generally selects for larger harvestable plant structures, usually the fruit, potentially disrupting both bottom-up and top-down controls on insect herbivores – of particular interest here is the negative effect on the herbivore’s natural enemies (e.g., Lil & Marquis 2001). Insect herbivores can find protection, or enemy-free space, within protected structures such as fruits and galls (e.g., Leyva, Browning, & Gilstrap 1991; Sisterson & Gould 1999; Stiling & Rossi 1997). Chen and Welter (2007) recently provided evidence that crop domestication created a structural refuge for a native sunflower moth, *Homoeosoma electellum* Hulst, from a specialized larval parasitoid. The moth larvae are more abundant on the enlarged cultivated flower heads than on wild plants, but parasitism is higher on the smaller wild form. In a study on a herbivore that switched from its original wild host (the small hawthorn fruit) to cultivated apples, Feder (1995) showed that the apple maggot fly, *Rhagoletis pomonella* Walsh, gained a structural refuge where its specialized parasitoids are unable to reach a significant proportion of maggots feeding inside apple fruit.

No study to date has simultaneously examined both bottom-up and top-down effects of crop domestication on a herbivorous insect and the consequences for biological control. Here, we address effects of fruit enlargement on the olive, *Olea europaea* L. (Oleaceae), the specialist olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), and its larval parasitoids. The olive-fly–parasitoid system is an ideal model for addressing the ecological consequences of crop domestication on herbivores and their natural enemies, and the resulting effects on pest management. Olives have been cultivated for approximately 6000 years.

The domestic olive, *O. europaea europaea*, probably originated from *O. europaea cuspidata* (Wall ex G. Don) which is a distinct subspecies of wild olive in many ‘Mediterranean type’ climate zones in Africa and south-central Asia (Zohary & Hopf 2000). The fruit of wild olive are small, compared with most cultivated olives. After domestication in North Africa, the olive was quickly introduced throughout the Mediterranean Basin, and other suitable areas worldwide (Zohary & Hopf 2000). It was brought to California, where this study was conducted, by way of Mexico about 250 years ago (Rice, Phillips, Stewart-Leslie, & Sibbett 2003).

The olive fruit fly has long been a major pest of cultivated olives throughout the Mediterranean Basin (Tzanakakis 2006). It reached California around 1998 (Rice et al. 2003). Recent molecular analysis of *B. oleae* populations suggests it may have evolved in Africa and followed the expansion of olive cultivation into the Mediterranean area and south-central Asia, with the California population recently derived from those in the Mediterranean Basin (Nardi, Carapelli, Dallas, Roderick, & Frati 2005). *B. oleae* lays its eggs just below the surface of the fruit, and larvae tunnel into and feed on pulp; the third instar pupates either inside pulp or drops to the soil (Tzanakakis 2006).
The parasitoids associated with *B. oleae* in its native range attack only the fly’s larvae. Four parasitoids are reported from Africa: *Psyttalia lounsburyi* (Silvestri), *Psyttalia concolor* (Szépligeti), *Utetes africanus* (Silvestri), and *Bracon celer* Szépligeti (all Hymenoptera: Braconidae) (Neuenschwander 1982); and another, *Psyttalia ponerophaga* (Silvestri), occurs in Pakistan and India (Narayanan & Chawla 1962). Of these, *P. lounsburyi*, *P. ponerophaga*, and *U. africanus* appear to be specialized on *B. oleae* (Narayanan & Chawla 1962; Neuenschwander 1982; Wharton & Gilstrap 1983). *P. concolor* was recorded from two other frugivorous tephritid genera (Wharton & Gilstrap 1983), and *B. celer* has been recorded from *Ceratitis* species (Copeland, White, Okumu, Machera, & Wharton 2004). These parasitoid species oviposit into larvae and the offspring complete development in the host puparium (Daane et al. 2008; Sime et al. 2006, 2007), except *B. celer* which oviposits and develops ectoparasitically on the mature third instar (Sime et al. 2006).

