CHAPTER 11

Biological Control of Olive Fruit Fly in California

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NON-TECHNICAL SUMMARY

The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), was first detected in 1998 in southern California. It was soon found in all olive-growing regions in California, becoming the most destructive olive pest in this region. Control relies on frequent insecticide applications, most commonly bait formulations that target adult flies. Pest control is hampered by the large number of unmanaged olive trees that can act as sources of flies moving into treated commercial orchards. To develop better sustainable olive fruit fly management, researchers in California conducted the largest modern exploration for natural enemies across the fly's native range, imported to quarantine laboratories, evaluated potentially suitable parasitoids, and developed information needed for USDA-APHIS permits for release of selected species. Two wasp species (*Psyttalia humilis* and *Psyttalia lounsburyi*, both Hymenoptera: Braconidae) were approved for field release. For *P. humilis*, 360,240 (Kenyan strain) and 42,591 (Namibian strain) were released in seven coastal and eight interior valley counties from 2006 to 2013. Although *P. humilis* showed initial promise in quarantine studies, permanent field establishment was not detected. For *P. lounsburyi*, 22,391 (South African strain) and 64,026 (Kenyan strain) were released in 12 coastal and four interior valley counties from 2006 to 2017. *Psyttalia lounsburyi* has permanently established and expanded its range along the coast, with the highest levels of fly larval parasitism reaching 39.9–73.5% within a few years of the initial release. However, *P. lounsburyi* has not yet been recovered in interior valley counties, and its densities in coastal areas appear to be falling. At present, it does not appear that *P. lounsburyi* will significantly suppress olive fruit fly populations, especially in the important agricultural regions in the interior valleys. Continued biological control efforts for olive fruit fly may seek parasitic wasp species or strains that have biological traits better suited to California's interior valley.

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HISTORY OF INVASION, NATURE OF PROBLEM

The Species Invasion

The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) is monophagous; its larvae feed exclusively in olives, both wild *Olea europaea* subsp. *cuspidata* and cultivated *O. europaea* subsp. *europaea* (Daane and Johnson, 2010). *Bactrocera oleae* likely originated in sub-Saharan Africa, where the wild olive is widespread and from which the domesticated olive was derived over 5,000 years ago (Zohary, 1994). Molecular analyses suggest that the fly followed olive cultivation, with an initial northward expansion from sub-Saharan Africa into North Africa and the Mediterranean Basin, and then a westward expansion through Europe and eventually to North America (Nardi et al., 2005, 2010). Population structure and genetic analyses suggest that the origin of olive fruit fly in California is most likely from the region encompassing Cyprus, Israel, and the neighboring coast of Turkey (Nardi et al., 2005; Zygouridis et al., 2009; Nardi et al., 2010).

In California, *B. oleae* was first detected in October 1998 in West Los Angeles (CDFA, 1998). Statewide surveys suggest the fly spread rapidly, and by 2002 it was reported in almost all Californian olive-growing regions (CDFA, 2002). Its widespread geographic expansion in California, along with abundant ornamental olive trees, made a statewide eradication program unfeasible. The fly's widespread detection within such a short period of time may indicate a pre-1998 introduction (Rice, 2000). The olive fruit fly has also recently invaded Hawaii (Matsunaga et al., 2019).

Nature of the Problem

Olives were introduced into California from Mexico by Franciscan monks in the late 1700s, and the fruit was primarily used for oil before 1900 (Connell, 1994). The table olive industry began in the early 1900s, and this market gradually increased as a percentage of olive production (Ferguson et al., 1994). Currently, about 40% of California's olive production is for table olives, with the total acreage at ~36,000 acres (~14,500 ha), production at ~67,700 tons (~61,400 metric tons), and annual crop value at ~$57.9 million (see www.nass.usda.gov/ca). California olive production accounts only for ~5.8% of the total U.S. olive consumption, suggesting a market potential that is still largely untapped. While olives are grown in other U.S. states (Arizona, Florida, Hawaii, Georgia, and Oregon), California accounts for the vast majority of olive production in the United States.

