United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Field Release of the Parasitoid *Diaphorencyrtus aligarhensis* for the Biological Control of the Asian Citrus Psyllid in the Contiguous United States

Environmental Assessment, October 2014

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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), Pest Permitting Branch is proposing to issue permits for release of the insect parasitoid[1] species *Diaphorencyrtus aligarhensis* (*D. aligarhensis*) (Hymenoptera: Encyrtidae). This organism would be used by the permit applicant for biological control of the nonindigenous Asian citrus psyllid (ACP) *Diaphorina citri* (Hemiptera: Liviidae) in the contiguous United States.

This environmental assessment[2] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act (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, *D. aligarhensis* to control ACP within the contiguous United States. This EA considers a "no action" alternative and the potential effects of the proposed action.

The applicant's purpose for releasing *D. aligarhensis* is to reduce infestations of ACP in California, although this agent could spread or be released anywhere in the contiguous United States. The ACP is an insect pest of citrus because it vectors a plant pathogenic bacterium, *Candidatus* Liberibacter asiaticus Jagoueix, Bove´ and Garnier, that causes a lethal citrus disease, known as huanglongbing (HLB) or citrus greening (Halbert and Manjunath, 2004; Bové 2006; Grafton-Cardwell et al., 2013). ACP and HLB are serious invasive species that cause immense economic loses in countries with important citrus industries.

In Florida, ACP was first detected in 1998 and HLB was discovered in 2005 (Hall and Hentz, 2011). Since that time, HLB has been found in commercial and residential sites in all counties of Florida with commercial citrus, and it is having an adverse economic impact on citrus production in that State (Hodges and Spreen, 2012). HLB has also been detected in California, Georgia, Louisiana, South Carolina, and Texas. Other States, including Alabama, Arizona, Hawaii, and Mississippi, are at risk for HLB because populations of ACP have been found in those States. See Appendix 1 for a map of national quarantine boundaries for ACP and HLB.

[1] In this case, small, stingless wasps that during their development, live in the body of a single host individual, eventually killing that individual.

[2] Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 *et seq.*) provide that an environmental assessment "[shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

Economic losses to growers in Florida attributable to HLB have been estimated at greater than $1.7 billion which translates to approximately a 16 percent reduction in grower revenues (Hodges and Spreen, 2012). An effective ACP management program is considered an important part of reducing the spread of HLB (USDA –APHIS, 2012).

All of the existing ACP management options (discussed below) are expensive, temporary, ineffective, 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 ACP in the contiguous United States, particularly for control of ACP in backyard citrus.

II. Alternatives

This section will explain the two alternatives available to APHIS–PPQ: no action (no issuance of permits) and issuance of permits for environmental release of *D. aligarhensis* in the contiguous United States. Although APHIS' alternatives are limited to a decision of whether to issue permits for release of *D. aligarhensis*, other methods are described that are currently used to control ACP in the United States. Use of these control methods is not an APHIS decision, and their use is likely to continue whether or not APHIS–PPQ issues permits for environmental release of *D. aligarhensis.*

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

A. No Action

Under the no action alternative, APHIS–PPQ would not issue permits for the field release of *D. aligarhensis* for the control of ACP — the release of this biological control agent would not occur, and current methods to control ACP in the United States will continue. Use of these methods is likely to continue even if APHIS–PPQ issues permits for release of *D. aligarhensis.* Presently, control of ACP in the United States is limited to chemical and biological control methods.

1. Chemical Control

There are many insecticides that may be applied to citrus as systemic and foliar sprays against ACP. Systemic insecticides are applied and

translocated throughout the tree. Systemic insecticides include imidacloprid, aldicarb, and thiamethoxam. Foliar sprays are applied to the leaves and include insecticides such as fenpropathrin, zeta-cypermthrin, chlorpyrifos, chlorantraniliprole, spirotetramat, abamectin, and carbaryl. Organic insecticide options are available to control ACP including Neem oil, pyrethrin, and narrow range oil. There are no chemical controls for HLB.

2. Biological Control

The parasitic wasp *Tamarixia radiata* has been released against ACP in Florida and California. It causes considerable mortality of ACP in some locations in Florida. Efficacy of *T. radiata* is still being evaluated in California, but it is not widely established there. APHIS prepared an environmental assessment and finding of no significant impact for environmental release of *T. radiata* in the contiguous United States (USDA−APHIS, 2010a).

D. aligarhensis originating from Taiwan and from China were previously released into Florida (McFarland and Hoy, 2001) but with mixed results (Halbert and Manjunath, 2004). It has not established in Florida, perhaps due to competition from *T. radiata,* generalist predators, and pesticides (Rohrig et al., 2012).

Native predators, such as spiders, coccinellids (lady beetles), lacewings, syrphids, and minute pirate bugs attack ACP (Quereshi and Stansly, 2009; UC-DANR, 2006). Coccinellids are important predators of ACP in Florida (Michaud, 2002; 2004).

B. Issue Permits for Environmental Release of *D. aligarhensis* (Preferred Alternative)

Under this alternative, APHIS–PPQ would issue permits upon request and after evaluation of each application for the field release of *D. aligarhensis* for the control of ACP in the contiguous United States. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

1. *D. aligarhensis* Taxonomic Information

(From Hoddle and Bistline, 2013).

Diaphorencyrtus aligarhensis is an endoparasitoid attacking second to fourth instar ACP nymphs. Oviposition (egg laying) by *D. aligarhensis* in the ACP nymph, and feeding of the larval *D. aligarhensis* inside of the ACP nymph eventually kills the pest.

a. Taxonomy.

Diaphorencyrtus aligarhensis (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae: Encyrtinae) was originally described from a specimen reared from "*Psylla* sp. on Citrus sp." that was collected in September 1968 in Aligarh, Uttar Pradesh, India (Shafee et al., 1975) and has been subsequently described multiple times (Ruggiero et al., 2011). The Universal Chalcidoidea Database (Noyes, 2013) lists the following synonomies:

Aphidencyrtus aligarhensis Shafee, Alam & Agarwal, 1975
Aphidencyrtus diaphorinae Myartseva & Trjapitzin, 1978
Aphidencyrtus sacchari Kaul & Agarwal, 1986
Diaphorencyrtus aligarhensis (Shafee, Alam & Agarwal, 1975)
Diaphorencyrtus diaphorinae (Lin & Tao, 1979)
Diaphorencyrtus diaphorinae (Myartseva & Trjapitzin, 1978)
Psyllaephagus diaphorinae Lin & Tao, 1979
Syrphophagus aligarhensis (Shafee, Alam & Agarwal, 1975)

b. Natural geographic range, other areas of introduction of *D. aligarhensis*.

Noyes (2013) lists the following countries from which *D. aligarhensis* has been recorded: India (Andhra Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh), China (Fujian [Fukien], Guangdong [Kwangtung], Guangxi [Kwangsi]), Philippines, Réunion, South Africa, Taiwan, United States (Florida), and Vietnam.

Diaphorencyrtus aligarhensis has been deliberately released for the biological control of ACP in Florida and Réunion (Rohrig, 2011). It established in Réunion (van den Berg, 1985) but failed to establish in Florida despite multiple releases of this species that were sourced from Taiwan (released 2000-2002) and China (released 2007-2009) (Rohrig et al., 2012). This parasitoid was deliberately released in South Africa (imported from Réunion in 1983) for control of the citrus pest *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) and failed to establish because it was too host specific (van den Berg, 1985). In Taiwan, where *D. aligarhensis* is native, it is not as effective as *T. radiata* (introduced into Taiwan over 1984-1988 from Réunion) possibly because *D. aligarhensis* suffers high levels of attack by hyperparasitoids (Chien et al., 1989).

In Saudi Arabia, *D. aligarhensis* is the dominant parasitoid attacking ACP nymphs in Mexican lime orchards (Al-Ghamdi and Faragalla, 2000a) where it achieves maximum parasitism rates of approximately 60−70 percent (Al-Ghamdi and Faragalla, 2000b). It was likely self-introduced

with ACP into Saudi Arabia, or it may be native having moved onto ACP from an unknown psyllid host.

c. Life history of *D. aligarhensis*

Diaphorencyrtus aligarhensis is a solitary endoparasitoid which can parasitize second through fourth instar ACP nymphs (Rohrig et al., 2012). An endoparasitoid completes all development inside the host. Besides parasitizing ACP, adult females also kill ACP by host feeding on nymphs (Skelley and Hoy, 2004); females insert their egg laying organ (ovipositor) into the ACP nymph to make a hole and then suck up the liquid that leaks out (Hoy and Nguyen, 2001). ACP has five nymphal instars but the fifth instar is unsuitable for parasitization or host feeding by *D. aligarhensis* (Skelley and Hoy, 2004). Adult females are capable of killing up to 280 ACP nymphs through host feeding and parasitism combined (Chien, 1995).

Development from when the *D. aligarhensis* egg is laid inside an ACP nymph to when *D. aligarhensis* emerges as an adult requires about 12-16 days at 25°C, depending on the nymphal instar (Rohrig et al., 2012). *Diaphorencyrtus aligarhensis* larvae attach the parasitized ACP nymph to plant material by chewing a hole through the top of the host; fluids ooze out through the hole and "glue" the parasitized ACP nymph to the plant. After development is completed inside the ACP host, an adult *D. aligarhensis* wasp emerges from the back end of the dead ACP host.

