2017 Biological Control of the Olive Fly
17-8506-1636-CA
Semi-Annual Accomplishment Report

Survey Report

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Annual Accomplishment Report	☐

Semi-Annual Accomplishment Report
Biological Control of the Olive Fruit Fly

For the Period of January 1 to July 31, 2017

Prepared by

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Objectives

1. Mass rear *Psytallia lounsburyi* (South Africa) that have been in culture and coming from recovery samples in California.
2. Field release and monitor the same permitted parasitoid at new locations in California to determine establishment and impact on olive fly populations.
3. Obtain approval for field release of a second promising parasitoid coming from the foothills of the Himalayas in Pakistan, *Psytallia ponerophaga*.

Olive fruit fly, *Bactrocera oleae*, is currently the most destructive olive pest in California. To develop sustainable management strategies, we have been evaluating biological control options since 2006 and have identified three promising larval parasitoids, *Psyttalia lounsburyi, P. humilis,* and *P. ponerophaga* for field release in California. Only *P. lounsburyi* has been recovered and *P. ponerophaga* has yet to be permitted for field release. Two strains of *P. lounsburyi* were released, one collected from South Africa, *P. lounsburyi* (ex. South Africa) and the second from Kenya (*P. lounsburyi* ex. Kenya). Based on DNA analyses, the former strain has been much more successful at surviving California coastal conditions. (Fig. 1). Therefore the mass rearing of *P. lounsburyi* this year will focus on the South African strain.

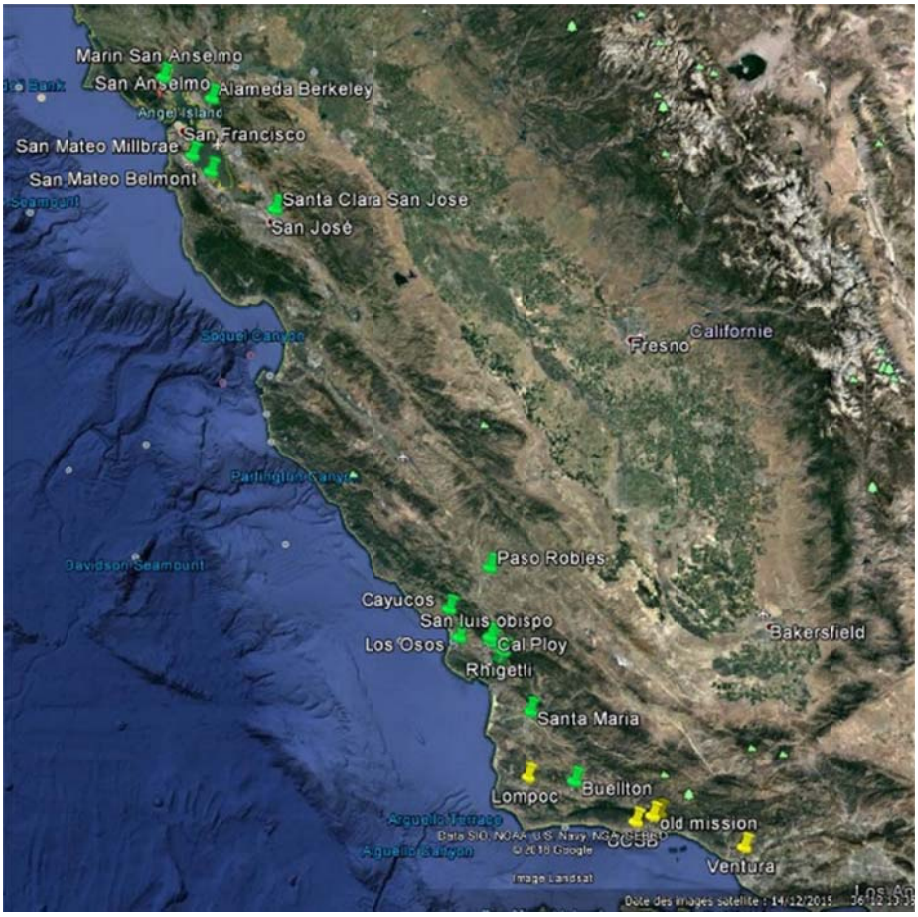

Figure 1. Distribution of the two maternal lineages (CO1) of *Psyttalia lounsburyi* recovered in California. (green = the South Africa lineage, and yellow = the Kenyan lineage. Image from Google Earth.)