Seasonal development of olive fruit fly begins with the adults that survive the winter or have emerged from overwintered pupae (Burrack et al., 2011; Yokoyama, 2015). Adult females begin to oviposit when fruit is ripe enough to support larval development, which ranges from May to early June depending on the cultivar and geographic location. Eggs are placed just beneath the fruit surface, and the newly hatched larvae feed and develop in the olive pulp. At 26°C (79°F), the fly can develop from egg to adult in 21 days, resulting in 3–5 generations per year in California (Burrack et al., 2011; Wang et al., 2012; Yokoyama, 2015). During the first fly generation of the fruiting season, larvae usually pupate inside fruit, while in later generations they often drop from fruits into the soil for pupation (Fletcher, 1987; Burrack et al., 2011). All life stages (**Fig. 1**) can be found during the winter if fruit is still present for oviposition and larval development (Burrack et al., 2011; Yokoyama, 2015). A female *B. oleae* can lay 200–500 eggs during her lifetime (Rice, 2000) and can live up to seven months under cool, humid conditions if food and water are available; however, adults live for only a few days when deprived of food and water or temperatures are high, as commonly occurs in California's interior valleys during the summer months (Wang et al., 2009a, 2013). Thus, fly populations are high in cool, humid coastal areas and low in hot, dry areas of the inland valley (Burrack et al., 2008, 2011). Eventually, climate warming could shift olive fruit fly populations northward beyond its current range (Gutierrez et al., 2009). Olive fruit flies are strong fliers and can disperse over 2 km (1.2 mi) in about 2 hours. Adults feed on available carbohydrate sources such as honeydew from black scale, *Saissetia oleae* (Hemiptera: Coccidae) (e.g., Wang et al., 2009b, 2011a).

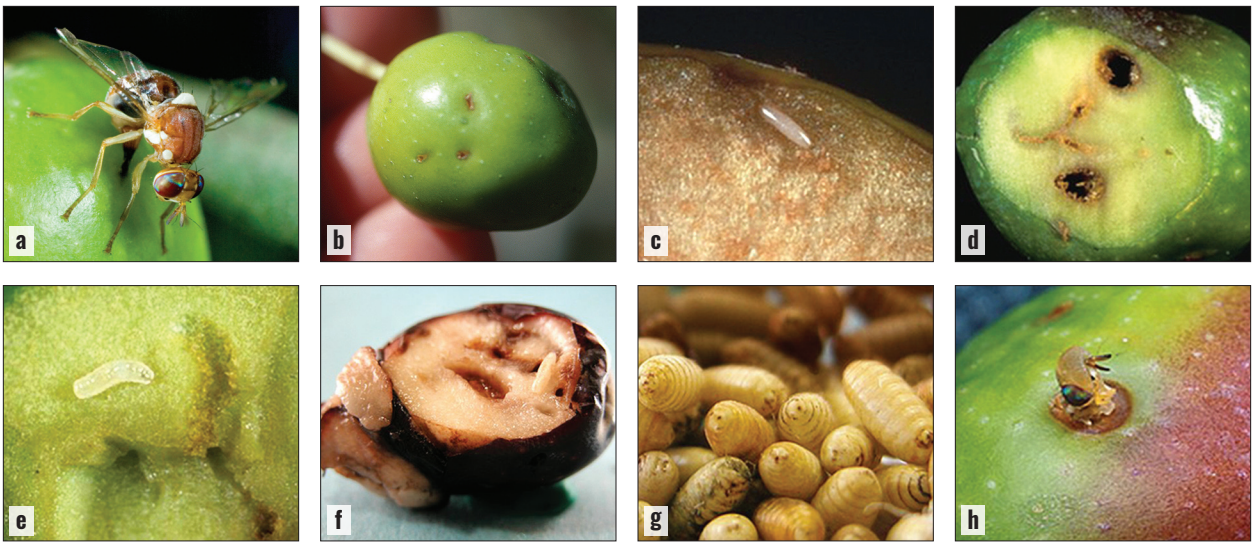

Figure 1. Life cycle of *Bactrocera oleae* and its damage on olives: (a) adult female fly; (b) oviposition stings; (c) egg; (d) feeding tunnel; (e) young larva; (f) mature larva; (g) fly puparia; and (h) adult emerging from fruit. (a–h: Marshall Johnson, UC Riverside)

Before the invasion of *B. oleae*, the major arthropod pests of olive in California were olive scale, *Parlatoria oleae* (Hemiptera: Diaspididae), and black scale. Olive scale is largely controlled by the introduced parasitoids *Aphytis maculicornis* and *Coccophagoides utilis* (both Hymenoptera: Aphelinidae) (Wilson et al., 2022), and black scale is partly controlled by a combination of natural enemies and cultural practices (Daane and Caltagirone, 1989). Therefore, *B. oleae* is now the primary threat to the olive industry. In addition, the olive psyllid, *Euphyllura olivina* (Hemiptera: Liviidae), is a species recently invasive in California with the potential to become an economical pest if it becomes established widely (Hougardy et al., 2020).