Populations of *D. aligarhensis* imported into Florida from Taiwan and China for biological control of ACP were thelytokous (only females are produced), and the production of all female offspring in both of these populations is likely the result of infection with *Wolbachia*, a sex ratio altering bacteria, for which both populations tested positive (Rohrig et al., 2012; Jeyaprakash and Hoy, 2000). In comparison, *D. aligarhensis* collected from Punjab Pakistan are bi-parental and the sex ratio is one male to one female. All host range experiments presented in this document were performed with this bi-parental population of *D. aligarhensis* that is maintained in quarantine at University of California Riverside, as described by Hoddle and Bistline (2013).

III. Affected Environment

A. Asian Citrus Psyllid

ACP is a pest of citrus and close relatives of citrus, and is the target of *D. aligarhensis*. Psyllids resemble miniature cicadas and are sometimes called jumping plant-lice. ACP damages plants through its feeding activities. New shoot growth that is heavily infested by ACP does not expand or develop normally, and is more susceptible to breaking off. While direct damage is

serious, there is even greater concern as ACP is an efficient vector of the bacterium that causes HLB.

ACP is found in tropical and subtropical Asia, Afghanistan, Saudi Arabia, Réunion, Mauritius, parts of South and Central America, Mexico, and the Caribbean. In the United States and its territories, this species is present in all or parts of Alabama, California, Florida, Georgia, Guam, Hawaii, Louisiana, Mississippi, Puerto Rico, South Carolina, and Texas.

ACP is known to develop only on members of the plant family Rutaceae including the genera *Aegle, Aelopsis, Afraegle, Atalantia, Balsamocitrus, Citropsis, Citrus, Clausena, Eretmocitrus, Fortunella, Limonia, Merrillia, Microcitrus, Murraya, Naringi, Pampurus, Poncirus, Severina, Swinglea, Toddalia, Vepris,* and *Zanthoxylum. Murraya paniculata* (L.) Jack (orange jasmine) and *Citrus* spp. are the preferred hosts of ACP (Aubert, 1987; Halbert and Manjunath, 2004; Yang et al., 2006).

B. North American Psyllid Species

Hundreds of psyllid species occur on both native and introduced landscape plants in the United States. Several psyllid species are pests of crops such as pear, potato, and tomato. Each kind of psyllid feeds on only one plant species or closely related group of plants. Some psyllids have been released for biological control of weeds, such as *Boreioglycaspis melaleucae*, released in 2002 in Florida against the invasive plant *Melaleuca quinquenervia*. California has the richest native psyllid fauna in North America, and the influence of climate and plant diversity in this region is considered key to the diversification of native psyllids. There are currently 165 psyllid species known from California. Most psyllids native to the United States are relatively uncommon and rarely become pests. Psyllid species in North America could possibly be attacked by *D. aligarhensis*.

C. Citrus Resources in North America

Citrus resources in the United States are at risk from ACP and HLB. The major citrus producing states in the United States include Florida, California, Texas, and Arizona. Of these states, Florida produced 63 percent of the total U.S. citrus crop in 2012, California produced 34 percent, and Texas and Arizona combined produced the remaining 3 percent (NASS, 2013). Florida is the largest producer of oranges, accounting for about 70 percent of total U.S. production, and of grapefruit, producing nearly 65 percent of total production (NASS, 2013). California is the largest producer of lemons, producing more than 92 percent of production, and of tangerines, accounting for about 80 percent of production (NASS, 2013).

In 2012, total U.S. citrus exports were valued at $1.0 billion, a 2 percent decrease from 2011. In terms of value, fresh oranges and orange juice were the major U.S. citrus export items, followed by fresh grapefruit, lemons and grapefruit juice (FAS, 2012). The top three overseas markets for U.S. citrus were Japan, South Korea and Canada (FAS, 2012).

There are many nursery operations in the United States that produce citrus trees, orange jasmine, curryleaf, and other ACP host plants and will be adversely affected by ACP and HLB. Many of these operations are identified by the Small Business Administration as small businesses.

IV. Environmental Consequences

A. No Action Alternative

1. Impact of ACP and HLB on Hosts of ACP and Citrus Resources

ACP damages citrus by feeding on young foliage, depleting the sap, and causing galling or curling of leaves. High populations feeding on a citrus shoot can kill the growing tip. ACP also causes damage by excreting honeydew that coats plant leaves, allowing the growth of sooty mold that reduces or inhibits sunlight penetration (Chien and Chu, 1996). However, the worst threat is that ACP is an efficient vector of the bacterium which causes HLB. This is the most serious disease of citrus in the world, causing reduced production of fruit and eventual death of the trees. HLB-infected trees show a blotchy, mottled condition of the leaves which results in the development of yellow shoots, the characteristic symptom of the disease. Trees are stunted, decline, and bear very few lop-sided, poor quality fruit. The fruit produced by infected trees is not suitable for either the fresh market or juice processing due to the significant increase in acidity and bitter taste. The lack of effective control measures to prevent the spread of HLB from sites of infestation to other areas and counties could lead to higher production costs and an increase in shortages of citrus fruits and plants to the general economy. This would potentially result in increased costs for survey, detection, and treatment for the control of ACP and HLB as they spread to other areas and counties.

United States citrus production for 2013/2014 is forecast down 11 percent to 6.7 million tons (FAS, 2014). In Florida, early dry weather and HLB have caused severe fruit drop (FAS, 2014). In California, production is down 5 percent and as a result of the December freeze, damaged fruit will be sent to be juiced (FAS, 2014). Between 2006 and 2012, HLB was estimated to have cost Florida's economy $4.5 billion and 8,257 jobs (Hodges and Spreen, 2012).

Insecticide usage to control ACP has increased significantly in Florida and other citrus-producing States since the introduction of HLB, with 6 or more applications per year. Prior to this, ACP was considered a minor pest. However, growers must apply both systemic soil drench treatments along with multiple foliar sprays (especially to protect newly planted and young (4 to 8-year old) trees that are more vulnerable to ACP attack because they flush more frequently. This pattern is similar to what has occurred in Brazil, South Africa, and other countries where HLB has been introduced. In Brazil, foliar applications of insecticides have steadily increased from 12 up to 24 per year, and soil treatments from 2 up to 4 per year. In an effort to reduce costs, pesticides with longer residual effects are being substituted. Pesticides also are being applied aerially over large areas to achieve greater control of ACP. ACP populations are normally higher in areas where there are commercial citrus plantings. When coordinated insecticide applications are completed on a majority of commercial citrus plantings in an area during a relatively short window of one or two weeks, ACP is easier to manage. In addition, many residential areas have citrus trees in private gardens that can harbor ACP. Homeowner resistance to the application of insecticides has prevented treatment of infested trees in some locations.

For nurseries in the United States that produce citrus trees, orange jasmine, and curryleaf, the effects of ACP and HLB will be adverse. For areas quarantined for HLB, nursery operations will suffer a complete loss of domestic market access. For areas quarantined only for ACP but not for HLB, nursery operations will incur costs associated with treatment prior to interstate movement of the articles. APHIS published regulations that allow the movement of regulated nursery stock, under specific conditions, from an area quarantined for ACP, but not for an area quarantined for HLB (USDA−APHIS, 2011).

2. Impact from the Use of Other ACP and HLB Control Methods

The continued use of chemical and biological control at current levels would result if the “no action” alternative is chosen, and may continue even if permits are issued for environmental release of *D. aligarhensis.*

a. Chemical Control

The environmental consequences of many of the insecticides used for control of ACP were analyzed by APHIS (USDA–APHIS, 2010b) and will not be discussed further in this document. However, an additional environmental consequence of the use of insecticides for control of ACP is a reduction in occurrence of natural predators of ACP, such as lady beetles, syrphids, and spiders. Many of the insecticides used to control ACP are

broad-spectrum insecticides which disrupt not only native predators of ACP but also of other citrus pests.

b. Biological Control

Native predators and previously released biological control agents (*Tamarixia radiata*) will remain in the environment and exert some level of control over ACP. However, these are not adequate to control ACP populations in the United States. Impacts of *T. radiata* have been modest at best, with parasitism levels often less than 10 percent on average, in commercial citrus production areas (Michaud, 2004; Qureshi and Stansly, 2009).

B. Biological control alternative (preferred alternative)

1. Environmental Impacts of the Proposed Release of *D. aligarhensis.*

a. Known impact on vertebrates including humans.

Diaphorencyrtus aligarhensis is an obligate parasitoid of psyllids, specifically ACP. As such, it will rarely come into contact with humans or other vertebrates, and if it does, it is incapable of stinging or biting. Encyrtid wasps have no known adverse impacts on humans or other vertebrates.

b. Direct impact of *D. aligarhensis* (e.g., intended effects on ACP, direct effects on non-targets).

To evaluate the effects of *D. aligarhensis* on non-target psyllid species, host specificity tests were conducted in the United States to determine possible direct effects on non-target psyllid species. Six non-target psyllid species were tested for their suitability as hosts for *D. aligarhensis* reproduction. Host range was assessed using seven experimental designs encompassing no-choice sequential tests, prolonged no choice and choice tests.

The non-target test species were comprised of:
(1) *Bactericera cockerelli* (the native pestiferous potato/tomato psyllid) (*n* = 480 total nymphs exposed);
(2) *Heteropsylla* sp. (the native Acacia psyllid) (*n* = 390 nymphs exposed);
(3) *Arytainilla spartiophylla* (a self-introduced natural enemy of the noxious weed scotch broom psyllid) (*n* = 380 nymphs exposed);
(4) *Euphyllura olivina* (the invasive olive psyllid) (*n* = 320 nymphs exposed);

(5) *Heteropsylla texana* (the native honey mesquite psyllid) (*n* = 395 nymphs exposed);
(6) *Dichlidophlebia fremontiae* (the native fremontia psyllid) (*n* = 375 nymphs exposed).