Objective 1. *Mass rearing of parasitoids*. Nearly all releases in 2017 will come from cultures produced at EBCL. In response to the observation that the South Africa strain of *P. lounsburyi* has become established at many sites in northern California (see Objective 2 below), we are shifting emphasis to produce only the South Africa strain for release in 2017. All colonies of *Psyttalia lounsburyi* are continuing to be maintained at EBCL, and the South African strain was increased starting in May, 2017. The first 2017 shipment for release in California was sent on July 11, containing 2,793 females and 1,136 males. Four more shipments are planned for 2017.

Analysis of 12 years of rearing records indicates that the South Africa strain generally had a higher female sex ratio: 46% females for South Africa vs. 39 % for the Burguret, Kenya strain (Fig. 2; *T* test: *p* value < 0.0001, df = 205). Furthermore, the South Africa colony was 66% more productive than the Kenya colony during mass rearing in 2016 (Fig. 3). Both of these characteristics indicate that this strain should be more efficient to rear than the Kenya strain.

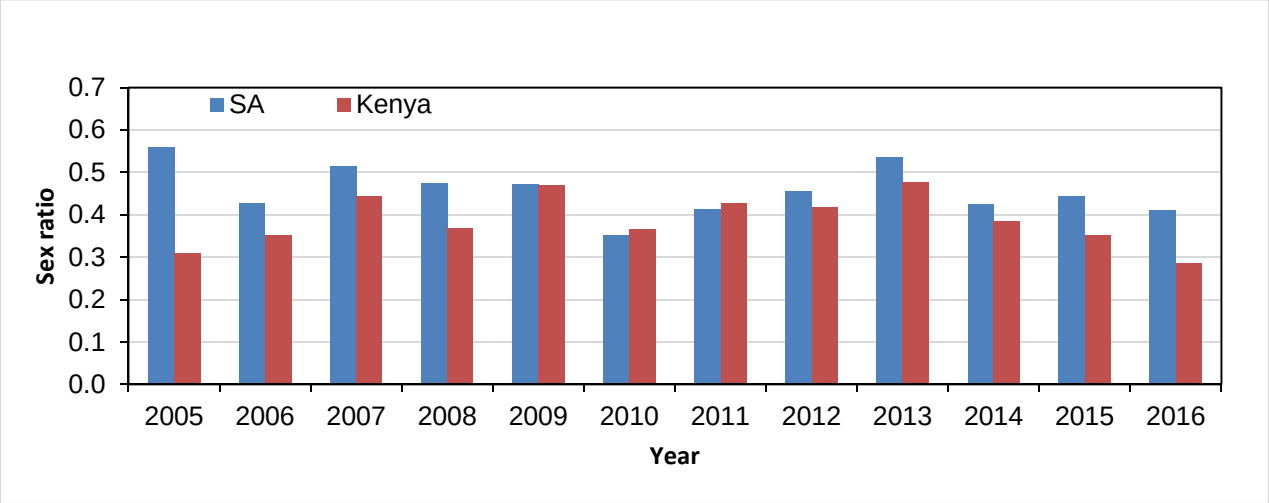

Figure 2. Comparison of the female sex ratio (proportion of progeny that were females) in EBCL laboratory colonies of *Psyttalia lounsburyi* from South Africa and Kenya.