Efforts to manage the olive fruit fly in southern Europe using classical biological control began in the early 20th century. As a testament to the astuteness of early proponents of classical biological control of the fruit fly, Latiere (1917) hypothesized the potential failure of African parasitoids to successfully establish on *B. oleae* in fleshier European cultivars based on the short ovipositors of parasitoids foraging in thin-fleshed, wild African olives. Although his prediction has been borne out – no parasitoids have successfully established in fleshier European olives – the mechanism of their failure has not been explained. Here, we provide an experimental test of Latiere’s hypothesis. We examined how variation in cultivated olive fruit size affects the performance of *B. oleae* and the efficiency of associated larval parasitoids using *P. lounsburyi* as an experimental model. Specifically, we (1) compared fruit sizes of wild olives and several common cultivars; (2) measured the ovipositor lengths of *B. oleae* parasitoids; (3) measured immature *B. oleae* depth in fruit pulp of various fruit sizes; (4) examined the fly’s ovipositional preference over a range of fruit sizes (within and across different cultivars) and the consequences of fruit size preference to measures of offspring fitness, and (5) determined the effects of fruit size on the performance of *P. lounsburyi*.

Materials and methods

Insects

Laboratory colonies of *B. oleae* and *P. lounsburyi* were established in a climate-controlled room (25±2 ºC, 16L:8D, 40–60% RH) at the University of California’s Kearney Agricultural Research and Extension Center (KREC) in Parlier, California, USA. Fly colonies were maintained on olive fruit following procedures described in Sime et al. (2007). The *P. lounsburyi* was derived from about 200 female and 200 male wasps imported in July 2006 from the USDA-ARS European Biological Control Laboratory in Montferrier, France. The original USDA-ARS colony was started from parasitized *B. oleae* collected in wild olives in the Burguret Forest, Kenya, from 2002 to 2005. The colony was initially maintained on *B. oleae* in olives, but was reared on *C. capitata* cultured on artificial diet for approximately 12 generations before arrival in California. At KREC, the colony was maintained on *B. oleae* feeding in olives following procedures described in Daane et al. (2008). Wasps used in experiments were from stock reared on *B. oleae* for at least one generation. The females were chosen randomly from holding cages where they had been housed with males for about 6d.

Olive fruit size

The size and pulp thicknesses of green and black olives were measured for wild olives, and for four major commercial cultivars grown in California. Wild olives grow throughout a large part of South Africa, and fruit collected for our studies were from wild olives in typical native habitats in three areas of the Western Cape Province: Ceres, Boshof, and Stellenbosch. In California’s Central Valley, four of the major commercial olive varieties (Ascolano, Manzanillo, Mission, and Sevillano) were available in a single olive orchard at KREC, each subjected to the same fertilization and watering treatments. Pulp thickness (i.e., distance from the epidermis to pit) was either averaged from three needle-probe measurements per fruit or calculated from measurements of fruit and seed width with calipers (to nearest 0.1 mm). Measurements were always taken from the mid-point along the longitudinal axis of a fruit, and were made on 20–50 fruit of each type or cultivar. In addition, the number of oviposition marks on each fruit was recorded for two different-sized wild olive types.

Parasitoid ovipositor length

Ovipositors were dissected from the abdomens of wasps reared from *B. oleae*, held fully extended, and the length of the first valvifers (tip to base) was measured under a stereo-microscope with an ocular micrometer. Twenty wasps were measured for each of five species, with the exception of 16 individuals for *U. africanus*.

Immature fly distribution within olive pulp

Infested olives were collected from the major olive growing areas in California and dissected to measure the
depth of immature stages in the pulp. To measure feeding depth across a wide range of fruit sizes, both small (Mission) and large (Sevillano) cultivars were sampled. Fruit samples were taken in July and August, 2003 and 2004, and included all stages (of ripeness) of Mission and Sevillano olives suitable for fly development. Sevillano is among the largest olives, while Mission represents the small- to medium-sized olives grown in California. Additionally, Sevillano fruit collected in December 2004 and January 2005 were used to rear \textit{B. oleae} in the laboratory to provide data from largest fruit.

Prior to dissection, the length of each fruit was measured with calipers, as length is a good predictor of pulp thickness over the entire life of the fruit (Burrack 2007). The fruit was then dissected under a stereomicroscope by lifting the flap of skin covering the egg and cutting away the pulp with a scalpel to locate the individual. The shortest distance from the olive epidermis to the egg, larva, or pupa was measured by marking the distance on a probe and reading it with calipers to the nearest 0.1 mm. In total, 1198 individuals were measured. The three instars were identified by the shape and color of the mandibular stylets (cephalopharyngeal skeleton). The first instar could be further categorized as young or late by the color of the stylets, which are brown on young first instars and partly black on older first instars. The relationship between fly depth and fruit size was analyzed using linear regression for each developmental stage of the fly in each cultivar.