Successful parasitism of a non-target species by *D. aligarhensis* was observed for only one species tested: *B. cockerelli*, the native and pestiferous potato psyllid, the vector of the zebra chip bacterium that causes significant economic losses in potatoes (Butler and Trumble, 2012). Observed parasitism rates ranged from 11−18 percent for the treatment types. Two of the treatment types exhibited moderate levels of parasitism, at 17−18 percent. When compared to *D. aligarhensis* ACP parasitism rates across all treatment types (27−45 percent), it is likely *B. cockerelli* is not a highly preferred host for *D. aligarhensis*, but it may be attacked by *D. aligarhensis* if released into the environment. In California, there is a guild of generalist predators and parasitoids that attack *B. cockerelli* (Butler and Trumble, 2012). See Appendix 2 for a more detailed discussion of ACP host specificity testing conducted by Hoddle and Bistline (2013).

In addition, studies have conclusively demonstrated that *D. aligarhensis* does not transmit *Candidatus* Liberibacter asiaticus, the pathogen that causes HLB in citrus (Hoy et al., 2001).

2. Uncertainties Regarding the Environmental Release of *D. aligarhensis.*

Once a biological control agent such as *D. aligarhensis* is released into the environment and becomes established, there is a possibility that it could move from the target insect (ACP) to attack nontarget insects, such as native psyllids. Based on host specificity testing conducted, *D. aligarhensis* only successfully parasitized one psyllid species other than ACP, *B. cockerelli,* the potato/tomato psyllid. The rate of parasitism was less on *B. cockerelli* than on ACP. 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 *D. aligarhensis*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *D. aligarhensis* generally spread without intervention by man. In principle, therefore, release of these parasitoids 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, *D. aligarhensis* may not be successful in reducing ACP 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 ACP populations by *D. aligarhensis* will not be known until after release occurs.

The environmental consequences discussed under the "no action" alternative may occur even with the implementation of the biological control alternative, depending on the efficacy of *D. aligarhensis* to reduce ACP populations in the contiguous United States.

3. Cumulative Impacts

"Cumulative impacts are defined as the impact 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).

Three USDA agencies have primary roles in combating HLB: APHIS, Agricultural Research Service (ARS), and National Institute of Food and Agriculture (NIFA). ARS and NIFA focus on research. APHIS focuses on survey and detection, regulatory action, and the development of data and protocols the citrus industry can use to manage, suppress, and slow the spread of the disease.

Specifically, APHIS' Citrus Health Response Program (CHRP) funds the administration of domestic regulations, pest surveys, coordinated area-wide suppression of ACP, and several initiatives in cooperation with State regulatory agencies and the citrus industry in all of the major citrus-producing States: Arizona, California, Florida, and Texas. APHIS also conducts a public outreach and education program to increase the urgency about the risk of moving citrus plants in States and Territories with Federal citrus quarantines. ARS conducts HLB-related research. Currently, ARS is using a multi-faceted approach aimed at the three components of the disease: resistance in the citrus host, suppression of the ACP vector, and control of the HLB causal organism. NIFA supports research on HLB, including ways to limit the spread of the disease and innovative management techniques to address the ACP vector.

States are heavily involved in implementing a variety of response activities to ACP and HLB, such as conducting ACP and HLB surveys and ACP treatments, entering into compliance agreements with packing houses to ensure that fruit packing processes meet certain standards to minimize disease spread, and certifying citrus products. The States use their regulatory authority to establish intrastate HLB quarantine areas that parallel the Federal quarantine areas.

The citrus industry works closely with USDA and State departments of agriculture to combat HLB. The industry coordinates its HLB and ACP management efforts based on USDA- and State-provided data on ACP and HLB detections and population distribution. Industry-funded initiatives include operational activities, research, and outreach and education. Growers in the citrus-producing States conduct management programs for ACP in commercial citrus; remove HLB-infected commercial and residential citrus; and conduct outreach to homeowners.

Release of *D. aligarhensis* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to ACP. Effective biological control of ACP would have beneficial effects for ACP and HLB management programs, and may result in a long-term, non-damaging method to assist in the control of ACP and HLB, and prevent their spread into other areas potentially at risk from invasion. Suppression of ACP in citrus located in residential areas will not be achieved as it is in orchards where insecticides are routinely used. The primary means of suppressing ACP in residential citrus will be through biological control (USDA−APHIS, 2012).

4. 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 or endangered species or result in the destruction or adverse modification of critical habitat.

APHIS has determined that, based on the host specificity of *D. aligarhensis,* there will be no effect on any listed insect in the contiguous United States. In host specificity testing, the biological control organism preferred ACP and only successfully parasitized one non-target psyllid species, *B. cockerelli*, the potato/tomato psyllid. No psyllids are federally listed threatened or endangered insects (FWS, 2014). In addition, no listed species is dependent on ACP as a food source. Although certain federally listed plants occur in the family Rutaceae and may serve as hosts of ACP, release of *D. aligarhensis* will not benefit any of these species because all occur outside of the contiguous United States (Hawaii, Puerto Rico, or the Virgin Islands). *D. aligarhensis* will only be released within the contiguous United States.

V. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income

Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects from the field release of *D. aligarhensis*, and their release will not have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, "Protection of Children From Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of the field release of *D. aligarhensis*.

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 sent letters of notification and requests for comment and consultation on the proposed action to tribes in California, Arizona, Florida, Louisiana, Alabama, Mississippi, and Texas. 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 APHIS. The addresses of participating APHIS units, cooperators, and consultants follow.

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VII. References

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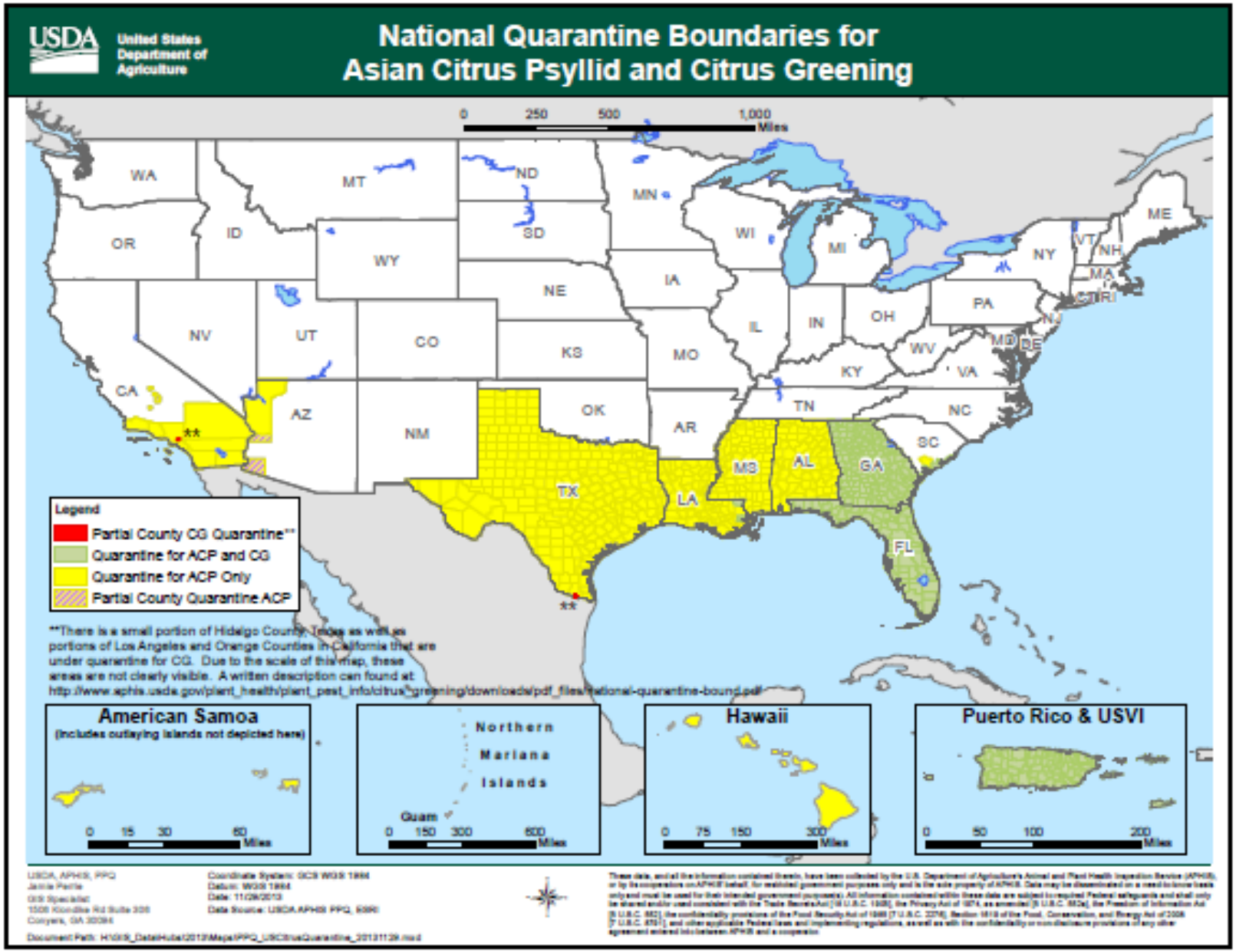
Appendix 1. National Quarantine Map for ACP and HLB.