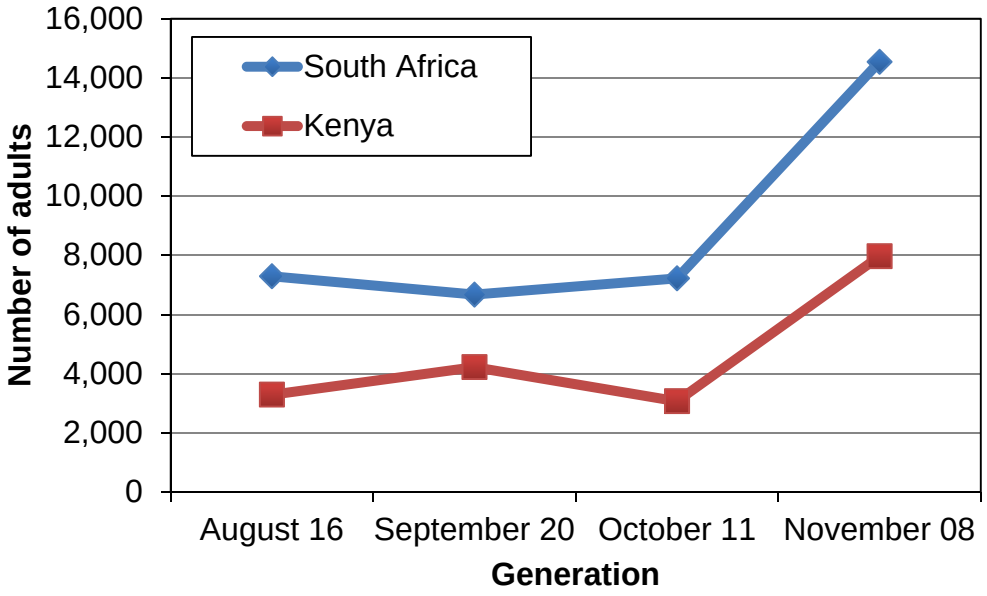

Figure 3. Production of the South Africa strain was 66% higher than the Kenya strain in 2016.

Objective 2. *Field releases*. In 2017, we plan 5 releases, once per month from July to December along the California coastal olive growing regions from San Diego County to Mendocino County and some interior olive growing regions (e.g. Napa County). Since fruit matures earlier in Southern than Northern California, the release will start from Southern California, then move northward with time. The first release for this year was made in late July 2017 at 7 different locations in San Diego, Riverside and Los Angeles counties. A total of 2,793 females and 1,136 males of the South African strain of *P. lounsburyi* were shipped from the USDA ARS European Biological Control Laboratory in France. The second release will be planned in Ventura, Santa Barbara and San Luis Obispo Counties while the third release will be conducted in Santa Cruz and South Bay Area. The fourth and fifth releases will be conducted in Norther Bay Area, and other Northern California olive growing regions.

In 2017 we will also conduct extensive samples of olives in all previous release sites and nearby regions to continually monitor the establishment, dispersal and seasonal patterns of parasitism in different regions. With these data (current and previous years), we will determine the effects of environmental (e.g. habitat types, climate) and biotic (host density, release size etc.) on the establishment and dispersal of *P. lounsburyi*.

Following reports of establishment of *Psyttalia lounsburyi* at many sites in California, EBCL conducted a molecular genetic evaluation to determine which strain (Kenya or South Africa) was established. We analyzed the sequence variation of the mitochondrial barcode (*CO1*) in one specimen from each of 25 populations of *P. lounsburyi* recovered in California in 2015 and compared them to historical specimens collected in Kenya and South Africa, as well as specimens from the colonies currently mass reared at EBCL. We clearly confirmed that all populations recovered in California are *Psyttalia lounsburyi*. Furthermore, we observed two distinct maternal lineages among the recovered populations. The most predominant ones (20 out of 25 populations analyzed) corresponded to the South Africa lineage that was mass-reared at EBCL. This maternal lineage is widely distributed from northern California (San Anselmo) to southern California (Buelton) (Fig. 1). The other lineage occurred in only 5 populations, and matched the historical populations collected in Kenya (mostly at Burguret Forest), and which was mass-reared at EBCL. This maternal lineage was found only in southern California, near Santa Barbara and Ventura.

In order to determine if there has been hybridization between the South African and Kenyan strains following introduction and establishment in California, we have started to conduct an analysis using eight microsatellites on 189 females recovered from California to compare them with historical populations and the EBCL colonies. This analysis is still in progress.