WHY CONTROL THIS INVASIVE SPECIES?

Olive crop production can be lowered by *B. oleae* oviposition or feeding on immature fruit, which may be aborted before harvest (Yokoyama, 2015). In mature fruit, *B. oleae* maggots also introduce pathogens that result in fruit deterioration. For the oil market, feeding reduces oil quality and value due to increased acidity (Rice et al., 2003). For table olives, there is a "zero tolerance" such that the presence of any maggots or pupae inside any fruit, or even oviposition marks can lead to the rejection of a grower's entire crop by table olive processors (**Fig. 1**) (Johnson et al., 2006). Rejected table olives may be used for oil production, which has a tolerance level of 10–15% infestation, although maggots in fruit can reduce oil quality and value. In areas of the world where the olive fruit fly is established, it can be responsible for loss of up to 80% of oil value and 100% of the crop for some table olive cultivars (Daane and Johnson, 2010). Non-treated olives can reach 100% infestation in California, and if olive fruit fly populations are not effectively and sustainably managed, the entire olive industry in California could be lost (Johnson et al., 2006, 2011).

Management strategies for *B. oleae* rely on frequent insecticide sprays, most commonly of an insecticidal bait (GF-120 NF Naturalyte Fruit Fly Bait, Dow AgroSciences LLC) (containing 0.02% spinosad) that attracts and kills adult flies (Johnson et al., 2006; Wilson et al., 2022). Other methods for suppressing *B. oleae* may include the use of attract-and-kill traps, particle film sprays (kaolin clay) to reduce adult oviposition, and sanitation of overwintered fruit (Wilson et al., 2022), although these techniques are not easily implemented on larger commercial operations and kaolin residues on the crop can reduce its value. The possibility of using

sterile insect technique has been proposed but has not been developed due to the inability to economically rear millions of *B. oleae* individuals on an artificial diet in California or other regions (Johnson et al., 2006).

California olive growers spend $60–120 per acre ($148–297/ha) for *B. oleae* control, typically spraying the spinosad bait GF-120 once weekly or twice monthly from two weeks before olive pit hardening (early June) until fruit is harvested in the fall (for table olives) or winter (for oil production) (Wilson et al., 2022). This added expense has forced some California farmers out of olive production. Furthermore, repeated applications of GF-120 have led to resistance to spinosad in some areas (Kakani et al., 2010). GF-120 bait can also harm biological control agents (e.g., green lacewing adults and scale parasitoids) if they feed on foliar residues of the bait (Nadel et al., 2008; Wang et al., 2011b). Although studies indicate that several important tephritid fruit fly parasitoids such as *Fopius arisanus*, *Diachasmimorpha tryoni*, *Psyttalia fletcheri*, and *P. humilis* (all Hymenoptera: Braconidae) do not feed directly on fresh GF-120 residues, when the insecticide was directly applied topically to beneficial insects, high mortality resulted (Wang et al., 2005).

THE ECOLOGY OF THE PROBLEM

Insecticide-based management strategies for *B. oleae* are further hampered by movement into commercial orchards of flies from ornamental olive trees and abandoned orchards, which are often not treated (Collier and van Steenwyk, 2003). Insecticides are difficult to apply to ornamental trees in residential and public areas as well as to abandoned or volunteer trees in rough terrain. These unmanaged olives provide the fly with breeding and overwintering sites, resulting in a refuge for reinvasion to nearby commercial crops. Natural enemies, especially self-perpetuating parasitoids, may play a unique role not only because they can attack immature flies in the fruit (where pesticides are less effective), but also can reduce fly densities at the landscape level. Biological control, especially in such refuge areas, could provide a valuable ecosystem service, improve environmental quality, and lower growers' management costs.

