Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Release of *Psyllaephagus euphyllurae* (Hymenoptera: Encyrtidae) for Biological Control of Olive Psyllid, *Euphyllura olivina* (Hemiptera: Liviidae), in the Contiguous United States

Final Environmental Assessment, May 2022

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Final Environmental Assessment, May 2022

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I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the insect *Psyllaephagus euphyllurae* (Hymenoptera: Encyrtidae). This organism would be used by the permit applicant for biological control of olive psyllid, *Euphyllura olivina* (Hemiptera: Liviidae), in the contiguous United States.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities.

This environmental assessment (EA) was prepared to be consistent with USDA–APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of the parasitoid wasp, *P. euphyllurae,* to control olive psyllid in the contiguous United States. A parasitoid is an insect whose immature stages (larvae and pupae) live as parasites that eventually kill their hosts (typically other insects). This EA considers a "no action" alternative and the potential effects of the proposed action. Notice of this EA was made available in the Federal Register on March 31, 2022 for a 30-day public comment period. One comment was received on the EA by the close of the comment period. The comment was in favor of the proposed release of *P. euphyllurae*.

The applicant's purpose for releasing *P. euphyllurae* is to reduce the severity of damage to olives from infestations of olive psyllid in California. The olive psyllid, *Euphyllurae olivina* (Heteroptera: Liviidae) is native to southern Europe and was first reported in North America in 2007. By the time this psyllid was found on olives in southern California, it was widespread in the region. This pest feeds exclusively on the flower blossoms and growing tissue of olive, causing reductions in fruit set, with reductions in fruit yield as high as 60 percent reported in some parts of the Mediterranean Basin (the region of lands around the Mediterranean Sea in Europe, Africa, and Asia) (Jardak et al.,1984). Serious damage has also been reported in other north African and Middle Eastern countries (Gentry, 1965).

Current olive psyllid control programs rely primarily on cultural control and insecticides. These methods (discussed below) are expensive, temporary, have not been effective, and/or include non-target impacts. For these reasons, there is a need to identify and release an effective, host-specific biological control organism against olive psyllid in California.

II. Alternatives

This section will explain the two alternatives available to PPBP: no action (no issuance of permits) and issuance of permits for environmental release of *P. euphyllurae* into the contiguous United States. Although APHIS' alternatives are limited to a decision of whether to issue permits

for release of *P. euphyllurae*, we describe other methods currently used to control olive psyllid in California. Use of these control methods is not an APHIS decision, and their use is likely to continue whether or not PPBP issues permits for environmental release of *P. euphyllurae*.

The PPBP considered a third alternative but will not analyze it further. Under this third alternative, PPBP would issue permits for the field release of *P. euphyllurae*. The permits, however, would contain special provisions or requirements concerning release procedures or mitigating measures, such as limited releases of *P. euphyllurae* in the contiguous United States. There are no issues raised indicating that special provisions or requirements are necessary.

A. No Action

Under the no action alternative, the PPBP would not issue permits for the field release of *P. euphyllurae* for the control of olive psyllid — the release of this biological control agent would not occur, and current methods to control olive psyllid in California will continue at current levels. Use of these methods is likely to continue even if PPBP issues permits for release of *P. euphyllurae*. Presently, control of olive psyllid in the United States is limited to chemical and cultural control methods.

1. Chemical Control

Insecticides can be used to control olive psyllids, including spinetoram. Neem oil, horticultural oil, and insecticidal soap. Control measures should be taken before psyllids start secreting their heavy waxy coating, which protects them from insecticides.

2. Cultural Control

Pruning can be used to reduce olive psyllid populations. Growers can prune infested areas, mainly suckers, along with center limbs to improve air circulation that increases heat exposure to olive psyllids (Johnson, 2009; Kabashima et al., 2014; Linn and Gillett-Kaufman, 2016).

B. Issue Permits for Environmental Release of Psyllaephagus euphyllurae

Under this alternative, PPBP would issue permits for the field release of *P. euphyllurae* for the control of olive psyllid in the contiguous United States. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures. *Psyllaephagus euphyllurae* is specific to olive psyllid.

1. *P. euphyllurae* Taxonomic Information

Insect Taxonomy
Order: Hymenoptera
Family: Encyrtidae
Genus: *Psyllaephagus*

Species: *euphyllurae* (Masi)
Common name: none

This parasitoid was originally described by Masi (1911) as *Encyrtus euphyllurae*, then later redescribed by Mercet (1921) as *Psyllaephagus euphyllurae*. Voucher specimens have been deposited in the University of California (UC) Riverside Entomology Research Museum. Specimens were also placed in the California State Collection of Arthropods, California Department of Food and Agriculture, Sacramento, California.

2. Biology of P. euphyllurae

Psyllaephagus euphyllurae is a thelyotokus (females are produced from unfertilized eggs) parasitoid wasp of olive psyllid. The adult wasp inserts its ovipositor (egg laying organ) and lays an egg into olive psyllids. The *P, euphyllurae* egg hatches inside the olive psyllid, and over time, eventually consumes the olive psyllid host as it progresses through its development. *P. euphyllurae* is active during spring months; it stops reproducing in July and aestivates (a period where it spends a hot or dry period in a prolonged state of dormancy) as a preadult (inside its host's mummy) until the following spring.

3. Geographic Range of *P. euphyllurae*

There are no known published records for *P, euphyllurae* being introduced to other countries, within or outside of its natural distribution in the Mediterranean Basin. Published records show that it has been collected and reared only from olive psyllid in France (Chermiti et al., 2006, Pickett et al., 2019), Greece (Pickett et al., 2019), Portugal (Gahan and Waterston, 1926), Spain (Mercet, 1921; Triapitsyn et al., 2014), and Italy (Masi, 1911).

4. Potential Range of P. euphyllurae in North America

Based on climate matching data (Sutherst and Maywald, 1999) and known current distribution in southwestern Europe, this parasitoid should be able to establish throughout most areas of California where olive is grown and olive psyllid is established, e.g. elevations below 1,000 meters. This would include southern California, coastal areas through Marin County, and central California north to Butte County. Psyllids are highly host plant specific (Percy et al., 2012). Published host records, and specificity tests show that the parasitoid is restricted to the olive psyllid, which in turn is limited almost entirely to olive. Olive psyllid has also been reported on *Phillyrea latifolia* (mock privet) so it is possible the parasitoid could be found reproducing on olive psyllid attacking this plant. *Phillyrea* spp. are native to the Mediterranean Basin and the plant is in the same family (Oleaceae) and tribe (Oleae) as olive.

5. Impact of *P. euphyllurae* on Olive Psyllid

Psyllaephagus euphyllurae should have a direct impact on the target, olive psyllid, causing its population to decline and eventually be of little to no economic concern to olive production.

III. Affected Environment

A. Olive Psyllid

1. Olive Psyllid Taxonomic Information

The common name 'olive psyllid' refers to several species occurring throughout the Mediterranean Basin, *Euphyllura olivina* (Costa), *Euphyllura phillyreae* Foerster, *Euphyllura straminea* Loginova, and *Euphyllura pakistanica* Loginova (Hemiptera: Liviidae). They all primarily attack olive trees and can be found in different regions of the Mediterranean. The species invading California, *Euphyllurae olivina*, is considered the most important of all the species and is found in North Africa, Spain, and southern France (Tzanakakis, 2006). DNA sequencing of *E. olivina* shows that the California population is only two mutations apart from the Spanish population where collecting of parasitoids for testing was carried out. No other psyllids occur on olive trees in California.