Fly oviposition preference and offspring fitness

Choice tests were conducted to determine ovipositional preference and offspring fitness for different-sized Sevillano fruit and across the four cultivars (Ascolano, Manzanillo, Mission, and Sevillano), each of different fruit sizes. Observations were conducted in clear acrylic screen cages (15 cm × 15 cm × 20 cm). Fruit for the tests were collected on the same day from the KREC olive orchard, using green fruit on which the fly’s oviposition mark is easily visible. Fruit size in Sevillano, the variety with the largest fruit, was variable enough to create three different-sized groups of fruit collected on the same date. Thus, this cultivar was chosen to test the within-cultivar response by the fly to different-sized fruit. All fruit for this test were collected from a single tree.

In the first test, three different-sized Sevillano fruit were randomly arranged 2 cm apart in a Petri dish (8.5 cm diameter) elevated 5 cm above the cage floor on a vial (50 ml) in the middle of the cage. A female fly was held in a 50 ml vial for 1 h, and then placed in the cage to freely leave the vial. Once she exited the vial, she was observed continuously and the number of eggs laid on each fruit was recorded until she left the experimental arena (the interior of the Petri dish) for more than 2 min. Egg-laying behavior is characterized by the fly undergoing a motionless period while depositing a single egg (Girolami, Vianello, Strazpazzon, Ragazzi, & Veronese 1981). Each fly visited all fruit within 1 h and typically started switching frequently among fruit before exiting the experimental arena. In total, 31 females were observed. All observations were conducted between 10:00 and 16:00 h. Following each observation, fruit were individually held in plastic containers (100 ml) and checked daily to determine the developmental time and sex of emerging offspring. Survival rate was calculated from the number of emerged flies and the number of oviposition scars counted on each fruit. The head width of both sexes was measured with an ocular micrometer from samples of freshly killed individual flies reared from different-sized fruit.

The procedure for the second test across different cultivars was similar to the first, but the fly was offered one fruit of each of the four commercial cultivars. Observations were conducted on 30 females. Four of these observations were excluded from the analysis because the flies left the experimental arenas without visiting all four fruit.

To distinguish whether fruit size alone, or some other factor associated with cultivar, influences egg density per fruit, the fly’s oviposition preference was compared not only based on the number of eggs deposited per fruit, but also per cm$^2$ of surface area. The surface area (SA) of the ellipsoid (often egg-shaped) olive fruit with semi-length \( L \) (cm) and semi-width \( W \) (cm) was calculated using the formula: \( SA = 2 \pi \frac{W^2}{2} + 2 \pi \times L \times W \) arccos\((E)/E\), where \( E = (1 - W^2/L^2)^{1/2} \) (the angular eccentricity of the ellipse).

Parasitoid performance on different-sized fruit

Two choice tests were conducted to determine the relationship between fruit size and \textit{B. oleae}’s oviposition preference and \textit{P. lounsburyi}’s parasitism success: first, on different-sized fruit within one cultivar (Sevillano) and, second, across four cultivars (Ascolano, Manzanillo, Mission, and Sevillano). Simultaneously, the effect of host density on parasitoid performance was tested for different-sized fruit using two host density categories: first, a ‘variable host distribution’ where tested fruit were exposed to female flies to freely lay eggs and, second, a ‘uniform host distribution’ where host density was manipulated to be as similar as possible across fruit sizes. Fruit for the tests were also collected from the KREC olive orchard, using the same methods as described in the fly’s ovipositional preference tests above.