Previously, the natural enemies attacking *B. oleae* in California were largely ineffective and consisted mainly of the generalist ectoparasitoid *Pteromalus* nr. *myopitae* (Hymenoptera: Pteromalidae) (Kapaun et al., 2010) and generalist predators such as ants (Orsini et al., 2007). This situation is similar to the Mediterranean Basin where indigenous parasitoids attacking *B. oleae* are generalist ectoparasitoids that do not sufficiently suppress fly populations (Daane and Johnson, 2010). For this reason, classical biological control in Europe has been investigated since the early 1900s. Surveys in the 1910s and later found solitary endoparasitoids, including *Bracon celer*, *Psytallia concolor*, *Psyttalia lounsburyi*, and *Utetes africanus* (all Hymenoptera: Braconidae) in collections from South Africa, Kenya, and Ethiopia (reviewed in Neuenschwander et al., 1982; Daane and Johnson, 2010; Hoelmer et al., 2011). More recent explorations showed the presence of a parasitoid complex that was closely associated with *B. oleae* and collectively contributed to *B. oleae* suppression in sub-Saharan Africa (Wang et al., 2021a). To improve sustainable management for this invasive pest, members of the University of California, California Department of Food and Agriculture, and the USDA-ARS European Biological Control Laboratory initiated a modern classical biological control project for *B. oleae* (Daane and Johnson, 2010; Daane et al., 2011, 2015; Wang et al., 2021a).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The first major attempt to introduce co-evolved parasitoids to suppress *B. oleae* populations dates to the early 1900s with the exploration in Africa for olive fruit fly natural enemies that were later released in Italy (reviewed in Wharton, 1989; Daane and Johnson, 2010). However, this initial work was constrained because imported parasitoids were difficult to rear, resulting in only small numbers being released in Italy without any documented establishment. One species, *P. concolor* obtained from Tunisia, has been repeatedly introduced into southern Europe since the early 1900s, but it has established only in the southernmost parts of Mediterranean Europe and does not provide effective control (Raspi et al., 2007; Miranda et al., 2008).

The invasion of *B. oleae* in California renewed interest in the classical biological control of olive fruit fly and led to the initiation of an importation and evaluation program in 2003 (Daane et al., 2011). Researchers conducted the largest modern exploration for olive fruit fly parasitoids, investigating sites across sub-Saharan Africa (Kenya, Namibia, and South Africa), some adjoining regions with olive fruit fly populations (Canary Islands, Morocco, Réunion Island, and Tunisia), and parts of southwestern Asia (Bon et al., 2015; Wang et al., 2021a). In sub-Saharan regions, four braconids were collected from wild olives (*B. celer*, *P. humilis*, *P. lounsburyi*, and *U. africanus*); *P. humilis* was dominant in hot semi-arid areas of Namibia, *P. lounsburyi* was dominant in more tropical areas of Kenya, and *U. africanus* was most prevalent in Mediterranean climates of South Africa. Mean parasitism levels were 30.1, 41.9, and 21.6% in Kenya, Namibia, and South Africa, respectively (Wang et al., 2021a). Collectively, these co-adapted parasitoids have contributed to maintaining a low fruit infestation rate (generally <15%) in its native range such as South Africa (Wang et al., 2021a). *Psyttalia concolor* was the only species found in the Canary Islands, Morocco, or Tunisia, while *Diachasmimorpha* nr. *fullawayi* was the only species collected from the island of Réunion (Wang et al., 2021a). In addition, *Psyttalia ponerophaga* appeared to be an effective *B. oleae* parasitoid in Pakistan, where the fly infestations are scarce, with parasitism rates of up to ~60% in Punjab Province (Bon et al., 2015). *Psyttalia humilis* is morphologically identical to *P. concolor*, but a later study suggests that all sub-Saharan populations are considered as *P. humilis* (Rugman-Jones et al., 2009), although they have been referred to as *P.* cf. *concolor* or *P. concolor* in some earlier publications (e.g., Rehman et al., 2009; Rugman-Jones et al., 2009).

All of the five major parasitoids (*B. celer, P. humilis*, *P. lounsburyi*, *P. ponerophaga*, and *U. africanus*; **Fig. 2**) were imported and evaluated at the University of California, Berkeley, quarantine facility (Daane et al., 2011). Also evaluated were three other tephritid fruit fly parasitoids, *Fopius arisanus*, *Diachasmimorpha kraussii*, and *Diachasmimorpha longicaudata* (**Fig. 2**), supplied by Dr. Russell Messing at the University of Hawaii (Sime et al., 2006abc, 2007, 2008; Nadel et al., 2009). *Fopius arisanus* is the only parasitoid that oviposits in host eggs, and all others are larval parasitoids. All species emerge as adults from host puparia. Under conditions in quarantine, *D. longicaudata* was considered the most effective. It is a generalist parasitoid of fruit flies that have been introduced from Asia into Hawaii and many other regions for biological control of various tephritids. *Diachasmimorpha kraussii* is an Australian species that was introduced into Hawaii for the control of Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) (Wang et al., 2021b).