2. Life History of Olive Psyllid

The olive psyllid produces multiple generations a year and passes through five nymphal instars (immature stages). Nymphs produce honeydew (a sweet, sticky substance) and a white flocculent wax (resembling tufts of wool) as they feed and develop. The actual number of generations per year is unclear but varies from two to six. Egg and nymphal olive psyllids develop in 8.2 and 23 days, respectively (Kumral et al., 2008). The adults are 2.5 to 2.8 millimeters (mm) long, a light green to light brown. They jump when disturbed, hence the common name jumping plant lice. Although winged, adults are poor fliers. Females lay eggs onto plant tissue. Most commonly, eggs are deposited near growing tissue such as leaf and flower bud axils. As with most psyllids, olive psyllid has a restricted host plant range. It is primarily reported on olive, but has also been reported feeding on another member of the Oleaceae, *Phillyrea latifolia*, in southern Europe (Tzanakakis, 2006).

B. Areas Affected by Olive Psyllid

1. Native and Worldwide Distribution

Olive psyllid has been reported occurring throughout the olive growing areas of the Mediterranean Basin, but there is uncertainty if the correct identification has been made for all populations because there are at least four closely related species in this genus distributed throughout this region. *Euphyllura olivina* is today considered dominant in southwestern Europe (France and Spain), northern Africa, and possibly Jordan (Tzanakakis, 2006).

2. Present Distribution in North America

In California, olive psyllid was first reported from Newport Beach, Orange County in June 2007. Now it can be found throughout Orange, San Diego, Riverside, Los Angeles, Santa Barbara, and Monterey counties.

3. Olive Psyllid Hosts

The olive psyllid feeds almost exclusively on olive trees. However, they may be found on *Phillyrea latifolia* (Oleaceae) that is found in southern Europe and is closely related to the olive tree.

C. Insects Related to Olive Psyllid and Psyllaephagus euphyllurae in the United States

1. Insects Related to Olive Psyllid

Information regarding insects taxonomically related to olive psyllid is included because closely related insect species have the greatest potential for attack by *P. euphyllurae* if it is released in the United States.

The olive psyllid in California is most closely related to the native *Neophyllura* found on manzanita (*Arctostaphylus* spp.) and on *Arbutus menziesii*. They share the same tribe, Euphyllurini, within the family Liviidae. Although the exotic invasive Asian citrus psyllid, *Diaphorina citri*, is placed in the same subfamily as olive psyllid, Euphyllurinae, some question this relationship. There are no other representatives of the *Euphyllura* in North America. This is an Old World group limited to the Mediterranean Basin north to Russia, Middle East, and North Africa (Tzanakakis, 2006). The olive psyllid's natural distribution in southern Europe overlaps that of the French broom psyllid, *Arytinnis hakani*, yet there are no records for it infesting French broom, *Genista monospessulana*. This psyllid was chosen for host specificity testing (discussed later in this document) because it was recently under consideration for release in California as a biocontrol agent for the invasive weed species, French broom. Only *Phillyrea latifolia* has been reported as an alternate host in Europe. The candidate parasitoid *Psyllaephagus euphyllurae* has been reared out from the psyllids *Euphyllura olivina* and *Euphyllura phillyreae* Foerster (Triapitsyn et al., 2014).

2. Insects Related to *P. euphyllurae*

California has a rich diversity of Psylloidea (superfamily of true bugs including olive psyllid) with 164 species representing 35 genera (Percy et al., 2012). Consequently, there have been a large number of parasitoids reported attacking psyllids in California. Jensen (1957) in California reared parasitoids from 30 species of psyllids representing 11 genera. *Psyllaephagus* and *Prionomitus*, both encyrtids, show a strong preference for psyllids. Jensen (1957) reared out 10 and 9 undescribed species of *Psyllaephagus* and *Prionomitus*, respectively, attacking native psyllids from California, in addition to several more described species.

IV. Environmental Consequences

A. No Action

1. Impact of Olive Psyllid on the Environment

Although it is not known for certain that olive psyllid will become a serious pest in commercial production regions of central and northern California, it is spreading and is considered an economic pest of olive growers in southern Europe. Olive psyllid naturally occurs throughout the Mediterranean Basin, both coastally and inland, and exclusively attacks the flower blossoms and growing tissue of olive (Tzanakakis, 2006). The olive psyllid is reproductively active during spring months when nymphal populations can cause significant reductions to the olive fruit set. Spring infestations have been reported reducing fruit yields by up to 60 percent in some parts of the Mediterranean Basin (Jardak et al., 1984; Tzanakakis, 2006). Serious damage has been reported in other north African and Middle Eastern countries (Gentry, 1965).

2. Impact from the Use of Other Control Methods

The continued use of chemical and cultural controls at current levels would result if the "no action" alternative is chosen and may continue even if permits are issued for environmental release of *P. euphyllurae.*

a) Chemical Control

Insecticide applications to control olive psyllid may negatively impact beneficial insects and pollinators.

b) Cultural Control

Cultural control can be useful in reducing olive psyllid damage, but alone is not effective in eliminating olive psyllid.

These impacts from the use of other control methods may have environmental consequences even with the implementation of the biological control alternative, depending on the efficacy of *P. euphyllurae* to reduce olive psyllid infestations in the contiguous United States.

B. Issue Permits for Environmental Release of Psyllaephagus euphyllurae

1. Impact of *P. euphyllurae* on Non-target Insects

Host specificity of *P. euphyllurae* to olive psyllid has been demonstrated through scientific literature and host range testing. If the candidate biological control agent only attacks one or a few insect species closely related to the target insect, it is considered to be very host-specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

a) Scientific Literature

Only *Psyllaephagus euphyllurae* has been reported emerging from the olive psyllid, *Euphyllura olivina* (Mercet, 1921; Aversenq et al., 2005; Chermiti et al., 2006). However, this parasitoid has been collected from olive psyllid mummies collected in Greece (Pickett et al., 2019). No adult psyllids were identified with the collection; therefore, the host possibly was the sister species *Euphyllura phillyreae*, which is referred to as the olive psyllid in Greece because it is the dominant psyllid attacking olive there.

b) Host Specificity Testing

Both native and non-native psyllids were chosen for host specificity testing. Species selected including relatedness to the olive psyllid, occurrence in habitat similar to, and near where commercial olives are grown, and their availability. California has a rich diversity of these insects associated with its native vegetation (Percey et al., 2011). However, only a few representatives can be chosen for testing because there are over 165 species. Two of the psyllids were selected due to relatedness to the target olive psyllid. *Neophyllura arctostaphyli*, native to California, and *Diaphorina citri*, introduced, are in the same subfamily Euphyllurinae as olive psyllid. If *P. euphyllurae* is unable to attack and develop on a related psyllid, then it is unlikely to develop on the native, more distantly related psyllids. Other non-targets include psyllids associated with native plants common to foothill regions in central and northern California, such as *Ceanothus* spp. and *Rhus trilobata* (Table 1). Associated psyllids on *Ceanothus* were *Ceanothia ceanothi* and *Euglyptoneura robusta*, and on the plant *Rhus trilobata* is a *Calophya* species psyllid. Another candidate for host specificity testing was the biological control agent *Arytinnis hakani* which feeds on French broom (*Genista monospessulana*) a relatively new invasive weed to California.