To create a variable host density for the parasitoid, the three different-sized Sevillano fruit were exposed to a
single female fly for 2 h in clear acrylic screen cages (15 cm \times 15 cm \times 20 cm). Host density on different-sized fruit was estimated by the easily visible oviposition marks on the green fruit; the actual host density was determined from rearing after the experiments. To obtain a uniform host density, Sevillano fruit in each size category were exposed to female flies for 1–2 h, examined, and those with three to four oviposition marks per fruit were selected. Similar methods, as described above, were used to create variable and uniform host densities on fruit from the four different cultivars. Through these methods, we created four categories: different-sized Sevillano fruit with variable and uniform host densities, and different-sized cultivars with variable and uniform host densities. Because larval–pupal parasitoids of \textit{B. oleae} prefer to attack second to third instars (Daane et al. 2008; Sime et al. 2006, 2007), the addition of \textit{P. lounsburyi} to each fruit size and host density category was made 8–10 d after infestation, when larvae were in second to young third instars. Observations were conducted in acrylic screen cages (30 cm \times 30 cm \times 30 cm), provisioned with a freshly cut olive branch bouquet to stimulate parasitoid host searching behavior, and water and honey. Two female wasps were released into the cage 1 d before each test for an acclimation period. On the second day, one infested olive of each of the three different sizes of Sevillano fruit was randomly distributed in a 15-cm-diameter Petri dish placed on a 10 cm high container in the middle of the cage. The fruit were exposed to the wasps for 36 h. There were 33 and 29 replicates for variable and uniform host densities, respectively.

The parasitoid’s response to the four cultivars of different-sized fruit was tested in a similar manner with the following exceptions: four female wasps were released into the cage and were offered two infested fruit of each of the four cultivars for 24 h. There were 16 and 30 replicates for variable and uniform host densities, respectively.

Following exposure to \textit{P. lounsburyi}, fruit were individually isolated until flies or wasps emerged, which were recorded. Finally, all exposed fruit were dissected to calculate fly survival and overall parasitism rates. Dead puparia were reconstituted in water for 1–2 d and were dissected under a stereo-microscope to determine presence or absence of immature parasitoid cadavers and pharate adults. Percentage parasitism was estimated based on eclosed wasps, flies, and dissection of dead puparia.

**Data analysis**

Results are presented as means per treatment (± S.E.). Proportional data were arcsine square-root transformed and data on fly depth were log transformed \((\log(x + 1))\) as needed to stabilize the variance. Data analyses, including linear regression, one-way and two-way ANOVA for comparisons of means, and Tukey’s HSD test for multiple comparisons of mean values were performed using JMP (V 6.0.3, SAS Institute, Cary, NC).

**Results**

**Olive sizes**

Average fruit pulp in wild olives in Africa ranged from 1.1 to 1.9 mm, while the commercial olives in California were substantially larger, with pulp thickness ranging from 4.1 to 8.4 mm (Table 1). The four Californian-grown cultivars ranged greatly in pulp thickness in the following order: Sevillano > Ascolano > Manzanillo > Mission (Table 1).

**Parasitoid ovipositor length**

The five larval parasitoids naturally associated with \textit{B. oleae} have average ovipositor lengths ranging from 0.9 to 2.9 mm, with four distinctly different lengths \((F_{5,91} = 101.7, P < 0.001)\) (Table 2). Among them, the specialists \textit{U. africanus}, \textit{P. lounsburyi}, and \textit{P. ponerophaga} have ovipositors <2 mm, which corresponds to the pulp thickness of wild olives (Table 1). The more polyphagous \textit{P. concolor} and \textit{B. celer} have significantly longer ovipositors.

**Immature fly distribution in fruit pulp**

Eggs were found <1 mm below the fruit surface, regardless of fruit size within or among cultivars (Fig. 1A; Table 3). The mobile larval stage, however,
tunneled deeper with larval age, and with increasing fruit size both within and among cultivars (fly stage: \( F_{5,1288} = 345, P < 0.001 \); cultivar: \( F_{1,1288} = 150, P < 0.001 \); fly stage \times\) cultivar: \( F_{5,72} = 40.9, P < 0.001 \) (Table 3). Young first instars were found mainly \(< 2\) mm from the surface (Fig. 1B) and their depth was not related to fruit size. Older first instars (Fig. 1C) and second instars (Fig. 1D), however, were found deeper in fruit pulp than young first instars and their depth increased with fruit size of both Sevillano and Mission. Third instars (Fig. 1E) were found deeper in the pulp of Sevillano than Mission olives, but no significant relationship was found between depth and fruit size within each cultivar. Pupation occurred mostly 2–3 mm below the fruit surface (Fig. 1F), and there was no difference in different-sized fruit within or among different cultivars (Table 3).