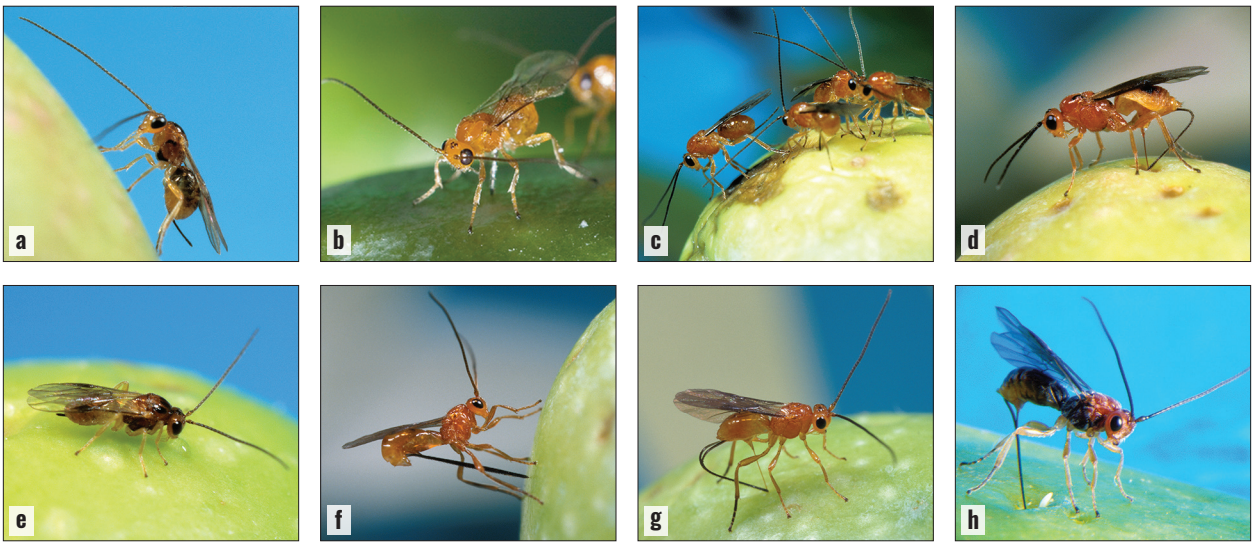

Figure 2. Parasitoids imported into California for quarantine studies include braconid parasitoids reared from wild olive fruit fly: (a) *Psyttalia lounsburyi*; (b) *P. humilis*; (c) *P. ponerophaga*; (d) *Bracon celer*; and (e) *Utetes africanus*, as well as braconid parasitoids reared on other fruit fly species, including (f) *Diachasmimorpha longicaudata*; (g) *D. kraussii*; and (h) *Fopius arisanus*. (a–h: Kent Daane [UC Berkeley] and Marshall Johnson [UC Riverside])

These parasitoids were assessed for their potential to attack several fruit fly species, including the fruit-feeding native black cherry fly (*Rhagoletis fausta*), the flower-head feeding *Chaetorellia succinea* (imported for the control of yellow starthistle, *Centaurea soltitialis*), and the gall-forming *Parafreutreta regalis* (imported for the control of stem galls in Cape ivy, *Delairea odorata*) (Daane et al., 2011). Quarantine evaluations showed that *P. lounsburyi* was the most specialized parasitoid of olive fruit fly (Daane et al., 2008). *Psyttalia ponerophaga*, *P. concolor*, and *P. humilis* were excluded because they could attack the beneficial weed biocontrol agent *P. regalis* (Daane et al., 2011), and *B. celer* could also attack and reproduce on *P. regalis* and probed other host species offered (Nadel et al., 2009). *Utetes africanus* was difficult to rear in either the target or various non-target hosts during the tests, but the literature indicates that it has been reared from several other fruit fly species. *Diachasmimorpha longicaudata* and *D. kraussii* were the most aggressive of the quarantine-screened parasitoids, probing nearly all host species presented and producing offspring from non-target, fruit-infesting species as well as from the beneficial species. These species have been reared from more than 20 fruit fly species (Wang et al., 2021b) and, therefore, they were considered not suitable for introduction. These two species are, however, already established in Hawaii and may, in the future, attack *B. oleae* there. *Fopius arisanus* was not attracted to any of the beneficial non-target species tested, which is consistent with earlier studies that showed that *F. arisanus* only attacks fruit-feeding tephritids such as *C. capitata* in quarantine tests in Hawaii (Wang et al., 2004). Most importantly, *F. arisanus* did not develop well on *B. oleae* (Sime et al., 2008).