Appendix 2. Host-specificity testing from Hoddle and Bistline, 2013.

MATERIALS AND METHODS FOR HOST SPECIFICITY TESTING

A. REARING

Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae): Asian citrus psyllids (ACP) used for host range assessment experiments were taken from colonies established at the University of California, Riverside (UCR), in Insectary-level secure laboratories at the Insectary and Quarantine Facility (IQF) (Facility Number 93). Colonies were maintained under California Department of Food and Agriculture (CDFA) Permit No. 2870, and in accordance with designated CDFA and USDA protocols. All ACP used were from colonies initiated from California collected material and confirmed to be huanglongbing (HLB) free.

Host plant production for ACP colonies: Seedlings of *Citrus volkameriana* used as host plants for rearing ACP were obtained either through Willits and Newcomb Inc. citrus nursery (Arvin, CA), or from the CDFA rearing facility at Mt. Rubidoux Field Station (Rubidoux, CA). ACP were maintained on *C. volkameriana* seedlings which ranged from less than one year to approximately two years of age, potted in 4 inch diameter pots. Success of ACP reproduction, regardless of plant age, is strongly stimulated by the presence of young budding flush ("feather flush") and leaf axils, on which ACP females lay eggs. To maintain constant numbers of ACP for experimental use, it is critical to have plants producing feather flush on a regular basis. All *C. volkameriana* seedlings were maintained in greenhouses at UCR Agricultural Operations (AgOps) outside of quarantine, and were grown in UCR III type soil and watered and fertilized as needed.

Developing new flush for ACP oviposition: To promote numerous lateral branching points that would bear flush growth, *C. volkameriana* seedlings were initially pruned to within 15-20 centimeters (cm) above soil level in pots. In addition, the lower trunks of these seedlings were stripped of all developing buds, leaving only three to five possible branching points concentrated within the upper third of the trunk. After this initial round of intensive pruning, plants had to be regularly pruned in order to induce continuous flushing on developing branches. In order to ensure a continuous supply of plants with plentiful feather flush for ACP reproduction, a pruning schedule was developed and implemented. Seedlings were grouped into approximately ten batches, dependent upon the total number of plants in circulation at any one time. Plants were pruned twice weekly, with one batch each being pruned either Monday or Friday.

Feather flush suitable for ACP egg-laying was attained approximately 10-12 days after plant pruning, under AgOps greenhouse conditions (27±2°C; 50% RH; L:D under natural day length). Seedlings with sufficient clusters of feather flush were moved from AgOps greenhouses into an Insectary-level laboratory in the UCR IQF and inoculated with ACP adults. Feather flush on plants not utilized for ACP rearing was allowed to develop into mature leaves and grow for an

additional four to five weeks, after which the plant was subsequently pruned back to encourage the development of additional flushing points. Such a cycle of plant re-use reduced the number of waste plants, and ensured seedlings would fit into the acrylic ACP rearing cages once they were ready for use.

ACP rearing cages in Quarantine: Colonies of ACP were reared on the Insectary level of UCR IQF using a double-cage system (i.e., a cage within a cage) in order to prevent escape of psyllids. Survivability of ACP outside of rearing cages was further reduced by there being no other available plant material in the rearing rooms on which escapees could feed. The outer cage was comprised of a large Bugdorm 2400 (Megaview Science, Taiwan). Within the Bugdorm were the primary rearing cages constructed of acrylic risers (construction described below) containing a potted *C. volkameriana* and psyllids. This outer cage contained up to a maximum of 16 smaller rearing cages.

ACP rearing cages were constructed by modifying two transparent acrylic risers (SW Plastics, Riverside, CA) which each measured 15 cm x 15.3 cm x 15.3 cm (h x w x d). The open back face was covered with white no-see-um mesh netting (Skeeta, Bradenton, FL), and the front face was covered with a sleeve approximately 30cm in length sewn from no-see-um which provided access to the cage's contents.

ACP inoculation schedule of host plants: Approximately 10-12 days after initial pruning, *C. volkamariana* seedlings displaying adequate amounts of feather flush were selected from UCR AgOps greenhouses and brought to an IQF Insectary laboratory for ACP inoculation. Prior to the introduction of ACP, seedling pots were prepared by placing a length of nylon stocking over the top of the pot (covering the soil), secured around the base of the plant with a plastic twist-tie. This nylon barrier was put in place to reduce the number of soil-dwelling fungus gnats emerging into rearing cages, but was porous enough to allow for the plant to be watered without first removing it. Plants were also stripped of a majority (> 75 percent) of mature leaves, so as to force ACP females to utilize feather flush for feeding and ovipositing. Plants were then placed into primary rearing cages with a saucer to contain excess water draining after watering.

An average of 18 cages (six each every Monday, Wednesday and Friday) of *C. volkameriana* bearing feather flush were set up each week for ACP oviposition in quarantine. Approximately 15−20 ACP adults were released into each cage and allowed to oviposit for 2−4 days, after which they were removed and transferred into a new cage needing inoculation. Any ACP adult mortality was mitigated by supplementing additional adults sourced from HLB-free ACP California strain lab colonies. Plants were watered three times per week (Monday, Wednesday and Friday).

THE SELECTION OF NON-TARGET PSYLLID SPECIES IN CALIFORNIA FOR HOST SPECIFICITY TESTING OF *DIAPHORENCYTRUS ALIGARHENSIS* SOURCED FROM PUNJAB PAKISTAN

California has the richest native psyllid fauna in North America, and the influence of climatic and floristic diversity in this region is considered key to the diversification of native psyllids. There are currently 165 psyllid species (~5−6% of the world's fauna) known from California and

a revision of the psyllid fauna of California was used to guide selection of psyllid species for host specificity testing of *D. aligarhensis* sourced from Punjab Pakistan (Percy et al., 2011). Obviously, it would be an almost impossible job to test all of California's psyllid species for suitability as hosts to *D. aligarhensis*. A basic assumption in conducting host range tests is that native species most closely related to the target pest are likely to be more at risk from biological control agents under consideration for use against the target pest. There are no native representatives in the genus *Diaphorina* or the tribe Diaphorininae in California to which ACP belongs which means the closest relatives that should be selected for host range testing should come from Family level representation. However, in addition to taxonomic relatedness, other factors could influence host location and use by parasitoids including cues released by native plants related to citrus, or exploitation of sub-optimal hosts under conditions when the preferred target, ACP, is not available. In consultation with Dr. Diana Percy, a world authority on psyllid taxonomy and phylogenetics, the testing strategy shown in Table 1 was designed to select pertinent California psyllid species for host range testing in quarantine.

Table 1. Experimental selection criteria and representative species of non-target California psyllids used in host specificity testing of *Diaphorencyrtus aligarhensis* at UCR IQ.

Criterion	**Selected Representative Species**
Target pest species	*Diaphornia citri* (Asian citrus psyllid [invasive pest] [Liviidae: Euphyllurinae])
Phylogenetic proximity to ACP	*Dichlidophlebia fremontiae* (fremontia psyllid [native] [Liviidae: Liviinae]) *Euphyllura olivina* (olive psyllid [invasive pest] [Liviidae: Euphyllurinae])
High probability for occurrence environmentally in native vegetation	*Heteropsylla texana* (honey mesquite psyllid : native [Psyllidae: Ciriacreminae]) *Heteropsylla* sp. (acacia psyllid [native] [Psyllidae: Ciriacreminae])
Native psyllid species (pest)	*Bactericera cockerelli* (potato psyllid [native] [Triozidae: no sub-family designations exist for Triozidae])
Biological control agent of invasive weed	*Arytainilla spartiophylla* (scotch broom psyllid [self-introduced] [Psyllidae: Psyllinae])

Non-target psyllid sources used for initiating colonies: In addition to the various native psyllids throughout California, there are also many known natural enemies associated with each species (Percy et al., 2011). To avoid contamination and potential influence of specificity trials, all psyllid species native to California were collected from field populations and brought to UCR IQF. From these initial collections, colonies were set up and reared in an IQF Insectary

laboratory. Establishment and maintenance of colonies allowed positive confirmation that all colonies and subsequently individual nymphs used in host specificity trials were free of parasitoids. Broom psyllid (*A. spartiophylla*) and olive psyllid (*Euphyllura olivine*) were field collected for use in experiments. Both of these exotic psyllids lack a parasitoid fauna associated with the nymphs.

Non-target psyllid rearing:

Host plants: Successful rearing of non-target psyllids was based primarily on having a continuous supply of host plants (specific to the species being reared) with adequate feather flush for oviposition. Since each non-target psyllid species was highly specific to their own host plant, colonies needed to be maintained on species-appropriate host plants (mainly California native plants). In host specificity trials, each non-target species was maintained on seedlings of their choice host plant (with the exception of *Bactericera cockerelli*, the pestiferous polyphagous potato psyllid, which was reared on eggplant seedlings but tested on *Capsicum* seedlings). Table 2 lists the host plant associated with each representative psyllid species.

Table 2. Host plants and associated psyllid species which were used for maintaining colonies of selected non-target psyllid species for testing against *D. aligarhensis* in UCR IQF.

Non-target Psyllid Species	**Host Plant**	
[1]*Bactericera cockerelli*	Egg plant	*Solanum melongena*
[1]*Heteropsylla sp.*	Sweet acacia	*Acacia farnesiana*
[1]*Heteropsylla texana*	Honey mesquite	*Prosopis glandulosa*
[1]*Dichlidophlebia fremontiae*	Fremontia bush	*Fremontodentron sp.*
[2]*Euphyllura olivina*	Olive	*Olea europaea*
[2]*Arytainilla spartiophylla*	Scotch broom	*Cytisus scoparious*

[1]Colony maintained in Quarantine, UCR IQF. [2]Colony not maintained in Quarantine, insects were collected from wild populations and transferred to potted host plants for experiments.