Table 1. Non-target psyllid species, selection criteria, and host plants in host specificity testing.

Psyllid Species	Selection Criteria	Host Plant
French broom psyllid, *Arytinnhakani*	Beneficial insect attacking a noxious weed (in California)	*Genista monospessulana*
Potato psyllid, *Bactericera cockerelli*	Native pest psyllid	*Solanum melongena*
Ceanothia ceanothi	Natural habitat near olive production	*Ceanothus integerrimus*
Asian citrus psyllid, *Diaphorina citri*	Relatedness	Citrus species
Neophyllura arctostaphyli (El Dorado Co.)	Native, related to olive psyllid	*Archtostaphylos sp.*
Calophya nigrella	Native, natural habitat nr. olive production	*Rhus trilobata*
Euglyptoneura nr. robusta	Native, natural habitat nr. olive production	*Ceanothus integerrimus*

Summary of host specificity results.

From 212 individual nymphs exposed to *P. euphyllurae*, representing four non-target species during the first round of testing at UC Riverside, no reproduction was recorded. Over the same period of time 17.5 percent of olive psyllid nymphs exposed to the parasitoid produced *P. euphyllurae*. Host specificity testing done at UC Berkeley produced similar results as at UC Riverside. Forty-three non-target nymphs were exposed to *P. euphyllurae* using no-choice and choice exposures. During testing at UC Berkeley, no reproduction on non-targets was reported again during the most conservative testing, no-choice exposures. Most importantly, no reproduction occurred on *Neophyllura arctostaphyli*, the most closely related psyllid to the target psyllid, suggesting that attack and reproduction on more distantly related psyllids native to California is even less likely. See Appendix A for a complete description of host specificity testing and results.

2. Impact of *P. euphyllurae* on Olive Psyllid

Psyllaephagus euphyllurae is the most commonly collected and recorded primary parasitoid attacking olive psyllid in Spain and southern France, a region most likely the source for the olive psyllids that invaded California. Host specificity tests and its field biology show that it is a specialist parasitoid, using the olive psyllid as its host. Olive psyllid was first reported infesting olive trees in southern California in 2007 and is now found north to Monterey County. Although not currently reported as an economic pest of olive trees in California, its continued spread in this state and natural distribution in the Mediterranean basin suggests that with time it will soon be reported from commercially grown olives, affecting fruit set. Surveys show that olive psyllid in California lacks a specialist natural enemy. Absence of such a parasitoid will result in higher numbers of olive psyllid and will trigger additional pesticide applications in California, increasing the production costs for growers and pollution to the environment. Release of *P. euphyllurae* will also provide an environmentally friendly strategy for the organic production of olives, a rapidly expanding market in California. Classical biological control is a potentially useful management strategy for an invasive pest species whenever effective resident natural enemies are lacking in the new distribution range.

3. Impact on Human and Animal Health

Psyllaephagus euphyllurae is a tiny, stingless wasp. Like all parasitic wasps, the immature stages develop as parasitoids of arthropods where, in this case, feeding of the wasp larva inside the host olive psyllid eventually kills it. This insect poses no risk to humans, livestock, or wildlife.

4. Uncertainties Regarding the Environmental Release of *P. euphyllurae*

Once a biological control agent such as *P. euphyllurae* is released into the environment and becomes established, there is a possibility it could move from the target insect (olive psyllid) to attack nontarget insects. Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other insect species were to be attacked by *P. euphyllurae*, the resulting effects could be environmental impacts that may not be easily reversed. Biological

control agents such as *P. euphyllurae* generally spread without intervention by man. In principle, therefore, release of this parasitoid at even one site should be considered equivalent to release over the entire area in which potential hosts occur and in which the climate is suitable for reproduction and survival.

In addition, these agents may not be successful in reducing olive psyllid populations in the contiguous United States. Approximately 12 percent of all parasitoid introductions have led to significant sustained control of the target pests, but the majority of introductions have failed to provide control of the pest (Greathead and Greathead, 1992) either because introduction did not lead to establishment or establishment did not lead to control (Lane et al., 1999).

Actual impacts on olive psyllid populations by *P. euphyllurae* will not be known until after release and establishment occurs. Monitoring will be conducted by the permittee to determine the establishment of *P. euphyllurae* (Appendix B). The environmental consequences discussed under the no action alternative may occur even with the implementation of the action alternative, depending on the efficacy of *P. euphyllurae* to reduce olive psyllid in the contiguous United States.

5. Cumulative Impacts

"Cumulative impacts are defined as the impacts on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Release of *P. euphyllurae* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to olive psyllid. Effective biological control from introduced *P. euphyllurae* may not only provide safe, effective, and long-term control of olive psyllid, but the parasitoid may also result in reduced use of insecticides against olive psyllid.

No other agents have been released in the contiguous United States for biological control of olive psyllid; therefore, no competitive interactions between agents are expected. Release of *P. euphyllurae* would not affect the ability of growers to continue to control olive psyllid using other methods. Based on host specificity testing, it is also not expected to attack other psyllids released for biological control of invasive plants, so will not have an adverse effect on other control programs.

6. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

APHIS has determined that, based on the host specificity of *P. euphyllurae,* there will be no

effect on any listed species or designated critical habitat in the contiguous United States. In host specificity testing, *P. euphyllurae* is specific only to olive psyllid. There are no federally listed psyllid species, and there are no federally listed species known to depend on or use olive psyllid.

V. Other Issues

A. Equity and Underserved Communities

In Executive Order (EO) 13985, Advancing Racial Equity and Support for Underserved Communities Through the Federal Government, each agency must assess whether, and to what extent, its programs and policies perpetuate systemic barriers to opportunities and benefits for people of color and other underserved groups. In EO 12898, Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations, Federal agencies must identify and address disproportionately high and adverse human health or environmental impacts of proposed activities.

Consistent with these EOs, APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. APHIS did not identify any disproportionately high or adverse environmental or human health effects from the field release of *P. euphyllurae.* The preferred action will not have disproportionately high or adverse effects to any minority or low-income populations.

Federal agencies also comply with EO 13045, Protection of Children from Environmental Health Risks and Safety Risks. This EO requires each Federal agency, consistent with its mission, to identify and assess environmental health and safety risks that may disproportionately affect children and to ensure its policies, programs, activities, and standards address the potential for disproportionate risks to children. Consistent with EO 13045, APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No aspects of the proposed field release of *P. euphyllurae* could be identified that would have disproportionate effects on children.

B. Tribal Consultation and Coordination

EO 13175, "Consultation and Coordination with Indian Tribal Governments", was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications…." Consistent with EO 13175, APHIS will continue to consult and collaborate with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests, in accordance with EO 13175.

VI. Agencies, Organizations, and Individuals Consulted

This EA was prepared and reviewed by personnel from APHIS and California Department of Food and Agriculture. The addresses of participating APHIS units and any applicable cooperators are provided below.