### Table 2. Ovipositor length (means±S.E.) for five species of larval B. oleae parasitoids

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>n</th>
<th>Ovipositor length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utetes africanus</td>
<td>16</td>
<td>0.94±0.01 a</td>
</tr>
<tr>
<td>Psyttalia lounsburyi</td>
<td>20</td>
<td>1.79±0.04 b</td>
</tr>
<tr>
<td>Psyttalia ponerophaga</td>
<td>20</td>
<td>1.85±0.04 b</td>
</tr>
<tr>
<td>Psyttalia concolor</td>
<td>20</td>
<td>2.46±0.09 c</td>
</tr>
<tr>
<td>Bracon celer</td>
<td>20</td>
<td>2.88±0.10 d</td>
</tr>
</tbody>
</table>

Means followed by different letters are significantly different (Tukey HSD test, \( P < 0.05 \)).

### Table 3. Depth (means±S.E.) of various B. oleae immature developmental stages within fruit pulp of Mission and Sevillano

<table>
<thead>
<tr>
<th>Fly stage</th>
<th>Depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mission</td>
</tr>
<tr>
<td>Egg</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td>0.50±0.01 a</td>
</tr>
<tr>
<td>Young first instar</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>1.06±0.06 b</td>
</tr>
<tr>
<td>Later first instar</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>2.47±0.16 c</td>
</tr>
<tr>
<td>Second instar</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>2.91±0.19 c</td>
</tr>
<tr>
<td>Third instar</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>2.80±0.16 c</td>
</tr>
<tr>
<td>Puparium</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>2.32±0.09 c</td>
</tr>
</tbody>
</table>

Means were compared for different fly stages within the same cultivar, or for the same fly stage between two cultivars, and different letters within each row or column indicate significant differences (Tukey HSD test, \( P < 0.05 \)).

Fig. 1. Distributions of B. oleae (A) eggs, (B) young first instars, (C) later first instars, (D) second instars, (E) third instars, and (F) pupation sites within the pulp of different-sized olive cultivars. The relationship between B. oleae depth (\( y \)) and fruit pulp thickness (\( x \)) was not significant for eggs, young first instars, third instars, and pupae, regardless of olive cultivars (all \( P > 0.05 \)), but was significant for later first instars (Mission: \( y = -1.957 + 1.993x, r^2 = 0.331, P < 0.001, df = 58 \); Sevillano: \( y = -0.371 + 1.398x, r^2 = 0.320, P < 0.001, df = 57 \)) and second instars (Mission: \( y = -1.618 + 2.014x, r^2 = 0.237, P < 0.001, df = 44 \); Sevillano: \( y = -0.632 + 1.380x, r^2 = 0.105, P < 0.05, df = 86 \)).
Adult fly preference and offspring fitness

The sizes (length in cm) among small (1.73 ± 0.02), medium (2.41 ± 0.03), and large (3.14 ± 0.03) Sevillano fruit were significantly different ($F_{2,87} = 653$, $n = 30$, $P < 0.001$). B. oleae laid more eggs per fruit on the large than either the small- or medium-sized fruit ($F_{2,90} = 17.0$, $P < 0.001$) (Fig. 2A); there was no difference per cm² of fruit surface ($F_{2,90} = 0.4$, $P = 0.683$) (Fig. 2B). Male and female offspring developed at similar rates across Sevillano fruit sizes (fruit size: $F_{2,340} = 1.5$, $P = 0.200$; sex: $F_{1,340} = 2.9$, $P = 0.087$; fruit × sex: $F_{2,340} = 0.1$, $P = 0.886$) (Table 4). Offspring size was not affected by fruit size, but females were larger than males (fruit size: $F_{2,74} = 1.9$, $P = 0.160$; sex: $F_{1,74} = 217$, $P < 0.001$; fruit × sex: $F_{2,74} = 0.6$, $P = 0.549$) (Table 4). There was no difference in percentages of female offspring emerged from Sevillano fruit categories of small (59.2 ± 4.8%), medium (51.5 ± 5.5%) and large (50.1 ± 4.9%) ($F_{3,76} = 1.1$, $P = 0.358$), or percentages of all offspring that survived in different fruit size categories (small: 82.2 ± 5.8%; medium: 86.5 ± 6.5%; large: 84.5 ± 3.9%) ($F_{3,76} = 1.1$, $P = 0.358$).