Potted *F. californicum* seedlings were acquired from a local California native plant nursery (Moosa Creek Nursery, Valley Center, CA). Eggplants used in colony maintenance were matured at AgOps from commercially-available seedlings ("Long Purple" variety, Botanical Interests, Broomfield, CO). Seedlings of *Acacia farnesiana*, and *P. glandulosa* were provided by CDFA (Mt. Rubidoux Field Station) once per week on an as-needed basis for use in experiments. Scotch broom seedlings were harvested from wild populations in El Dorado Co., and transplanted into experimental pots in Quarantine. Potted olive seedlings were provided by CDFA. Broom and olive plants were used to maintain reproducing populations of the associated psyllid. All host plants were maintained in greenhouses at UCR AgOps and provided necessary fertilization and water until use in colony cages. Plants were pruned whenever necessary to maintain a small size to fit within the colony Bugdorms, as well as to promote growth of feather flush for psyllid oviposition.

Non-target psyllid rearing: Non-target psyllid colonies were maintained in Insectary-level laboratories of UCR IQF in Bugdorms (model 2120 MegaView Science, Taiwan). Based on the status of the psyllid species (i.e., pre-testing; currently undergoing testing; post-testing), colonies were expanded (usually encompassing three to five Bugdorms) or reduced (one Bugdorm) as needed. Mature potted seedlings of native host plants obtained from CDFA were maintained at UCR AgOps until time of use. Plants were selected for use based on amounts of feather flush present, and were subsequently moved from AgOps to IQF. Fresh plants were placed into Bugdorms already containing their particular non-target psyllid on older plants, and old, dying, or infested (usually by plant-feeding mites) plants were removed and disposed of according to the corresponding species' CDFA permit instructions. In this manner, native psyllid colonies were continually "refreshed" and kept robust. All psyllids kept in colony, with the exception of *B. cockerelli*, utilized young feather flush for oviposition. Nymphs would emerge and mature through the first and second instars within the feather flush, then move down onto the plant trunk or mature leaf surfaces to feed as later instars. In contrast, *B. cockerelli* oviposits directly onto the leaf lamina and along the margin of the leaf, with nymphs maturing and moving to feed along leaf veins. This psyllid doesn't require flush growth to stimulate oviposition.

Developmental notes of non-target psyllids: Nymphs of *B. cockerelli* (potato psyllid) developed on mature leaf surfaces (as this is where eggs are oviposited by females) and ranged from nearly transparent (early instars) to a very light yellow-green color which was most obvious in fourth and fifth instars. Psyllids retained a round, dome-like body shape until the final molt to adulthood.

Heteropsylla sp. (acacia psyllid) and *Heteropsylla texana* (honey mesquite psyllid) had early instars which were light yellow in color, subsequently developing to green as they matured. *Dichlidophlebia fremontia* nymphs were white after first emerging, and matured to a very light whitish-green. They formed small clusters which were often covered with dense, white cottony wax.

Scotch broom psyllid, *A. spartiophylla*, and olive psyllid, *E. olivina*, were not reared in the laboratory. Because these exotic psyllids lack parasitoids in California, they were collected from wild populations and tested on their respective host plants at UCR IQF without concern that nymphs had been previously parasitized by species other than *D. aligarhensis* to which they were exposed to in quarantine. *A. spartiophylla* nymphs were a nearly uniform light yellow in color, and were found feeding on both the leaves and stems of host plant cuttings. This species is very active and will move readily with very little stimulus, making them easy to transfer from cuttings to experimental seedlings.

E. olivine nymphs, much like *D. fremontia*, are white and produce a fluffy white wax, which shelters clusters of young instars. Once older, *E. olivine* nymphs tend to be more solitary, however, they remain protected under waxy exudates. This species is also very active and, once found by removing the wax around the bases of leaf axils or along young branches, nymphs were readily transferred onto experimental seedlings.

B. HOST RANGE TESTING

Young seedlings used as host plants in *D. aligarhensis* exposure trials (*Capsicum*, *Acacia farnesiana*, *Prosopis* sp., and *Olea europaea*) were grown by CDFA at their Mt. Rubidoux Field Station in outdoor greenhouses. Capsicum seedlings were the California Wonder variety (Ferry Morse Seed Company, Felton KY). *Fremontodendron* seedlings were obtained from Moosa Creek Nursery (Valley Center, CA). Scotch broom seedlings were harvested from wild populations in northern California, with assistance from the CDFA Biocontrol Program, Sacramento (Dr. Kris Godfrey now at UC Davis), and transplanted into experimental cones in Quarantine at UCR IQF. A summary of host plants involved in *D. aligarhensis* testing is provided in Table 3.

Table 3. Host plants used for testing target and non-target psyllid species. All host plants used for testing were seedlings; however the size of plant varied.

Non-target Psyllid (Testing)	**Host Plant Seedling**	**Cone-tainer or D40?**
Diaphorina citri	Volkamer lemon (*Citrus volkameriana*)	Cone-tainer
Bactericera cockerelli	Sweet pepper (*Capsicum annum*)	Cone-tainer
Heteropsylla texana	Honey mesquite (*Prosopis glandulosa*)	Cone-tainer
Heteropsylla sp.	Sweet acacia (*Acacia farnesiana*)	Cone-tainer

Test plant preparation: *Prosopis*, *Acacia*, *Olea* , and *Capsicum* were obtained from the CDFA Mt. Rubidoux Field Station as seedlings already in cone-tainers (SC7 Stubby, low density, 3.8 cm diameter, 114 mL capacity, Ray-leach, Stewe and Sons Inc., OR). Mature leaves on these seedlings were removed, leaving only the youngest leaves at the apex of the plant. A piece of circular upholstery foam lined with netting was placed within the cone over the surface of the soil to reduce fungus gnat emergence, as well as aiding in the recovery of test insects that could fall onto the soil (this was important for determining the ultimate outcome of target psyllid's fate [i.e., alive vs. dead]). The bottom of the cone-tainer was passed through a plastic lid with a hole cut out, which was secured near the top of the cone-tainer with two thumb tacks. Finally, a ventilated plastic vial was inverted over the test plant and secured to the lid, confining the psyllids and *D. aligarhensis* on the test plant. Standard experimental setup of these cone-tainers is illustrated below in Fig. 1.

Fig. 1. Preparation of experimental seedlings in cone-tainers with test psyllid species for *D. aligarhensis* exposure trials. 1) original seedling in cone-tainer; 2) a trimmed seedling, with mature foliage removed; 3) netted upholstery foam disc placed over soil (top of foam is level with top of cone-tainer); 4) lid fitted around the top of the cone-tainer and secured with thumb tacks; 5) ventilated vial inverted and placed over the test plant, secured to the lid from step 4.

We were unable to maintain scotch broom seedlings, as they are classified as a quarantined noxious weed, so seedlings used for testing were harvested from wild populations in northern California (the same location the psyllids were collected), shipped under CDFA permit (no. 2977), and transplanted into cone-tainers in Quarantine at UCR IQF. All other experimental setup procedures for scotch broom seedlings were identical to the process described above. *Fremontia* seedlings purchased from local California native-plant nurseries were larger than expected upon arrival. The above preparation process was modified to accommodate the larger growing cones ("D40") the *Fremontia* seedlings arrived in (Fig. 2). Paper discs 7mm in diameter, cut from waxy paper plates, were fitted on top of the cone and around the plant stem, with the same purpose of the netted foam from the cone-tainer setup, to prevent fungus gnat emergence. The D40 growing cones were fitted with an 11mm diameter clear plastic beverage lid, with a rubber band (size 32, ¼ lb) fitted underneath for support against slipping and to the reinforce seal. A 16-ounce capacity clear plastic beverage cup, with the bottom removed and replaced with no-see-um netting for ventilation, was then inverted and secured over the plant, to restrict the *Fremontia* psyllids (*Dichlidophlebia fremontiae*) and *D. aligarhensis* to a small area surrounding the test plant.

Fig. 2. Adapted experimental setup for *Dichlidophlebia fremontiae* on *Fremontodendron* sp. (Fremontia) in D40 growing cones.

Transferring psyllid nymphs to test plants: It took an average of approximately 7−8 days post-oviposition for ACP nymphs to reach the early third instar (a preferred host stage of *D. aligarhensis*) under experimental conditions in the Insectary rearing room at UCR IQF (26.7 ± 1° C and 40 ± 5% RH). Native non-target psyllid species that were reared in colonies were maintained in rearing rooms separate from ACP at a slightly lower temperature of 25±1°C and 40 ± 5% on the Insectary level of IQF, because this slightly lower temperature improved colony vigor.

A total of five third-instar psyllids on a single host cone-tainer seedling comprised a treatment which was exposed to a single female *D. aligarhensis*. To achieve this precise number of psyllids on each testing plant, a method of transferring nymphs from large colony plants onto smaller cone-tainer seedlings was developed. Nymphs of the appropriate age/life stage were located on large colony plants and disturbed by gentle tapping with a fine-haired paintbrush to encourage nymphs to become mobile. Once psyllids ceased feeding and/or clinging to the host plant and began walking, they were gently lifted off of the colony plant and individually placed onto the young flush of the test plant. All nymphs were moved onto testing plants the morning before their exposure to *D. aligarhensis*, so they had no additional time to mature and were guaranteed to be in an appropriate life stage for *D. aligarhensis* to parasitize. Because psyllids were very young at the time of transfer (sometimes as young as late second instar) the trauma of plant transfer caused some mortality, but overall this transfer method achieved a success rate of over 80% survivorship.