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Policy and Program Development
Environmental and Risk Analysis Services
4700 River Road, Unit 149
Riverdale, MD 20737

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Pests, Pathogens, and Biocontrol Permits
4700 River Road, Unit 133
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VII. References

Aversenq, S., C. Gratradu, and C. Pinatel. 2005. Olive tree pests and beneficials: a summary of three years of monitoring in S.E. France. Phytoma. 586: 32–36.

Chermiti, B., N. Hawlitzky, C. Boulay, and J.C. Onillon. 2006. Quelque caractéristiques du développement de l'endoparasite *Psyllaephagus euphyluurae* (Hymenoptera, Encyrtidae) et exploitation de son hôte Euphyllura olivine (Homoptere, Psyllidae). Entomophaga. 32: 351–361.

Ferguson, L., G.S. Sibbett, and G.C. Martin. 1994. Olive Production Manual. University of California, Division of Agriculture and Natural Resources, Oakland, CA. Publication 3353.

Gentry, J. W. 1965. Crop Insects of Northeast Africa-Southwest Asia. USDA. Washington, D.C.

Greathead, D., and A.H. Greathead. 1992. Biological control of insect pests by parasitoids and predators: the BIOCAT database. Biocontrol News and Information. 13: 61N−68N.

Hoddle, M.S., and R. Pandey. 2014. Host range testing of *Tamarixia radiata* (Hymenoptera: Eulophidae) sourced from the Punjab of Pakistan for classical biological control of *Diaphorina*

citri (Hemiptera: Liviidae: Euphyllurinae: Diaphorinini) in California. Journal of Economic Entomology. 107: 125–136.

Jardak, T., H. Smiri, M. Moalla, and H. Khalfallah. 1984. Tests to assess the damage caused by the olive psyllid *Euphyllura olivina* Costa (Homoptera, Psyllidae): preliminary data on the harmfulness threshold. *In:* R. Cavallora A. Crovetti (eds.) Integrated Pest Control in Olive – Groves. Proceedings of the CEC /FAO/IOBC International Joint Meeting/Pisa/3–6 April 1984. Published for the Commission of the European Communities by A. A. Balkema/Rotterdam/Boston/1985.

Jensen, D.D. 1957. Parasites of the Psyllidae. Hilgardia. 27: 71–99.

Johnson, M.W. 2009. Olive psyllid, *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae). Center for Invasive Species Research. University of California Riverside.

Jones, M.L.J., C.H. Pickett, S.V. Triapitsyn, and M.S. Hoddle. 2016. New host record for *Psyllaephagus pulchellus* (Mercet, 1921) (Hymenoptera, Encyrtidae) as a parasitoid of *Euphyllura olivina* (Costa, 1839) (Hemiptera, Liviidae), in Spain. Boletín Asociación Española de Entomolgía. 40: 67–84.

Kabashima J.N., T.D. Paine, K.M. Daane, and S.H. Dreistadt. 2014. Psyllids: Integrated pest management for home gardeners and landscape professionals. University of California, Agriculture and Natural Resources. Pest Notes Publication 7423.

Kumral, N.A., and B. Kovanci, and B. Akudak. 2008. Using degree-day accumulations and host phenology for predicting larval emergence patterns of the olive psyllid, *Euphyllura phillyreae*. Journal of Pest Science. 81: 63–69.

Linn, S. and J.L. Gillett-Kaufman. 2016. Featured Creatures.Publication No. EENY-656. University of Florida, Institute of Food and Agricultural Sciences. Available: http://entnemdept.ufl.edu/creatures/FRUIT/TROPICAL/olive_psyllid.htm. Accessed: February 5, 2021.

Littell, R. C., W. W. Stroup, and R. J. Freund. 2002. SAS for Linear Models., fourth edition.

Louda, S.M., R.W. Pemberton, M.T. Johnson, and P.A. Follett. 2003. Nontarget effects—the Achilles' heel of biological control. Retrospective analyses to reduce risk associated with biological control introductions. Annual Review of Entomology. 48: 365–396.

Masi, L. 1911. Contribuzioni all conoscenza dei Calcididi italiani. (Parte IV). Boll. Lab. Zool. Gen. Agr. R. Scuola Super. Agric. Portici. 5: 140–171.

Mercet, R.G. 1921. Fauna Ibérica. Himenópteros Fam. Encírtidos. Museo Nacional de Ciencas Naturales, Madrid: I–XI, 732 pp.

Percy, D.M., A. Rung, and M.S. Hoddle. 2012. An annotated checklist of the psyllids of

California (Hemiptera: Psylloidea). Zootaxa. 3193: 1–27.

Pickett, C.H., J.M. Jones, S. Triapitsyn, and E. Hougardy. 2019. A petition for release of *Psyllaephagus euphyllurae* (Hymenoptera: Encyrtidae) Collected in Eastern Spain for the Biological Control of Olive Psyllid, *Euphyllura olivina* (Hemiptera: Liviidae, formerly Psyllidae) in California. Report submitted to USDA APHIS. California Department of Food & Agriculture, and University of California, Riverside and Berkeley. 55 pp.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd Edition. Freeman and Company, NY. 859 pp.

Sutherst, R.W., and G.F. Maywald. 1999. CLIMEX: Predicting the effects of climate on plants and animals. CSIRO Publishing. Collingwood, Victoria, Australia.

Triapitsyn, S. V., J. M. L. Jones, C. H. Pickett, M. L. Buffington, P. F. Rugman-Jones, and K. M. Daane. 2014. Description of the male of *Psyllaephagus euphyllurae* (Masi) (Hymenoptera, Encyrtidae), a parasitoid of the olive Psylla, *Euphyllura olivina* (Costa) (Hemiptera, Liviidae), with notes on its reproductive traits and hyperparasitoids. Journal of Entomological and Acarological Research. 46: 112–118.

Tzanakakis, M.E. 2006. Insects and Mites Feeding on Olive, Distribution, Importance, Habits, Seasonal Development and Dormancy. Koninklijke Brill NV, Leiden, The Netherlands. 182 pp.

VIII. Appendix A. Host Specificity Testing (Pickett at al., 2019).

Plants and insect cultures. Plants used in studies were started from seeds or cuttings in the California Department of Food and Agriculture (CDFA) greenhouses at the Meadowview Campus in Sacramento and transferred to Riverside or University of California (UC) Berkeley. Olive plants were all started from cuttings, of the Mission variety, following methods of Ferguson et al. (1994). After rooting, plants were transferred to one-gallon pots filled with Supersoil Palm and Cactus Mix® blend soil, and maintained inside a temperature-controlled glass greenhouse with an upper threshold of 27°C. A strict regime was established to prevent any contaminants from feeding on potted olive plants. The olive psyllid was maintained on the potted olive plants that were transferred into a quarantine facility at CDFA's Meadowview campus in south Sacramento and were used for all tests. Plants in the greenhouse were routinely surveyed for contaminants that were removed by hand. Prior to placement in the quarantine facility, plants were washed with a soap solution and observed for several days for any scale (Coccidae) and ant activity. Olive psyllid-infested plants were held inside 45-centimeter (cm) x 90 cm x 45 cm rectangular Bugdorm© cages (Figure 1), with two side walls of clear plastic and the remainder constructed of synthetic organdy.