Across cultivars, B. oleae laid more eggs per fruit in larger than smaller cultivars ($F_{3,100} = 15.1$, $P < 0.001$) (Fig. 3A). However, there was no difference in the number of eggs per cm² of fruit surface, with the exception that fewer eggs were on Mission than Manzanillo ($F_{3,100} = 4.7$, $P = 0.004$) (Fig. 3B). Offspring developmental rate was influenced by cultivar (cultivar: $F_{3,114} = 17.9$, $P < 0.001$; sex: $F_{1,114} = 0.3$, $P = 0.590$; cultivar × sex: $F_{3,114} = 0.9$, $P = 0.433$) (Table 4). Offspring size was also influenced by cultivar and females were larger than males (cultivar: $F_{3,80} = 12.2$, $P < 0.001$; sex: $F_{1,80} = 20.8$, $P < 0.001$; cultivar × sex: $F_{2,80} = 0.02$, $P = 0.985$) (Table 4).

### Table 4. Developmental time and adult size (mean ± S.E.) of B. oleae reared from three different-sized Sevillano fruit or olives of different cultivars

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Developmental time (days)</th>
<th>Adult size (head width, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Sevillano</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>23.1 ± 0.15 (56) a</td>
<td>23.4 ± 0.11 (72) a</td>
</tr>
<tr>
<td>Medium</td>
<td>23.5 ± 0.22 (44) a</td>
<td>23.7 ± 0.16 (49) a</td>
</tr>
<tr>
<td>Large</td>
<td>23.4 ± 0.14 (58) a</td>
<td>23.6 ± 0.16 (67) a</td>
</tr>
<tr>
<td>Cultivars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mission</td>
<td>26.4 ± 0.59 (5) a</td>
<td>27.3 ± 0.58 (7) a</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>25.9 ± 0.41 (13) ab</td>
<td>25.3 ± 0.42 (15) a</td>
</tr>
<tr>
<td>Ascolano</td>
<td>24.9 ± 0.31 (22) bc</td>
<td>24.1 ± 0.33 (19) ab</td>
</tr>
<tr>
<td>Sevillano</td>
<td>23.6 ± 0.41 (21) c</td>
<td>23.2 ± 0.42 (20) b</td>
</tr>
</tbody>
</table>

Data from three different-sized Sevillano fruit and among different cultivars were separately compared. Values followed by different letters within each column are significantly different (Tukey HSD test, $P < 0.05$). Figures in the parentheses represent number of replicates. Data from male flies reared from Mission were excluded from the analysis due to small sample size.
female flies developed faster and attained a larger size when in the largest cultivar. Percentage of female offspring was not significantly different among flies that emerged from Mission (51.8 ± 7.9%), Manzanillo (43.3 ± 7.4%), Ascolano (55.3 ± 7.4%), or Sevillano (44.8 ± 7.4%) (F3,75 = 0.7, P = 0.549). Percentage of survival was lowest from Mission (50.0 ± 9.8%) (F3,87 = 8.8, P < 0.001), but not significantly different among Manzanillo (70.8 ± 9.5%), Ascolano (81.9 ± 4.2%), and Sevillano (78.9 ± 5.2%).

Among 50 of either large or small type of wild olive fruit from collections in Stellenbosch, South Africa (see Table 1), 25 contained only one oviposition mark, while the rest had no oviposition mark, suggesting that B. oleae lays only one egg on wild olive fruit.

**Parasitoid preference and performance**

Within the Sevillano cultivar, host density per fruit was higher in the larger fruit size categories in the variable treatment (F2,96 = 23.6, P < 0.001) (Fig. 4A), while host density in the uniform treatment was similar across fruit size categories (F2,84 = 1.9, P = 0.156) (Fig. 4B). However, parasitism of B. oleae by P. lounsburyi was higher on small fruit than on medium or large fruit in both variable (F2,96 = 40.4, P < 0.001) and uniform host distributions (F2,84 = 69.5, P < 0.001) (Fig. 4A and B). Larvae in medium and large fruit were equally parasitized in both host distributions.