Objective 3. *Field release permit for Psyttalia ponerophaga* . There are two reasons we continue to pursue a field release permit for *P. ponerophaga*: its ability to survive in a mountainous, temperate environment, and secondly its high degree of host specificity. *Psyttalia ponerophaga* from Pakistan likely is better adapted to extreme temperate environments than *P. lounsburyi*. It is more cold tolerant and appears to diapause at low temperatures. This characteristic may make *P. ponerophaga* more capable of surviving the winter in central California especially in colder olive growing regions than either of the strains of *P. lounsburyi*.

Observations on the requirements to successfully culture *P. ponerophaga* at EBCL, compared to *P. lounsburyi* currently being released in California, indicate it is highly specific to olive fruit fly. However, initial testing at UC Berkeley in 2012 suggested it readily attacks a native fruit fly. Therefore last year the USDA ARS European Biological Control Laboratory contracted with CABI in Pakistan, the source of these parasitoids, to conduct field and laboratory studies measuring the host specificity of *P. ponerophaga*. Both their field and lab results showed a high degree of host specificity for olive fruit fly by this parasitoid. This triggered a review of previous work on host specificity testing at UC Berkeley. The initial test results showed successful reproduction by *P. ponerophaga* on the western cherry fruit fly, *Rhagoletis indifferens* larvae infesting bitter cherry (*Prunus,* probably *emarginata*). As an initial step in reviewing earlier testing, the DNA for parasitoids emerging from the fruit flies was examined (Marie-Claude Bon, EBCL). Two of 9 specimens were in fact *P. ponerophaga*, however the other 7 were North American species of *Utetes* (Braconidae) The latter most likely had already attacked the cherry fruit fly in the field, prior to collection of fruit used in testing. Without closely examining the species identification of emerging parasitoids, all were assumed *P. ponerophaga*, biasing the results, and explaining the high proportion of non-targets reported attacked by this parasitoid. We plan to repeat this test this year, add that data to above results and submit a petition for a field release permit. Last year unusual climatic conditions precluded the collection

of the black cherry fruit fly, *Rhagoletes fausta* (a trypetine frugivor that infest fruit of bitter cherry, *Prunus emarginata* (Douglas ex Hooker) Eaton (Rosaceae) in the mountains of California). A permit to release *P. ponerophaga* has already been drafted already and should be available soon.

From January through March of 2017, 1,150 olives and 500 olives were collected at Cañada College (Redwood City, CA) and Watsonville, CA, respectively. Annual parasitism by *P. lounsburyi* at Cañada College peaked in January (Fig. 4) at 50%. The degree of parasitism decreased at the end of fruiting season (March), while degree of fly infestation appeared to increase. At this time of year, there are only a few fruit remaining on the tree. Emerging adult flies have few host infested fruit to develop on.

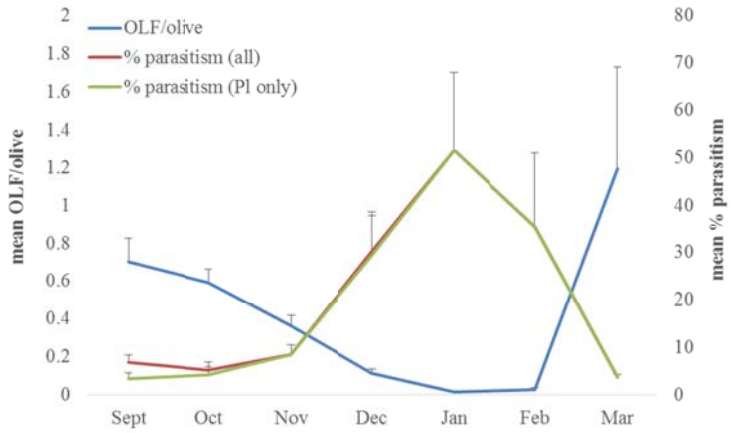

Fig. 4. Seasonal fluctuation in numbers of olive fruit flies and percent parasitism at the Cañada College study site, fall 2016 to spring 2017.

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Signatures

9/28/17

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