Figure 1. Olive saplings infested with olive psyllid, UC Riverside

Studies conducted at UC Riverside used cultured citrus, potato, and olive host plants. The native plant, *Ceanothus integerrimus,* was started from seed or purchased seedlings. The French broom (*Genista monospessulana*) seedlings were obtained from the USDA Agricultural Research Service (ARS), in Albany, California. Native, non-target insects were collected in the field. For studies conducted at UC Berkeley, non-target plant cuttings were collected from the field, while olive saplings, along with olive psyllid were cultured at CDFA.

Methods for Testing at UC Riverside, 2013 to 2015

The first round of host testing was done in a laboratory inside the UC Riverside Quarantine and Insectary facility using host specificity testing procedures following Hoddle and Pandey (2014). Rearing rooms were kept at 22.8°C, with 50 percent relative humidity (RH) for the olive psyllid colony and 20.5°C in an experimental room. Each room had a constant photoperiod of 14 hours light, 10 hours dark. Exotic non-targets and associated plants came from cultures at UC Riverside and the USDA-ARS facility in Albany, California. Only late instar fourth or fifth instar nymphs were used in testing. All tested psyllids produce a waxy flocculent substance which was removed just prior to testing so that both the target and non-target psyllids could appear as similar as possible in size and chemistry (this was not done for the second set of testing done at UC Berkeley). Nymphs were transferred to the test plant using a damp paint brush on the day of trial. The candidate parasitoid *P. euphyllurae* was collected in eastern Spain in 2013 and 2014. A total of 221 parasitoids emerged from mummies in 2013, with 60.6 percent being the candidate, primary parasitoid *P. euphyllurae*, and 39.4 percent hyperparasitoids. These were composed of *Apocharips trapezoidea* (Figitidae) and *Pachyneuron* sp. (Pteromalidae). In 2014, 632 parasitoids emerged from mummies with 45.7 percent primary parasitoids and 54 percent hyperparasitoids. Most of the primary parasitoids were the candidate *P. euphyllurae* (85.8 percent) followed by *P. pulchellus*, (14.2 percent). The hyperparasitoids were *A. trapezoidea* (73.7 percent) and *Pachyneuron* (26.2 percent). Due to the difficulty in rearing *P. euphyllurae* most individuals used in testing originated from mummified psyllids collected from infested, abandoned olive trees in rural areas of the provinces Catalonia, Valencia, and Murcia. Cultures were maintained on potted olive plants infested with olive psyllid originating from San Diego County or Monterey County. When not in use, parasitoids were stored individually in 2 milliliter (ml) conical microcentrifuge tubes streaked with honey. These tubes were kept in an incubation chamber (13.5–14.8°C; 14 hours light/10 hours dark). Cuttings used in testing were enclosed in a 50-dram inverted plastic vial fit with a 2 cm diameter screened openings secured to the top side of a ‘cone-tainer’ (Figure 2). The vial, with a large hole cut in its lid, was inserted over the top end of a cone-tainer supporting a plant cutting. Foam rubber was cut to fit over the soil in the cone-tainer to prevent fungus flies from emerging into cage. The arena used for choice tests was constructed of acrylic material (15 cm x 15 cm x 15 cm). One side of the cage was sealed with a synthetic organdy (72x72 strands per inch), while the opposite side was fitted with similar synthetic organdy but as a sleeve for entry to the cage (Figure 3).

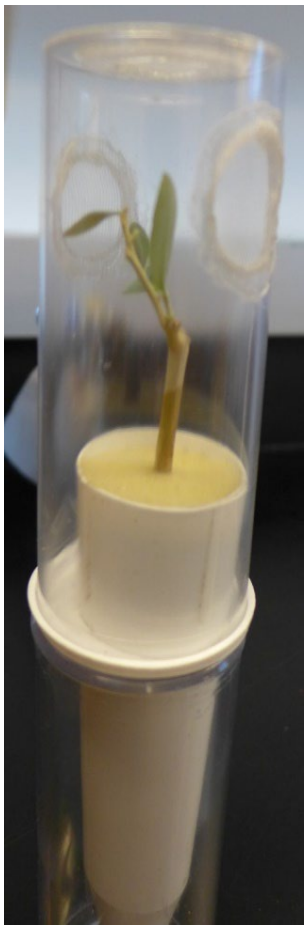

Figure 2. Cone-tainer arena for no-choice tests, UC Riverside.

Figure 3. Cage used for choice tests, UC Riverside

Host specificity testing methods. Three tests were used to determine the preference of the parasitoid for the target pest, olive psyllid, versus non-target psyllids (Table A-1). A sequential, no-choice test (T1) started with a naïve female parasitoid placed inside a cone-tainer containing four nymphs on a host plant. After four hours the parasitoid was removed and placed into a second cone-tainer with the non-target psyllid for four hours. The same parasitoid was then removed to a 2-millimeter (mm) honey-streaked glass vial and allowed to rest for 16 hours after which it was presented to psyllids in a reverse order (T2) using the same exposure times as in T1. After the end of these two tests, the parasitoid was removed and the psyllids inside cone-tainers were left to incubate at 23°C ± 2°C, 50 percent RH in the quarantine facility at UC Riverside for a few weeks after which the plant material was examined to determine the number of parasitized nymphs (mummies) and the presence of adult wasps. These are by far the most conservative testing scenarios, whereby the female parasitoid has only one choice of host to deposit its eggs. The last test determined whether a choice of hosts, the target olive psyllid vs. non-target, will affect the female parasitoid's preference for depositing its eggs (T3). All tests were run in parallel with a host only, parasitoid absent, control. The control was needed to determine the impact of naturally occurring mortality under the conditions of the test experiments. Three to nine replicates were conducted for each test: sequential T1 or T2 (no-choice), and the T3 choice test. Parasitism was measured as the number of mummies and adult *P. euphyllurae* that emerged from mummies at time of recording.

Table A-1. Types of specificity treatments detailing *P. euphyllurae* exposure psyllids.
[1]Parasitoids were returned to the individual 2 ml vials and placed in an incubation chamber for the entire duration of the resting period.

Treatment type	Duration of exposure to psyllids
T1. Sequential no-choice (non-target to target)	4 h non-target → 4 h target →16 h rest[1] →4 h non-target→ 4 h target
T2. Sequential no-choice (target to non-target)	4 h target → 4 h non-target →16 h rest[1] →4 h target→ 4 h non-target
T3. Choice	4 h of simultaneous exposure to target and non-target
Control	No parasitoid exposure

Not all non-target psyllids were exposed to all tests (T1–T3) at the same point in time due to lack of availability of all insects. GLM procedures with SAS software (Littell et al., 2002) were used to analyze results when comparing survivorship between the target and non-target nymphs when exposed to the candidate parasitoid, and when comparing mortality of non-target psyllids exposed to the parasitoid to their no-exposure control.

Methods for Testing at UC Berkeley, 2018

The *P. euphyllurae* adult wasps used in these non-target tests originated from mummies collected in Spain in May 2018. A total of 1,503 psyllid mummies were shipped to the Insectary and Quarantine Facility at UC Berkeley where the emerging parasitoids were then collected. Among the 741 wasps that emerged, 426 (57 percent) were the primary parasitoid *P. euphyllurae* and 315 (43 percent) were identified as hyperparasitoids. Among them, 126 individuals (40 percent) were identified as *Pachyneuron* sp. (Pteromalidae) and 189 individuals (60 percent) were figitid species.