Test cage setup: Clear plastic vials of 148 mL capacity (Thornton Plastic Co, Salt Lake City, Utah) with three ventilation holes 12 mm in diameter (two on opposite sides, one on the bottom)

and covered with an ultra-fine white organza mesh were inverted to fit into the vial lid that was firmly secured around the cone-tainer. This ventilated inverted vial enclosed the test plant and psyllids, and confined *D. aligarhensis* with the psyllid species of interest.

Diaphorencyrtus aligarhensis: Adult *D. aligarhensis* (this population is bi-parental) originally collected from the Punjab of Pakistan, were sourced from colonies maintained in Quarantine greenhouses at UCR IQF. Mating pairs of 1 male and 1 female were collected in 200 µL microcentrifuge tubes with honey added for feeding, and allowed to mate for no less than 24 hours. Immediately before day 1 testing commenced, female *D. aligarhensis* were separated into a 0.6 mL microcentrifuge tubes, from which they were introduced into the inverted ventilated cages described above. At the end of the prescribed duration of exposure to test psyllids, the vial was opened within an observation cage and the parasitoid was recaptured in the microcentrifuge tube. Each *D. aligarhensis* female was used only once for choice and prolonged no-choice tests over the indicated 2-day period. In sequential no-choice tests, the same female was tested four consecutive times.

Cone-tainer setup: Experimental blocks were set up in shoebox-sized plastic storage boxes, with three rows of five (15 in total per lid) holes cut into the plastic lid of the box with a diameter the same as that of the testing cone-tainers, and cone-tainer seedlings were placed through the holes which supported them in a vertical position. Approximately two to three inches of tap water was added to the box to allow for continuous watering of testing plants (through absorption through the exposed soil tip at the base of each cone-tainer) without the need for removing the ventilated vials at the top of the cone-tainer during the testing period (Fig. 3).

Fig. 3. One experimental block of cone-tainer seedlings, showing constant plant access to water provided at the bottom of the testing tray.

Host specificity test methods: Host specificity testing for *D. aligarhensis* was conducted under choice and no-choice conditions involving sequential and prolonged exposure experiments over the course of two days. *D. aligarhensis* used in short-term no-choice sequential treatments were allowed to rest overnight between day one (d1) and day 2 (d2) replicates (Table 4). *D.*

aligarhensis used in prolonged exposure treatments were given a 24 hour exposure window on each d1 treatment before being immediately transferred to d2 treatments.

Sequential no choice tests: Short-term no-choice sequential exposure treatments followed one of two sequences. In the first treatment (T1), *D. aligarhensis* were exposed to ACP for four hours, then immediately transferred to a plant with a non-target psyllid (NTP). The second treatment (T2) was the reciprocal, introducing *D. aligarhensis* first to NTP for four hours, then being moved to ACP for an additional four hours. After the second four-hour exposure (whether on ACP or NTP), *D. aligarhensis* were removed and transferred into a 200 µL microcentrifuge tubes with honey, and stored overnight in a temperature control cabinet at 13.2-14.4°C. Day 2 replicates were set up identically to day 1 replicates (i.e.: the *D. aligarhensis* used for T1d1 was then used for T1 day 2). The second-day exposure (day 2) was conducted to measure the effects of previous psyllid exposure and host-feeding on parasitism rates on both ACP and NTP.

Prolonged exposure no choice tests: This design consisted of four different treatment types, which varied the order of host psyllid species presented to *D. aligarhensis* under prolonged exposure. Each prolonged exposure treatment consisted of a day 1 24-hour exposure to either ACP or NTP, before being moved immediately onto a secondary day 2 plant containing either ACP or NTP for another 24-hour exposure. This experimental design was implemented to determine attack rates on NTP when *D. aligarhensis* repeatedly encountered NTP, or when *D. aligarhens*is was able to host-feed on or parasitize ACP before or after encountering NTP, and was intended to more closely mimic conditions in a natural California habitat should *D. aligarhensis* find itself in areas with only non-target psyllids to attack.

Choice tests: Choice tests (T3) were conducted by simultaneously exposing *D. aligarhensis* to ACP and NTP on their respective host plants and allowing the parasitoid to forage freely and choose between psyllid species for attack. These treatments were conducted using the same acrylic riser cages constructed for use in colony maintenance (see above), giving *D. aligarhensis* a greatly expanded area of foraging and host encounters. Treatments were set up on day 1 and left to run for 24-hour, after which *D. aligarhensis* was captured, the day 1 plants removed, and day 2 plants with fresh psyllids placed into the choice cage. *D. aligarhensis* was subsequently released and given access to day 2 plants for an additional 24-hour before final removal.

Environmental test conditions in quarantine: The Insectary laboratories where NTP colonies were maintained were regulated at 25ºC, 40% RH and 14:10 L:D cycle. In order to promote increased ACP reproduction for testing, the specific ACP rearing laboratory was maintained at constant environmental conditions of 28.33ºC, 40% RH, and 14:10 L:D. The quarantine testing room where trials took place was maintained at a mid-range temperature, with environmental conditions set at 26.7ºC, 40% RH, and 14:10 L:D throughout all testing phases.

Experimental Execution: A single mated adult female *D. aligarhensis* (approx. 2−7 days of age) was introduced into a test cage for a 4 hour exposure period in sequential no choice (T1, T2) treatments, or for 24 hours for choice (T3) and prolonged sequential no-choice (T4-7) treatments. Baseline mortality rates of psyllid nymphs dying from natural causes were derived from Control treatments (T8), in which a set of psyllids was set up on test plants in an identical manner to those used in experiments and maintained under the same quarantine conditions, but were not

exposed to parasitoids. Experiments were set up in blocks comprised of T1-T8 treatments, which were run in eight two-day replications for each test species and treatment type.

Sequential no-choice tests (T1, T2). During initial setup, two *D. aligarhensis* females were released into one of two test vials, one containing ACP (T1) and the other containing NTP (T2). After a period of 4 hours, parasitoids were removed and transferred immediately to a second vial containing the reciprocal psyllid type (NTP for T1, ACP for T2) for an additional 4 hours. At the end of this 8-hour rotation, females were removed and transferred into a 200 μL microcentrifuge tube with honey, and rested overnight at 13−14°C. The same test sequences were repeated for female *D. aligarhensis* on day 2.

Choice tests (T3). Ventilated inverted vials on individual test plants were removed in order to expose test psyllids on their respective host plants. Two cone-tainers, one containing ACP and the other NTP, were placed inside an acrylic riser cage (identical to those constructed for use in colony maintenance [see above]), giving *D. aligarhensis* a greatly expanded area of foraging. Treatments were set up on day 1 and run for 24 hours, after which *D. aligarhensis* was captured, the day 1 plants removed, and day 2 plants with fresh psyllids placed into the choice cage. *D. aligarhensis* was subsequently released and given access to day 2 plants for an additional 24 hours. After removal, ventilated vials were replaced over each individual test plant and its psyllids, and the cone-tainer was returned to its respective block's watering tray.

Prolonged sequential exposure test (T4-7). These 24-hour sequential no-choice treatments aimed to evaluate whether *D. aligarhensis* females would attack NTP under an exposure period longer than 4 hours, and with varying host sequence exposure. It was theorized that *D. aligarhensis* attacks on NTP would be possible if the exposure time was lengthened, either because females would have more time to act on NTP or because there was an extensive period during which the parasitoid was restricted to a single non-choice species and in the absence of the preferred host, ACP, oviposition on less desirable hosts may result. This setup also evaluated whether the order of exposure to the preferred (ACP) or less desired (NTP) host had any effect on attack rates on either species.

The first treatment (T4) had *D. aligarhensis* exposed to ACP on day 1, then moved to ACP on day 2. The second treatment (T5) had *D. aligarhensis* exposed to ACP on day 1, then moved to NTP on day 2. The third treatment (T6) had *D. aligarhensis* exposed to NTP on day 1, then transferred to ACP for day 2. In the final treatment (T7), *D. aligarhensis* were exposed to NTP on day 1, and transferred to NTP for day 2. In this manner, all combinations of exposures were addressed.

Control (T8). One set of five ACP or five NTP nymphs on their respective host plants in cone-tainers were set up and maintained in a manner identical to that for test psyllids exposed to *D. aligarhensis* for each treatment day (day 1, day 2). Control psyllids of each test species were not exposed to any predator or parasitoid. These control cages were used to provide estimates of naturally-occurring mortality due to the process of transferring psyllids onto seedlings used in testing, and subsequent maintenance in quarantine testing room conditions to determine developmental fate (i.e., death from unknown causes or development to adult psyllids).

Table 4. Treatment summary for exposure tests of female *D. aligarhensis* to Asian citrus psyllid (ACP) and non-target psyllid (NTP) species.