In 2006, a USDA-APHIS permit was granted to release *P. lounsburyi* (Daane et al., 2008, 2015) and subsequently for *P. humilis*. *Psyttalia lounsburyi* populations (originating in Kenya or South Africa) and *P. humilis* (originating in Namibia) were mass-reared in France and Israel on *C. capitata* (Chardonnet et al., 2019) that had been reared on an artificial diet. Parasitoid production in 2008, 2009, and 2013–2017 was conducted at the USDA-ARS European Biological Control Laboratory in Montferrier-sur-Lez, France and in 2009–2012 at the Israel Cohen Institute of Biological Control in Bet Dagan, Israel. Adult parasitoids (1–2 weeks old) were shipped via overnight courier to California for field release (led by K. Daane and C. Pickett). All live parasitoids were fed honey and water and released within 1–2 days after their arrival. Similarly, *P. humilis* (originating in Kenya) was mass-reared on *C. capitata* at the USDA-APHIS-PPQ, MOSCAMED Parasitoid Rearing Facility at San Miguel Petapa, Guatemala and shipped to California for release (led by V. Yokoyama). Maintaining large *B. oleae* colonies throughout the season proved difficult. For this reason, the parasitoids were reared on *C. capitata* produced on an artificial diet. Use of *C. capitata* as the rearing host precluded mass-rearing these parasitoids in California, where *C. capitata* is a quarantine pest.

Releases were conducted primarily in ornamental olives or abandoned olive groves that received no insecticide treatments. Releases were not made in commercial olive orchards because they were often treated with GF-120 bait sprays. The non-treated olives at our release sites were often heavily infested by *B. oleae*, making them ideal habitats for field colonization and initial establishment of introduced parasitoids. Pre- and post-release samples were primarily taken in the spring and fall, when olive fruit fly densities were highest (Yokoyama et al., 2008; Daane et al., 2015). Before 2013, most parasitoids released were *P. humilis*, which was easier to rear and gave higher levels of parasitism in both laboratory and pre-release field cage studies than did *P. lounsburyi* (Wang et al., 2009d, 2011a). About 360,240 (approximately 50% females) *P. humilis* (Kenyan strain) parasitoids were released across six coastal counties (Los Angeles, Napa, San Diego, San Luis Obispo, Santa Barbara, and Santa Clara) and six interior valley counties (Glenn, Kern, San Benito, Merced, San Joaquin, and Tulare) from 2006 to 2011 (Yokoyama et al., 2008, 2010, 2011, 2012). Similarly, 42,591 *P. humilis* (Namibian strain) parasitoids were released in five coastal counties (Napa, Sonoma, San Diego, San Luis Obispo, and San Mateo) and two interior counties (Butte and Yolo) from 2008 to 2013 (Daane et al., 2015). Both strains of *P. humilis* (Kenyan and Namibian) were recovered consistently within the same fruit seasons, and the highest levels of parasitism reached 30–40%. However, this parasitoid was not recovered in subsequent years (Daane et al., 2015). Because of its lack of establishment, releases of *P. humilis* ended in 2013.

A total of 22,391 (3,155 males) and 64,026 (9,728 males) of the South African and Kenyan *P. lounsburyi* were released, respectively, in 12 coastal counties (Alameda, Los Angeles, Marin, Mendocino, Napa, San Diego, San Luis Obispo, San Mateo, Santa Barbara, Santa Cruz, Sonoma, and Ventura) and 4 interior counties (Butte, Riverside, Solano, and Yolo), which included 42 coastal and four interior release sites from 2006 to 2017 and a total of 138 releases.

HOW WELL DID IT WORK?