Following their emergence, *P. euphyllurae* wasps were placed in glass vials and provided with honey until they were used in the non-target tests a few days later. All other parasitoids were preserved in 95 percent ethanol.

Non-target testing

Two sets of experiments were conducted to assess *P. euphyllurae* host range and possible non-target effects. In sequential no-choice tests, *P. euphyllurae* females were exposed to the target and non-target hosts - or in the reverse order – consecutively. In choice tests, *P. euphyllurae* females were presented with a choice of target and non-target hosts.

No-choice tests

In these tests, *P. euphyllurae* females were exposed to either the non-target or the target host first for 24 hours. Unlike the tests at UC Riverside, flocculants produced by hosts were not removed prior to exposure to the parasitoid. The researchers decided these waxes could provide volatiles unique to each species. Psyllid hosts were presented on bouquets of infested plant cuttings. The stems of cuttings were tightly fitted through the lid of small cup filled with water and the whole bouquet was then enclosed in a large ventilated deli cup, 14 cm x 10.5 cm in diameter (Figure 4). A honey/water solution was spread on the wall of the cages. One single *P. euphyllurae* female, naïve, one–five days old and held in a glass vial with honey and water since its emergence, was then released into each cage holding a non-target psyllid. After 24 hours, the female parasitoids were transferred to new cages containing the target hosts. After the end of the second 24-hour exposure, the psyllids were left to incubate at 23 °C ± 2 °C, 50 percent RH in the quarantine facility at UC Berkeley for a few weeks after which the plant material was examined to determine the number of parasitized nymphs (mummies) and the presence of adult wasps.

Figure 4. Container used in no-choice tests, UC Berkeley, 2018.

Two native psyllid hosts were tested: *Neophyllura arctostaphyli* found on *Arctostaphylos* spp. (manzanita) *and Euglyptoneura* nr. *robusta* found on *Ceanothus* sp. Infested plant material came directly from the field. Infested manzanita cuttings were collected in two different locations, one each in El Dorado and Napa counties. Infested *Ceanothus* sp. cuttings were collected in El Dorado County, and infested olive cuttings were collected near Carmel, California in Monterey County, or were generated at CDFA in Sacramento.

Choice tests

In these tests, one *P. euphyllurae* female was given a choice between the target host and one non-target host, and its behavioral response was recorded through direct observations using imaging. Three native psyllid species were tested: *Neophyllura arctostaphyli* found on *Arctostaphylos* spp. (manzanita)*, Euglyptoneura* nr. *robusta* found on *Ceanothus* sp., and *Calophya nigrella* found on *Rhus trilobata.* Infested plant material came directly from the field. Infested manzanita and *Ceanothus* sp. cuttings were collected in El Dorado County, while *R. trilobata* cuttings were collected in Siskiyou County. Infested olive cuttings were collected near Carmel, California in Monterey County and used to supplement olive psyllid produced by CDFA in Sacramento.

For each observation, two leaves (or plant parts), one infested with the target species and one infested with the non-target species, were placed in parallel 2 cm apart inside a small petri dish (50 mm diameter). Efforts were made to have two–three nymphs of mixed ages on each leaf. However, it was not always possible to determine the exact numbers and stages of the wax-covered psyllids before the observations because lifting or removing the wax could lead to the permanent displacement of the hosts. Leaves or plant parts bearing psyllid hosts were sometimes cut into smaller pieces to ensure that their sizes were similar between or across all replicates.

Preliminary observations were conducted to define these distinctive behaviors: (1) resting (sitting

motionless with the antennae stretched out); (2) grooming (repeatedly brushing ovipositor or wings with hindlegs, rubbing legs together, or any other actions taken to clean body parts); (3) walking (moving along the substrate at a relatively constant speed with the antennae stretched out); (4) antennating (palpating the substrate with the antennae held close together); (5) probing (quickly inserting ovipositor back and forth into the substrate); and (6) ovipositing (sitting motionless with ovipositor inserted into the host). An additional behavior was added to the list even though not observed: (7) host feeding (feeding on wound inflicted with the ovipositor, either by puncturing or ripping open the host cuticle).

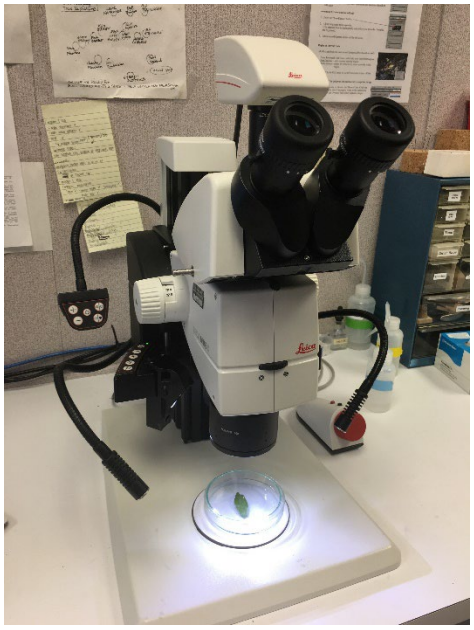

Figure 5. Leica microscope, camera set up.

A single *P. euphyllurae* female, naïve, 1–12 days old and held in glass vials with honey and water since their emergence, was released at equal distance from the two leaves. The released parasitoid was observed under a microscope (Leica 4EZW) and its behavior recorded using the Leica Acquire software (Figure 5). The parasitoid behaviour was continually recorded until either a psyllid was attacked, or the parasitoid left and rested outside of the host patch (i.e., the leaf or plant part) for at least two minutes. Observations where females that did not display a searching behavior (i.e., walking and antennating) after 20 minutes were discarded. Observations were repeated until there were 10 to 15 replicates with each non-target species. All the observations were conducted at 23°C ± 2°C, 50 percent RH in the quarantine facility at the UC Berkeley.

Behavioral data were summarized as follows: (1) first host plant species encountered, (2) time spent (= patch time) on target and non-target host plants, and (3) occurrence of probing, host finding, attacks, and oviposition on both target and non-target host plants. The occurrence of host feeding behavior was further investigated by conducting a small number of observations with older experienced wasps in addition to the ones with naïve wasps as described above. Advanced maternal age, host deprivation, and egg depletion are known factors prompting host feeding behavior in several parasitoid species.

Results

<u>*UC Riverside Tests*</u>. Four non-target psyllids were tested over a three-year period to determine the host specificity of *P. euphyllurae*. This parasitoid was unable to reproduce on any of the 212 exposed non-targets used in the three tests (Table A-2). Each test was replicated 5 to 9 times. Over the same testing period, 17.5% of olive psyllid nymphs produced adult *P. euphyllurae* (range 0 to 35%). Survivorship of the target olive psyllid nymphs was significantly lower than for non-targets exposed to *P. euphyllurae* (Table A-3). However, comparing the overall mortality of non-targets exposed to the parasitoid, it was greater for two of the four tested psyllids than for the target, olive psyllid (Table A-4). The *Arytinnis* and *Ceanothia* exposed to *P. euphyllurae* had equal mortality as with their controls, while *Diaphorina* and *Bactericera* exposed to this parasitoid had significantly higher levels of mortality than the non-exposed control nymphs.