Treatments		Day 1 (d1)		Night	Day 2 (d2)	
T1	Sequential (*D. citri* first)	[*D. citr*i]	►[NTP]	rest	►[D. citri]	► [NTP]
T2	Sequential (NTP first)	[NTP]	►[*D. citri*]	rest	► [NTP]	►[*D. citri*]
T3	Choice test	[*D. citri*+NTP]			[*D. citri*+NTP]	
T4	Prolonged sequential (ACP/ACP)	[D. citri]			► [D. citri]	
T5	Prolonged sequential (ACP/NTP)	[D. citri]			► [NTP]	
T6	Prolonged sequential (NTP/ACP)	[NTP]			► [D. citri]	
T7	Prolonged sequential (NTP/NTP)	[NTP]			► [NTP]	
T8	Control	[*D. citri*] / [NTP] *No parasitoid exposure to measure natural nymph mortality under experimental conditions*			[*D. citri*] / [NTP] *No parasitoid exposure to measure natural nymph mortality under experimental conditions*	

► *D. aligarhensis* movement to new psyllid hosts rest = containment of female *D. aligarhensis* in a 200 µL microcentrifuge tube with honey and no psyllid exposure for ~16 hours at 13.2-14.4ºC.

Data Recording: All treatments were observed at least twice to record psyllid developmental outcomes. Observation 1 was made 7−9 days post-day 1 exposure to *D. aligarhensis*. Observation 2 was completed 14−16 days post-day 1 exposure. If at this point there remained mummies or live psyllid nymphs, a third observation was taken 21 days post-day 1 exposure to determine developmental fate. Any intact mummies (i.e., no emergence of parasitoids) observed at this point were deemed dead and subsequently discarded. Counts were recorded of the number of psyllids that successfully matured to adulthood, psyllid nymphs found dead (physically accounted for), the number of successfully parasitized and mummified nymphs (observation 1 only), and number and sex of *D. aligarhensis* that emerged from hosts for each treatment.

RESULTS AND DISCUSSION

Six non-target psyllid species were tested for their suitability as hosts for *D. aligarhensis* reproduction. Host range was assessed using seven experimental designs encompassing no-choice sequential tests, prolonged no-choice and choice tests. The non-target test species were comprised of: (1) *Bactericera cockerelli* (the native pestiferous potato/tomato psyllid) (*n* = 480 total nymphs exposed); (2) *Heteropsylla* sp. (the native Acacia psyllid) (*n* = 390 nymphs); (3) *Arytainilla spartiophylla* (a self-introduced natural enemy of the noxious weed scotch broom psyllid) (*n* = 380 nymphs); (4) *Euphyllura olivina* (the invasive olive psyllid) (*n* = 320 nymphs); (5) *Heteropsylla texana* (the native honey mesquite psyllid) (*n* = 395 nymphs); and (6) *Dichlidophlebia fremontiae* (the native *Fremontia* psyllid) (*n* = 375 nymphs). A summary (mortality, parasitism, survival to adulthood, etc.) for each psyllid species is presented in Table 5.

Successful parasitism of a non-target species by *D. aligarhensis* was observed for only one

species tested: *B. cockerelli*, the native and pestiferous potato psyllid, the vector of the zebra chip bacterium which causes significant economic loses in potatoes (Butler and Trumble 2012). Observed parasitism rates ranged 11-18% for the four treatment types. Two treatment types (T2, the short no choice sequential trial in which NTP was presented first and T3 choice test) which exhibited moderate levels of parasitism, at 17-18%. Parasitism of *B. cockerelli* was lower in ACP-first sequential no-choice test and prolonged exposure tests (11- 13%). When compared to *D. aligarhensis* parasitism rates of ACP across all treatment types (27-45%), it is likely *B. cockerelli* is not a highly preferred host for *D. aligarhensis*. Because of these findings, as well as the fact that there is robust guild of generalist predators and parasitoids native to California attacking *B. cockerelli* (Butler and Trumble, 2012), it is unlikely that *D. aligarhensis* will pose a significant threat to *B. cockerelli* populations in the wild once released from Quarantine.

Elevated mortality levels classified as a result of "unknown causes" (dead nymphs which were *found* and confidently accounted for) were observed across all psyllid species exposed to *D. aligarhensis* (refer to Table 6 for the complete breakdown of confirmed mortality from unknown causes by psyllid species). This mortality increase was measured against control treatments consisting of host plant seedlings with NTP nymphs identical in setup to experimental seedlings and kept under identical conditions, but lacking exposure to *D. aligarhensis*. It is likely that elevated mortality when compared to controls was a combination of natural nymph mortality, trauma of moving early instars between plants, possible host feeding by *D. aligarhensis* (not confirmed), unsuccessful parasitism attempts (not confirmed), and (in some cases as discussed for *A. spartiophila*) unavoidable external conditions due to field collection, subsequent transportation, and set up in quarantine.

Mortality experienced by *B. cockerelli* nymphs (potato psyllid, tested on pepper [*Capsicum*]) undergoing *D. aligarhensis* exposure ranged from 3-27%, as compared to 4% control mortality. Mortality rates for ACP, excluding death caused by successful parasitism of *D. aligarhensis*, ranged from 4-11%. Control mortality for ACP was 6%.

No parasitism by *D. aligarhensis* was recorded for *Heteropsylla* sp. Increased mortality was observed for *Heteropsylla* sp. (acadia psyllid, tested on *Acacia* sp. seedlings) in the presence of *D. aligarhensis*. Control mortality for this species, was around 8%, but mortality ranged from 5-17% across treatments with *D. aligarhensis* (Table 5). These data suggest that *D. aligarhensis* could cause additional, albeit, minor increases in host-death when confined in small cages with this psyllid species. Observed mortality for ACP was between 20% and 23% (independent of parasitism), markedly higher than the mortality rate for *Heteropsylla* sp. (Table 5).

No parasitism by *D. aligarhensis* was recorded for *A. spartiophila* (Table 5). Of all non-target species tested, *A. spartiophila* (scotch broom psyllid, tested on transplanted scotch broom seedlings) had the highest overall nymph mortality rates (Table 6). All of the experimental treatment types, except T1 (sequential 4 hr exposure to ACP first) have lower mortality rates compared to the control (14-32% with *D. aligarhensis* exposure vs. 33% in control treatments lacking *D. aligarhensis* exposure [Table 5]). This elevated control mortality may be due to a number of external factors, including same day field-harvesting and transporting (via ice chest with psyllids flown Economy class from Sacramento to Riverside) of psyllids and low survivorship of field-harvested and transplanted scotch broom seedlings. There appears to be no

significant elevation in *A. spartiophila* mortality when *D. aligarhensis* is confined with this psyllid. This is very relevant as this particular psyllid is considered a fortuitous self-introduced biological control agent of invasive scotch broom in northern California. Further, collections of *A. spartiophila* were made from high elevation sites in northern California. These collecting areas are subject to snowfall, a climate type that is not conducive for year round persistence of *D. aligarhensis*. Observed ACP mortality rates in this experimental set ranged from 13-17%, and ACP control mortality was 19%.

No parasitism by *D. aligarhensis* was recorded for *E. olivina* (Table 5). *E. olivina* mortality in the presence of *D. aligarhensis* ranged 16-23% which was similar or slightly higher than control mortality which was recorded at 15% for olive psyllid (Table 6). In comparison, ACP mortality was similar, ranging 12-22%, and parasitism range 16-28%. ACP mortality for control treatments was 15%.

No parasitism by *D. aligarhensis* was recorded for *H. texana* (Table 5). *H. texana* experienced little or no increase in NTP mortality in the presence of *D. aligarhensis* (Table 5). Control mortality for *H. texana* was 21%, mortality when exposed to female *D. aligarhensis* ranged 8-24% for *H. texana* (Table 6). These data sets suggest that *D. aligarhensis* may not be significantly affecting mortality rates of this non-target species. In comparison, to *H. texana*, ACP mortality across exposure experiments ranged 16-23%, parasitism ranged 14-28%, and control mortality was 26%.

No parasitism by *D. aligarhensis* was recorded for *D.fremontiae* (Table 5). Control mortality for *D. fremontiae* was 3%, and *D. fremontiae* exposed to *D. aligarhensis* suffered mortality rates between 8% and 16%. In contrast, ACP mortality was 24% in control treatments and ranged 17-25% in exposure treatments and ACP parasitism ranged 8-24%. This only slight increase in non-target mortality under Quarantine conditions suggests that *D. aligarhensis* may affect natural death rate of *Fremontia* psyllids, but not significantly.

Table 5. Consolidated summary of data for exposure and control treatments pertaining to psyllid mortality and parasitism rates in the presence of a single mated *D. aligarhensis* female under each of seven different exposure treatments.