In California's Central Coast region, where releases were first made, *P. lounsburyi* was recovered in 2010 in San Luis Obispo County a year after it was first released, indicating that the parasitoid had successfully overwintered (Daane et al., 2015). Since then, *P. lounsburyi* has been recovered at many Central Coast sites in San Luis Obispo and nearby counties. For example, *P. lounsburyi* was found at 30 out of 31 sampled sites in Santa Barbara, San Luis Obispo, and Ventura Counties in 2015. The highest levels of parasitism were 63.0% in Santa Barbara County, 73.5% in San Luis Obispo County, and 36.9% in Ventura County. Across all sample sites in San Luis Obispo County, mean parasitism increased from 3.3% in 2012 to 32.2% in 2015 (Wang et al., unpub. data).

Further north near the San Francisco Bay Area, *P. lounsburyi* was first recovered in San Mateo County in 2011 after being released there in 2010 (Daane et al., 2015; Wang et al., unpub. data). Since then, the parasitoid has been found at many sites in each subsequent fruiting season, with mean parasitism increasing from 5.2% in 2011 to 12.7% in 2015; recoveries have been made at 23 out of 25 randomly sampled locations in San Mateo, Santa Clara, San Cruz, and San Francisco Counties, with the highest levels of parasitism being 34.6% in San Mateo and 37.3% in Santa Clara Counties. In other northern coastal counties, the parasitoid was recovered in Marin County in 2014 following its first release there in 2013 (Daane et al., 2015). By 2015–2016, *P. lounsburyi* was recovered from most sites surveyed in Marin (0–42.5% parasitism), Alameda (0–47.7% parasitism), Sonoma (2.8% parasitism) and Napa (0.9% parasitism) Counties, although only 1–3 sites were sampled in each region.

In southern California, early releases of *P. lounsburyi* started in 2010 in San Diego, and the parasitoid was first recovered in 2016 (0.1% parasitism) and again in 2017 (0.3% parasitism). *Psyttalia lounsburyi* was released for the first time in Riverside County in 2015 and in Los Angeles County in 2016, but post-release sampling in 2017 did not find this parasitoid established at these release sites. It was found, however, in one sampled non-release site in Los Angeles County (23.3% parasitism) that bordered Ventura County (Daane et al., 2015; Wang et al., unpub. data).

There are some release areas of California where *P. lounsburyi* has not been recovered. North of the San Francisco Bay Area, *P. lounsburyi* has not yet been recovered in Mendocino County, nor has it been recovered from any interior valley counties (Butte, Solano, and Yolo) after multiple years of releases (Daane et al., 2015; Wang et al., unpub. data).

These results demonstrate that *P. lounsburyi* has permanently established in California's coastal regions and may over time expand its range in this mild, temperate region. Surveys in 2018 showed that the overall numbers of recovered wasps and recovery sites were lower when compared to previous years, with highest parasitism being 26.7% in San Luis Obispo County and 29.5% in Alameda County (Wang et al., unpub. data). However, fly larval density was also lower, dropping from 0.43 to 0.25 larvae per fruit between 2010 and 2018. Surprisingly, *P.* nr. *myopitae* was found at most sampled sites, and its parasitism seemed to have increased over the years, with the highest rates of parasitism (42.8 and 50.9%) in August to September in San Luis Obispo and San Mateo Counties, respectively. Several other native parasitoids, including *Eurytoma* sp., and *Eupelmus* sp., were also reared from *B. oleae* (Daane et al., 2015; Wang et al., unpub. data).

In summary, many factors could have affected establishment of *P. humilis* and *P. lounsburyi* in California. The first consideration is likely the climatic adaptability of a species (and strain), as neither *P. humilis* nor *P.*

lounsburyi appears to have a winter diapause, and only *P. lounsburyi* was able to overwinter at any of the sampled sites (Wang et al., 2013). Laboratory studies suggest that *P. lounsburyi* is more cold-tolerant than *P. humilis*, and its low temperature tolerance is a better match with that of *B. oleae* than that of *P. humilis* (Wang et al., 2012; Daane et al., 2013). The coastal regions of California are characterized by year-round mild temperatures, while the interior valleys are characterized by hot, arid summers and cold winters (Wang et al., 2011b, 2013). In addition, the California olive fruit fly population appears to have originated from the Mediterranean Basin, and the long-term separation of the parasitoids released in this program from their host historically might have led to some divergence in thermal performance between the fly and these two African *Psyttalia* species (Wang et al., 2012). *Psyttalia ponerophaga* from Pakistan, as described previously, may be more cold-tolerant than these two tropical species (Daane et al., 2013) and could be an important addition that would complement the activity of *P. lounsburyi*. Second, seasonal host availability is another key factor given that in Africa *B. oleae* larvae are present throughout the year in many regions, which ensures a constant supply of hosts for parasitoids (Wang et al., 2021b). However, in California the phenology of domesticated olives, periodic drought, and other climate extremes may restrict the availability of *B. oleae* larvae to limited seasonal periods. For successful biological control, the introduced parasitoids must be able to find hosts year-round, and yet *B. oleae* larvae are scarce during summer and winter periods in many California olive regions.