Non target psyllid species	Treatment	% Total Parasitism Non-target psyllid	% Total Parasitism Olive psyllid	Number replicates	Total number exposed hosts T1, T2
Diaphorina citri	Sequential T1 (olive psyllid first), no-choice	0	30	5	20,20
	Sequential T2 (olive psyllid 2nd), no-choice	0	23	5	20,20
	Choice, T3	0	8	9	36,36
Bactericera cockerelii	Choice, T3	0	16.7	9	36,36
Ceanothia ceanothis	Sequential T1 (olive psyllid first), no-choice	0	35.0	6	24,24
	Sequential T2 (olive psyllid 2nd), no-choice	0	25.0	5	20,20
Arytinnis hakani (French broom psyllid)	Sequential T1 (olive psyllid first), no-choice	0	10.0	5	20,20
	Sequential T2 (olive psyllid 2nd), no-choice	0	12.5	5	20,20
	Choice, T3	0	0	5	20,20

Table A-2. Summary of psyllid parasitism in each exposure treatment, 2013 to 2015, UC Riverside.

Non-target species	Treatment tests	n reps= target, nontarget	% psyllid survivorship[1] Non-target	% psyllid survivorship[1] Target	GLM p-value	Model, error d.f.
Arytinnis	Sequential[1]	10,10	88.0_a	35.5_b	0.0002	1,18
	Sequential[2]	9,9	71.7_a	26.7_b	0.0051	1,16
	Choice	5,5	94.0_a	64.0_b	0.0077	1,8
Ceanothia	Sequential[1]	10,10	62.5_a	32.5_b	0.0024	1,18
	Sequential[2]	10,10	40.0_a	19.0_b	0.0023	1,18
	Choice	-	-	-	-	-
Diaphorina	Sequential[1]	10,10	73.0_a	32.5_b	0.0005	1,18
	Sequential[2]	10,10	58.0_a	26.5_b	0.0001	1,18
	Choice	9,9	76.7_a	38.3_b	0.0038	1,16
Bactericera	Sequential[1]	-	-	-	-	-
	Sequential[2]	-	-	-	-	-
	Choice	9,9	61.7_a	20.0_b	0.0004	1,16

Table A-3. Comparing survivorship of non-target to target psyllids when exposed to *P. euphyllurae*, during testing from 2013 to 2015 at UC Riverside.

[1]shown are back-transformed arc-sine values used in analyses. Dashes indicate incomplete test

Table A- 4. Mortality of non-targets, comparing nymphs exposed to P. euphyllura vs. a no-exposure control. From 2013 to 2015 at UC Riverside. Sequential tests are no-choice exposures.

Non target species	Treatment tests	n reps =target, nontarget	% Nontarget Mortality[1] Test	% Nontarget Mortality[1] Control	GLM, p-value	Model, Error d.f.
Arytinnis	Sequential[1]	10,10	12.0_a	21.0_a	0.4600	1,18
	Sequential[2]	9,10	28.3_a	21.0_a	0.5997	1,17
	Choice	5,3	6.0_a	0.0_a	0.4816	1,6
Ceanothia	Sequential[1]	10,10	$37.5.0_a$	42.0_a	0.6500	1,18
	Sequential[2]	10,10	60.0_a	42.0_a	0.0798	1,18
	Choice		-	-	-	-
Diaphorina	Sequential[1]	10,10	27.0_a	6.0_b	0.0143	1,18
	Sequential[2]	10,10	42.0_a	18.0_b	0.0006	1,18
	Choice	9,9	23.3_a	0.0_b	0.0017	1,17
Bactericera	Sequential[1]		-	-	-	-
	Sequential[2]		-	-	-	-
	Choice	9,9	38_a	0_b	0.0001	1,16

[1]LSD at α=0.05 tests were conducted when model p-values were less than 0.05.
[2]shown are back-transformed arc-sine values used in analyses. Dashes indicate incomplete test.

UC Berkeley

No-choice tests. Although efforts were made to have nymphs of mixed ages on each plant material, it was not always possible to determine the exact numbers and stages of the wax-covered psyllids before the tests because lifting or removing the wax could lead to the permanent displacement of the hosts. Unfortunately, it was later discovered that wax is not a good indicator of psyllid presence. Overall, no psyllids (dead or alive) nor even psyllid exoskeletons were found in 37 percent of the replicates at the end of the experiments. This percentage is the highest in the *Ceanothus* sp. plant replicates suggesting that *Euglyptoneura robusta* is either a highly mobile and/or easily disturbed psyllid species. In contrast, psyllids or traces of the presence of psyllids (exoskeletons) were always found in the olive replicates (Table A-5). Another drawback with this experimental set up was the difficulty of keeping the plant material alive for the duration of the parasitoid development (several weeks). Despite best efforts to keep the stems of the bouquets submerged in water, the cuttings tended to dry up quickly, especially the *Ceanothus* sp. foliage.

Despite these problems, seven *P. euphyllurae* adult wasps were reared from those tests: all emerged from olive replicates, none from any of the non-target psyllids *E. robusta* or *Neophyllura arctostaphyli* (Table A-5), showing this parasitoid can reproduce on the target, olive psyllid *Euphyllura olivina.* These results also show that the same parasitoid is unable to attack and reproduce on these non-target native psyllids. One taxon of native parasitoid emerged from both of the non-targets, a species of *Syrphopagus* sp. (Hymenoptera: Encyrtidae), one from *E. robusta*, (*Ceanothus* sp.) and one each from the El Dorado and Napa county populations of *N. arctostaphyli* (manzanita). Parasitoids were identified by Dr. Robert Zuparko, UC Berkeley.

Table A-5. No-choice, sequential tests for non-target species tested in 2018 at UC Berkeley. Shown are number of replicates (n=number psyllid nymphs) where no psylllids were found at the end of the experiments and number of adult *P. euphyllurae* emerging.

Non-target Species	Sequence	n	Host plant	No. of replicates with no psyllids	*P. euphyllurae*
Euglyptoneura robusta (*Ceanothus* sp.)	NT - T	20	NT	15 (75%)	0
	NT - T	20	T	0	2
	T - NT	18	NT	11 (60%)	0
	T - NT	18	T	0	2
Neophyllura arctostaphyli El Dorado Co. (manzanita)	NT - T	15	NT	0	0
	NT - T	15	T	0	0
	T - NT	8	NT	2 (25%)	0
	T - NT	8	T	0	1
Neophyllura arctostaphyli, Napa Co., manzanita	NT - T	6	NT	2 (33%)	0
	NT - T	6	T	0	2
	T - NT	10	NT	3 (30%)	0
	T - NT	10	T	0	0

Results of choice tests

A total of 62 observations were conducted but only 37 female parasitoids demonstrated a clear searching behavior. The target host plant was the first material encountered by the wasp (= first choice) in 69 to 73 percent of the observations (Table A-6). Time spent on the non-target host plants were significantly shorter than time spent on target host plants, compared using a paired t-test (Sokal and Rohlf, 1981) at least for two of the three non-target species tested: *E. robusta* ($t = 2.69$, $df = 6.7$, $P = 0.032$) and *R. trilobata* ($t = 4.57$, $df = 6$, $P = 0.004$). There was no significant difference in patch time when searching on manzanita vs. olive leaves ($t = 0.30$, $df = 8.4$, $P = 0.770$, Table A-6).

Table A-6. Total numbers of observations (n) for each choice test in 2018 at UC Berkeley. Shown are numbers of observations where target or non-target host plant was first encountered (first choice), and mean patch time in min (± SE) on target and non-targets.