Non-Target Psyllid Species	Test Type	% NTP Mortality	%NTP Parasitism	% ACP Mortality	%ACP Parasitism	%NTP Control Mortality	%ACP Control Mortality
Bactericera cockerelli (Potato/Tomato Psyllid) (PTP)	T1 (4hr, ACP first)	18	13	11	39	4	6
	T2 (4 hr NTP first)	3	18	7	42		
	Choice Test (T3)	13	17	4	27		
	Prolonged Exposure (T4-7)	27	11	1	45		
Heteropsylla sp. (*Acacia farnesiana* Psyllid) (AFP)	T1 (4hr, ACP first)	8	0	20	28	8	11
	T2 (4 hr NTP first)	5	0	23	21		
	Choice Test (T3)	17	0	21	21		
	Prolonged Exposure (T4-7)	10	0	23	32		
Arytainilla spartiophila (Scotch Broom Psyllid) (SBP)	T1 (4hr, ACP first)	41	0	16	18	33	19
	T2 (4 hr NTP first)	32	0	17	16		
	Choice Test (T3)	14	0	13	29		
	Prolonged Exposure (T4-7)	25	0	16	21		
Euphyllura olivina (Olive Psyllid) (OLV)	T1 (4hr, ACP first)	21	0	16	16	15	15
	T2 (4 hr NTP first)	23	0	22	25		
	Choice Test (T3)	16	0	19	21		
	Prolonged Exposure (T4-7)	19	0	12	28		
Heteropsylla texana (honey mesquite (Prosopis)	T1 (4hr, ACP first)	24	0	18	20	21	26
	T2 (4 hr NTP first)	13	0	9	20		

psyllid) (PRO)	Choice Test (T3)	8	0	23	28		
	Prolonged Exposure (T4-7)	19	0	16	14		
Dichlidophlebia fremontidae (Fremontia psyllid) (FRE)	T1 (4hr, ACP first)	11	0	25	8	3	24
	T2 (4 hr NTP first)	14	0	25	23		
	Choice Test (T3)	8	0	17	15		
	Prolonged Exposure (T4-7)	16	0	19	24		

All species of non-target psyllid nymphs experienced a modest increase in mortality due to unknown causes under experimental conditions when compared to control data. Elevated levels of unknown mortality are likely the result of a combination host feeding or unsuccessful parasitism by *D. aligarhensis*. However, the ecological importance of this is likely to be very low as *D. aligarhensis* was unable to reproduce on these hosts, and elevated levels of mortality (< 15% on average: *Heteropsylla* sp. = 14%; *A. spartiophila* = 3%; *E. olivina* = 6%; *H. texana* = 0%; *D. fremontidae* = 8%) could have possibly been an artifact of containment in small artificial arenas that prevented females leaving unsuitable host patches. In the unlikely event that these attack rates that should manifest themselves in nature, it is highly unlikely there would be any significant impact on non-target psyllid species. Additonally, these exposure trials in Quarantine do not take into account various other biotic and abiotic factors that would act as barriers to D. aligarhensis interfering with native populations (e.g., native natural enemy guilds, plant community composition, and the potential rarity of non-target species for most of the year). The only native psyllid species *D. aligarhensis* was able to reproduce on was the potato psyllid, *B. cockerelli*. This psyllid is a pest of tomatoes, egg plants, peppers (Capsicum), and potatoes (Butler and Trumble 2012). It is most destructive in potatoes because it spreads a phloem-limited bacterium, possibly *Candidatus* Liberibacter solanacearum, that causes zebra chip in potatoes and related maladies in other solanaceous crops. *D. aligarhensis* both increased unknown nymph mortality (by 24%) and successfully parasitized *B. cockerelli* nymphs at an average rate of ~15%. It is possible that *D. aligarhensis* could encounter *B. cockerelli* frequently under certain circumstances, particularly in urban environments, where tomatoes/potatoes/eggplants/peppers are grown in gardens co-inhabited with citrus. No adverse environmental impacts from attacks on *B. cockerelli* by *D. aligarhensis*, should they occur, are anticipated. Further, there is a guild of resident natural enemies already exploiting *B. cockerelli* in California (Casey and Trumble, 2012) and an exotic parasitoid that has relatively weak attack rates under optimal conditions is unlikely to compete effectively under field conditions.

CONCLUSIONS

Under stringent testing conditions that evaluated the host range of the bi-parental encyrtid parasitoid, *Diaphorencyrtus aligarhensis*, sourced from Punjab Pakistan under four different testing strategies against six non-target psyllid species, *D. aligarhensis* was unable to parasitize on five of these six species. Low rates of parasitism, averaging 15%, were observed on the native pestiferous potato psyllid *B. cockerelli*. In comparison, parasitism rates on the target, Asian citrus psyllid, ranged 8−50% (average 23% parasitism rate). Mortality rates due to unknown causes across all non-target psyllid species showed a slight elevation in the presence of *D. aligarhensis* as compared to control psyllids not exposed to *D. aligarhensis*. An exception was *H. texana* which exhibited no measurable differences in nymphal death rates between exposure and control treatments. It is concluded that it is highly unlikely that such low rates of increased mortality and a lack of parasitism would be sufficient to affect native populations of these non-target psyllids in California. Permission to release *D. aligarhensis* has been granted for Florida and releases of two strains of uniparental populations sourced from Taiwan and China as part of a biological control program targeting ACP have so far failed to establish (Rohrig et al., 2012). A different outcome from releasing *D. aligarhensis* may occur in California because the strain to be released is sexual (as opposed to being parthenogenic, whereby females reproduce asexually as is the case

in Florida) and a competing ACP parasitoid, *T. radiata*, is not yet widespread in California which affords *D. aligarhensis* the opportunity to establish and spread in areas lacking this competitor.

References:

Butler, C.D., and J.T. Trumble. 2012. Identification and impact of natural enemies of *Bactericera cockerelli* (Hemiptera: Triozidae) in southern Cailfornia. Journal of Economic Entomology. 105: 1509−1519.

Percy, D.M., A. Rung, and M.S. Hoddle. 2011. An annotated checklist of the psyllids of California (Hemiptera: Psylloidea). Zootaxa 3193: 1−27.

Rohrig, E., D.G. Hall, J.A. Qureshi, and P.A. Stansly. 2012. Field release in Florida of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), an endoparasitoid of *Diaphornia citri* (Homoptera: Psyllidae), from mainland China. Fla. Entomol. 95: 479−481.

Appendix 3. Response to Comments on the Draft Environmental Assessment.

Legal notice of the EA was made available in the Federal Register on September 18, 2014 for a 30-day public comment period. Sixteen comments were received on the EA (one was a duplicate). Thirteen were in favor and two were in opposition to the proposed release of *D. aligarhensis.* The two negative comments are addressed in this Appendix. No substantive issues were raised in the comments.

One commenter was concerned that *Diaphorencyrtus aligarhensis* can sting humans.

As stated on page 9 of the EA, *Diaphorencyrtus aligarhensis* is an obligate parasitoid of psyllids, specifically Asian citrus psyllid (ACP). As such, it will rarely come into contact with humans or other vertebrates, and if it does, it is incapable of stinging or biting. Encyrtid wasps have no known adverse impacts on humans or other vertebrates.

One commenter was concerned about what the wasp could destroy and if it will destroy necessary bacteria on plants.

Diaphorencyrtus aligarhensis will have no impact on plant bacteria. In addition, the EA discussed an evaluation of the possible effects of the release of *D. aligharensis* on the environment (pages 9-13 and Appendix 2), including specificity data, and impact on non-target species and humans.

Decision and Finding of No Significant Impact
for
Field Release of the Parasitoid *Diaphorencyrtus aligarhensis* for the Biological Control of the Asian Citrus Psyllid in the Contiguous United States
October 2014

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) Pest Permitting Branch (PPB), is proposing to issue permits for release of an insect, *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), in the contiguous United States. The agent would be used by the applicant for the biological control of the Asian citrus psyllid (ACP), *Diaphorina citri* (Hemiptera: Liviidae). Before permits are issued for release of *D. aligarhensis*, APHIS must analyze the potential impacts of the release of this organism into the contiguous United States in accordance with USDA APHIS National Environmental Policy Act implementing regulations (7 Code of Federal Regulations Part 372). APHIS has prepared an environmental assessment (EA) that analyzes the potential environmental consequences of this action. The EA is available from:

U.S. Department of Agriculture
Animal and Plant Health inspection Service
Plant Protection and Quarantine
Regulations, Permits, and Manuals
4700 River Road, Unit 133
Riverdale, MD 20737
http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Plant_Health%2FSA_Domestic_Pests_And_Diseases%2FSA_Environmental_Assessments%2F

The EA analyzed the following two alternatives in response to a request for permits authorizing environmental release of *D. aligarhensis*: (1) no action, and (2) issue permits for the release of *D. aligarhensis* for biological control of ACP. A third alternative, to issue permits with special provisions or requirements concerning release procedures or mitigating measures, was considered. However, this alternative was dismissed because no issues were raised that indicated that special provisions or requirements were necessary. The No Action alternative, as described in the EA, would likely result in the continued use at the current level of chemical and biological control methods of ACP. These control methods described are not alternatives for decisions to be made by the PPB, but are presently being used to control ACP in the United States and may continue regardless of permit issuance for field release of *D. aligarhensis*. Legal notice of the EA was made available in the Federal Register on September 18, 2014 for a 30-day public comment period. Fifteen comments were received on the EA. Thirteen were in favor and two were in opposition to the proposed release of *D. aligarhensis*. The negative comments are addressed in Appendix 3 of the final EA. No substantive issues were raised in the comments.

I have decided to authorize the PPB to issue permits for the environmental release of *D. aligarhensis*. The reasons for my decision are:

- This biological control agent is sufficiently host specific and poses little, if any, threat to the biological resources, including non-target insect species of the United States.

- The release will have no effect on federally listed threatened and endangered species or their habitats in the United States.

- *D. aligarhensis* poses no threat to the health of humans.

- No negative cumulative impacts are expected from release of *D. aligarhensis.*

- There are no disproportionate adverse effects to minorities, low-income populations, or children in accordance with Executive Order 12898 "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations" and Executive Order 13045, "Protection of Children from Environmental Health Risks and Safety Risks."

- While there is not total assurance that the release of *D. aligarhensis* into the environment will be reversible, there is no evidence that this organism will cause any adverse environmental effects.

I have determined that there would be no significant impact to the human environment from the implementation of the preferred alternative (issuance of permits for the release of *D. aligarhensis*) and, therefore, no Environmental Impact Statement needs to be prepared.

Emily Pullins
Director
Regulations, Permits, and Manuals
Plant Health Programs
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

October 24, 2014
Date