Similarly, host density increased with fruit size under variable host distribution (F3,60 = 27.8, P < 0.001) (Fig. 5A), and, despite our attempt to equalize eggs numbers per fruit, was higher on the largest cultivar under uniform host distribution (F3,116 = 7.3, P < 0.001) (Fig. 5B). However, parasitism of B. oleae by P. lounsburyi was higher on the two smaller cultivars, regardless of host density or distribution. Notably, very few host larvae were parasitized in the two larger cultivars (Fig. 5A and B).
increases the susceptibility of fruit to maggots feeding in wild olives. This study demonstrates that enlargement of cultivated olive fruit results in lower parasitism by a co-evolved parasitoid due to improved enemy-free space compared to small fruit. Our results suggest that the preference for larger fruit within a particular cultivar is mainly a result of increased surface area, which may be a cue used as part of an innate ‘spacing’ ability that no doubt evolved to prevent overexploitation of finite fruit resources. Across cultivars, however, Manzanillo is more attractive than Mission beyond the level predicted by relative fruit sizes. This suggests that other cues such as chemical stimuli may be involved in ovipositional preference (Dominici et al. 1986; Scarpati, Loscalzo, & Vita 1993), as cultivars clearly differ in susceptibility to a degree that is not entirely related to size (Dominici et al. 1986).

Although B. oleae offspring performance was not significantly influenced by fruit size within the Sevillano cultivar in our study, larger cultivars enhanced developmental rate, survival rate, and body size, suggesting that selection of large cultivars promotes B. oleae population increase. This result also suggests that in addition to differing in fruit size, olive cultivars differ in nutritional properties that affect the fly’s fitness (Dominici et al. 1986; Scarpati et al. 1993). Although it did not take into account other traits that might directly affect the fly’s behavior or performance, this study nonetheless provides strong evidence for the importance of fruit size in fly performance.

Reduced parasitism rates of B. oleae in larger fruit appear to result from deeper host tunneling by the larvae in larger fruit and, consequently, reduced accessibility to its larval parasitoids. The first and second instars appear to be driven by an instinct to tunnel deeper if given the opportunity, as shown by the relationship between tunnel depth and fruit size (Fig. 1). This tendency is not as apparent in the third instar, because the data were confounded by the need of mature third instars to return to the surface to exit and pupate inside. However, despite the variability introduced by this pre-pupation behavior, the average larval depth tended to increase with each successive instar, including the third, as revealed in Sevillano olives (Table 3), whose pulp is thick enough to allow choice of feeding depth. When parasitoid oviposition length is taken into account, B. oleae larvae should be accessible to its associated parasitoids in wild olives at all stages of development, but a large proportion of

Discussion

In natural environments, B. oleae populations are presumably limited by co-evolved parasitoids and the poorer quality of small fruit. Our results suggest that the larger fruit size of cultivated olives provides a better host resource for B. oleae maggots to burrow deeper into the pulp, resulting in lower parasitism by a co-evolved parasitoid due to improved enemy-free space compared with maggots feeding in wild olives. This study demonstrates that enlargement of cultivated olive fruit increases the susceptibility of fruit to B. oleae, yet reduces the efficiency of its specialized larval parasitoid, P. lounsburyi.

Ovipositional preference by B. oleae for larger fruit has been recorded both within cultivars (Dominici, Pucci, & Montanari 1986; Neuenschwander, Michelakis, Holloway, & Berchtold 1985) and among cultivars both in Europe (Neuenschwander et al. 1985) and California (Burrack 2007). In those studies, fruit weight was the principal determinant of oviposition preference compared with other fruit parameters examined (Tukey HSD test, $P < 0.05$).
the larval population is inaccessible in cultivated olives. The vulnerable stages are mostly early first instars, plus some third instars that may be vulnerable as they prepare for pupation. As hypothesized, the effectiveness of *P. lounsburyi* is inversely correlated with fruit size (Figs. 4 and 5). A similar pattern was noted in *P. ponerophaga*, an Asian specialist that possesses an ovipositor similar in length to *P. lounsburyi* (Sime et al. 2007).