Test	n	First choice	Number of observations	Patch time
Manzanita vs. olive	16	Target	11	15.9 ± 3.54
	16	Non-target	5	14.1 ±4.88
Ceanothus vs. olive	11	Target	8	16.6 ± 3.50
	11	Non-target	3	3.7 ± 3.26

Test	n	First choice	Number of observations	Patch time
Rhus vs. olive	10	Target	7	20.1 ± 4.38
	10	Non-target	3	0.1 ± 0.01

Encounters with the target host plant always led to the wasps searching its surface, while encounters with wax always triggered probing behavior (Table A-7). Searching and probing led to host discovery in 53 to 80 percent of the cases, and parasitoid attack always followed host discovery. However, attacks did not always end up in oviposition because these attempts sometimes caused the psyllid host to flee and successfully escape.

Probing on non-target host plants was observed in one test with manzanita (Table A-7). However, it did not lead to host discovery and eventually the wasp left to investigate the target host plant where it searched and probed the surface. However, that wasp was unsuccessful at locating a host because of the unusual thick layer of wax protecting them in this specific replicate. In the remaining observations on non-target host plants, probing was never observed (Table A-7).

Table A-7. Total number of observations (n) on target and non-target host plants with number of observations where female parasitoids were seen probing the substrate, finding, attacking and ovipositing in a psyllid host, 2018, UC Berkeley.

On Target

Test	n	Pro-bing	Host finding	Attack	Ovipo-sition
Ceanothus vs. olive	3	0	0	0	0
Rhus vs. olive	3	0	0	0	0

On non-target

Test	n	Pro-bing	Host finding	Attack	Ovipo-sition
Ceanothus vs. olive	3	0	0	0	0
Rhus vs. olive	3	0	0	0	0

Oviposition was very distinctive from probing in terms of duration and wasp movement. While probing was characterized by quick insertions (less than a second) of the ovipositor into the substrate (wax or host), oviposition lasted longer (2.21 ± 0.20 (SE) minutes, n = 16). Also, the wasp remained completely motionless during oviposition, in clear contrast with the restless activity during searching and probing. Oviposition attempts often resulted in the host fleeing the attack. However, once the ovipositor was inserted into the hosts, they seemed temporarily paralyzed during the duration of the oviposition but were usually able to walk away soon after the attack.

Host feeding behavior was never observed, above, for both young (1–12 days) and older females (Table A-7). Four 16- to 24-day old females, with previous oviposition experience on the target hosts and kept separately in a glass vial with just a water/honey for a week, were tested similarly. All of them were able to successfully find a host after searching and probing the target host plant. Three of them were able to successfully attack and oviposit in a host without exhibiting any host feeding behavior.

About 40 percent of the parasitoid females tested did not respond to either choice, spending most of their time motionless or grooming on the side or floor of the Petri dish. In the researcher's observations, non-responsive *P. euphyllurae* females tended to be younger (3.3 ± 0.7 days, n = 25) than responsive females (6.4 ± 0.8, n = 37; t = 2.67, df = 59.9, P = 0.0096). In many parasitoid species (usually synovigenic (parasitoids that do not have a full complement of eggs at eclosion and that continue to mature them throughout adult life; females require host-supplied nutrients for egg production) species), freshly emerged females require additional time (several days) to mature their eggs (preoviposition period). This result suggests that it might be the case for *P. euphyllurae* although there are numerous other physiological or environmental factors that could affect a parasitoid response to the presence of hosts.

References cited in this appendix are included in VII. References.

IX. Appendix B. Release and Post-release Monitoring (Pickett et al., 2019)

As a part of the post-release monitoring effort, the permit applicants have started measuring the degree of olive psyllid infestation prior to releases and surveying for their spread in the state. This will aid in measuring impact of the released biocontrol agent. Pre-release monitoring of olive psyllid started with survey work initiated by M. Johnson (2010, unpubl. data). Johnson's study was designed to determine the spread of olive psyllid while more recent work was designed to measure the population size and extant natural enemies associated with olive psyllid infestations. Two measurements were used to estimate psyllid density size: a visual ranking for the degree of canopy infestation, and secondly, stem cuttings to determine number of insects per cm stem where infestations were found.

Release of *Psyllaephagus euphyllurae*

Psyllaephagus euphyllurae will be released into heavily infested olive trees located on secured, managed properties. Trees will be free of insecticide treatments, harvesting, and trimming. The more heavily infested trees at this time are located near coastal locations in southern California. Potential sites include city and state parks where contact has been established with property managers. Each release site should have multiple infested trees. Pre-release sampling as described above, will be conducted at all potential release sites each year prior to releases.

Psyllaephagus euphyllurae parasitoids for release will be collected from original sampling locations described above and augmented in numbers by releasing onto lab cultured olive psyllids. Multiple releases will be made at each site, with at least 200 females released on each event. No sampling of trees will be done following releases to minimize loss of *P. euphyllurae* populations until just prior to year two of releases.

Monitoring of *Psyllaephagus euphyllurae*

If populations of *P. euphyllurae* can be recovered three consecutive years without additional releases, it will be declared established at that particular location. Post release monitoring to determine establishment will be done prior to each release and will include multiple samples during the prime time of parasitoid reproduction, March through July. Annual surveys will be done to measure the spread of the olive psyllid and then following releases of the parasitoid, to determine its spread. To determine the presence of parasitized nymphs during the early phases of population buildup (just prior to releases and after last release), cuttings of infested stems will be returned to the laboratory, placed in water picks or other media to maintain the longevity of the cut stems, allowing for nymphs to develop into adults or mummies. Only stems with high nymphal populations will be selected (randomly) for removal. The permit applicant anticipates the olive psyllid population becoming a minor problem unless P. *euphyllurae* populations are disrupted due to pesticide applications.

Populations of both the olive psyllid and *P. euphyllurae* will be monitored for at least 5 years once they have been permanently established. Infested olive trees within 100 meters will be

examined for parasitism as well. Once spread has been confirmed, trees several kilometers away will be examined. The most closely related psyllid tested, and associated with manzanita, is *Neophyllura arctostaphyli*. Manzanita is found throughout California. Although results from host specificity testing shows that under conservative testing conditions, the *P. euphyllurae* does not attack this native, efforts will be made in the field to confirm these findings. Populations of this psyllid within 1 kilometer of release sites will be collected and held for adult psyllid emergence to determine if *P. euphyllurae* is capable of attacking the manzanita psyllid, *N. arctostaphyli*. Similarly, other non-targets that could be collected, if near established populations of *P. euphyllurae*, will include *Calophya* sp. (on *Rhus trilobata*) and potato psyllid. Because there are no known parasitoids attacking olive psyllid in California, interactions with other natives are not anticipated.

Reference:

Pickett, C.H., J.M. Jones, S. Triapitsyn, and E. Hougardy. 2019. A petition for release of *Psyllaephagus euphyllurae* (Hymenoptera: Encyrtidae) Collected in Eastern Spain for the Biological Control of Olive Psyllid, *Euphyllura olivina* (Hemiptera: Liviidae, formerly Psyllidae) in California. Report submitted to USDA APHIS. California Department of Food & Agriculture, and University of California, Riverside and Berkeley. 55 pp.