A third potential factor is that domesticated olives are much larger than the wild olives present in the fly's native range, allowing fly larvae in commercial fruit to tunnel and feed deeper inside the fruit, thereby providing a refuge from parasitoids with a relatively short ovipositor (Wang et al., 2009d). In other words, co-adapted larval parasitoids (e.g., *P. humilis*, *P. lounsburyi*, *P. ponerophaga* and *U. africanus*) may have ovipositors too short to reach fly maggots feeding deep within the larger olives (Sime et al., 2007; Wang et al., 2009cd). Other larval parasitoids seeking to attack concealed maggots, such as *Anastrepha* spp. larvae in mangoes in Mexico, face the exact same issue with respect to accessing larvae in larger cultivated fruits vs smaller wild species of fruits (e.g., López et al., 1999; Sivinski et al., 2001). Although *D. longicaudata* and *D. kraussii* reproduced well on cultivated olives due to their very long ovipositors (Sime et al., 2006c), their host ranges are too broad to be considered for introduction.

The modern practice of classical biological control strongly emphasizes minimal non-target impacts of introduced agents, which consequently reduces the number of potential agents. Olive domestication may also alter other aligned or inherent tri-trophic relationships (e.g., presence of key chemical cues for parasitoid foraging), potentially disrupting the parasitoids' host-searching and host location success. A final factor potentially affecting this project's results may be interspecific competition among parasitoids. *Pteromalus* nr. *myopitae*, as an ectoparasitoid, may have a competitive advantage over larval endoparasitoids (such as species of *Psyttalia*) (Wang et al., unpub. data).

BENEFITS OF BIOLOGICAL CONTROL OF OLIVE FRUIT FLY

To date, the benefits of this program lie largely in the area of developing the groundwork, rather than having already achieved suppression of the target pest. This project has imported and evaluated several parasitoids of olive fruit fly, of which the three most promising larval parasitoids are *P. lounsburyi*, *P. humilis*, and *P. ponerophaga*. Both *P. humilis* and *P. lounsburyi* were approved and released widely, while *P. ponerophaga* has yet to be permitted for field release. Permanent establishment of *P. humilis* was not achieved, although augmentative field releases of *P. humilis* showed some impact on the pest (e.g., Yokoyama et al., 2010). However, it is unlikely that augmentative releases of *P. humilis* will ever be a commercially viable option due to costs and difficulties of mass-rearing this species. In contrast, *P. lounsburyi* has become permanently established in most coastal olive-growing regions. Genetic analyses of recovered specimens show that the South African strain of *P. lounsburyi* has established more widely than the Kenyan strain (Bon et al., 2017), even though the latter was released in greater numbers.

The permanent establishment of *P. lounsburyi* is a major step in the development of a successful classical biological control program against *B. oleae* in California and marks the first-ever establishment of a specialized *B. oleae* parasitoid worldwide outside of its native range (Daane et al., 2015). At present, it is too early to predict whether this parasitoid will significantly suppress *B. oleae*. To date, parasitoid populations have fluctuated strongly among sample years, perhaps due to recent California droughts, which reduce fruit availability in ornamental olives. Future work might consider establishing different commercial olive cultivars that have either fruiting periods that expand the seasonal availability of fly larvae or smaller fruit size that increases the accessibility of fly larvae in the pulp. This may be most feasible for olive oil production where fruits are relatively small, and some fly damage can be tolerated. For table olives, reducing olive fruit fly populations that act as sources of invasive flies entering commercial fields through biological control should help to make other integrated pest management strategies (e.g., trapping and killing, bait sprays) more efficient and economical. Therefore, continued biological control efforts against *B. oleae* should consider these ecological constraints and seek parasitoid species or strains with better inherent abilities to survive both climatic extremes and periods of low host density. There is also a need to introduce new parasitoids or strains that are better suited to California's interior valleys. The long-term economic benefit of the project would reduce pesticide use and enhance the California olive industry.

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