Deeper tunneling into fruit as larvae age is also well documented in other tephritid flies (e.g., Feder 1995). This behavior seems to be tied to a taxonomically widespread innate preference for frugivorous tephritids for large fruits, where the larvae are presumably at reduced risk of attack by parasitoids with short ovipositors (Feder 1995). Such structural refuges exist to some extent in natural systems and play an important role in sustaining multi-trophic interactions by preventing over-exploitation of hosts or prey by natural enemies (Dyer & Gentry 1999; Hawkins, Thomas, & Hochberg 1993). Enlargement of fruit through cultivation enhances this kind of enemy-free space by reducing parasitoid access, especially for those parasitoids that prefer to attack older instars that are deeply situated, as shown here for the *B. oleae* system. Although there is a low (but existing, see Table 1) variation in pulp thickness of wild olives, *B. oleae* still has the innate tendency to tunnel deeper and this became advantageous when olives were bred to be bigger.

Specialized tritrophic interactions presumably evolve through preference by natural enemies for host plants with traits that increase their success in exploiting herbivores (Hare 1992; Vet & Dicke 1992). Herbivores may escape specialized natural enemies by exploiting novel habitats. Among the Tephritidae and other herbivores with burrowing larvae, escape is especially likely to take the form of reduced accessibility to natural enemies. For example, Feder (1995) provides convincing evidence for a switch by a population of *R. pomonella* from the small native fruit of hawthorn to the introduced apple in North America, with subsequent reduction of parasitism by native parasitoids when feeding in apples. The spread of *R. pomonella* in apples was enhanced due to its evasion of various larval parasitoids possessing short ovipositors. A number of studies have shown that the success of parasitoid attack on concealed feeders depends greatly on the length of the parasitoid’s ovipositor relative to the depth of hosts feeding in plant organs (Feder 1995; Leyva et al. 1991; Sivinski & Aluja 2003). Considering the tight co-evolutionary linkage between *B. oleae*, its natural host, and its larval parasitoids, it is not surprising that its parasitoids possess short ovipositors. In contrast, generalist frugivorous fruit flies are typically exploited by parasitoids whose guilds are often regulated by a combination of interspecific differences in ovipositor length and preferences for specific sizes of host fruits (Rossi et al. 2006; Sivinski & Aluja 2003; Sivinski, Vultee, & Aluja 2001). The Mexican fruit fly, *Anastrepha ludens* (Loew), for example, which uses a range of fruit sizes, is attacked by diverse parasitoids with a broad range of ovipositor lengths (Sivinski et al. 2001). Although diverse parasitoids can potentially attack hosts in smaller fruits, those with longer ovipositors tend to be more common in hosts in larger fruits (Leyva et al. 1991; Lopez, Aluja, & Sivinski 1999).

To our knowledge, this study provides the first empirical support for Latiere’s (1917) hypothesis that biological control of *B. oleae* in Europe may be hampered by the short ovipositors of parasitoids reared from wild olives in Africa. Considerable efforts over nearly a century to introduce *P. concolor* from Africa to southern Europe for biological control of *B. oleae* have not proved successful (Wharton 1989). Some support for our work can also be found in the pattern of parasitoid distribution observed in South Africa. There, *P. lounsburyi* and *U. africanus* are the most abundant parasitoid species in wild olives, and parasitism by both species is significantly higher in wild olives than unsprayed commercial olive orchards (Walton et al., unpublished data). *Bracon celer* is consistently reported as the most abundant in commercial olives, attaining parasitism rates of over 80% and providing substantial control of *B. oleae* in orchards (Annecke & Moran 1982; Neuenschwander 1982). As our results indicate, the ovipositor of *B. celer* is nearly 2 mm longer than that of *U. africanus*, and over 1 mm longer than that of *P. lounsburyi*, which gives it an advantage in commercial olives. It is not clear, however, why *B. celer* is not also dominant in wild olives.

The impacts of crop domestication on trophic dynamics have received little attention (Chen & Welter 2007). This study provides an example where alteration of plant morphological traits through human selection has modified the relative strengths of bottom-up and top-down forces on a herbivore. As a result, trophic dynamics are expected to differ markedly between natural and agricultural systems. This finding offers some insights to challenges faced by biologically based pest management efforts in agricultural ecosystems. However, differences in tritrophic dynamics between agro-ecosystems and natural ecosystems are not based solely on crop morphology (Dyer & Gentry 1999). A better understanding of the dynamics under natural conditions should provide further invaluable clues to the processes that prevail in agricultural systems.

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