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

MARK S. HODDLE[1,2,3] AND RAJU PANDEY[1]

J. Econ. Entomol. 107(1): 125–136 (2014); DOI: http://dx.doi.org/10.1603/EC13318

ABSTRACT Tests evaluating the host range of *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae), a parasitoid of the pestiferous Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), sourced from the Punjab of Pakistan, were conducted in quarantine at the University of California, Riverside, CA. Seven nontarget psyllid species (five native and two self-introduced species) representing five families were exposed to *T. radiata* under the following three different exposure scenarios: 1) sequential no-choice tests, 2) static no-choice tests, and 3) choice tests. Nontarget species were selected for testing based on the following criteria: 1) taxonomic relatedness to the target, *D. citri*; 2) native psyllids inhabiting native host plants related to citrus that could release volatiles attractive to *T. radiata*; 3) native psyllids with a high probability of occurrence in native vegetation surrounding commercial citrus groves that could be encountered by *T. radiata* emigrating from *D. citri*-infested citrus orchards; 4) a common native pest psyllid species; and 5) a beneficial psyllid attacking a noxious weed. The results of host range testing were unambiguous; *T. radiata* exhibited a narrow host range and high host specificity, with just one species of nontarget psyllid, the abundant native pest *Bactericera cockerelli* Sulc, being parasitized at low levels (<5%). These results suggest that the likelihood of significant nontarget impacts is low, and the establishment of *T. radiata* in southern California for the classical biological control of *D. citri* poses negligible environmental risk.

KEY WORDS choice test, host range, host specificity, nontarget impact, quarantine

In August 2008, Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was detected for the first time in Imperial County and San Diego County in southern California. Initial detections were relatively close to the California–Mexico border (Anon 2010), suggesting that *D. citri* may have invaded California via northwards dispersal out of Mexico, where *D. citri* is widely established (Atkins 2010). In 2009, surveys by the California Department of Food and Agriculture (CDFA) detected large and widespread populations of *D. citri* in residential areas in Orange County and Los Angeles County (Grafton-Cardwell 2010). These populations, especially the heavy infestations in Los Angeles County, were disjunct and widely separated (>195 km) from initial *D. citri* detections close to the California–Mexico border. This suggested that a separate and undetected introduction of *D. citri* had occurred in Los Angeles, likely before 2008 (see *D. citri* distribution map in Anon 2010). The establishment of *D. citri* in southern California is generally viewed as the most serious threat to California's multibillion dollar citrus industry because of its capacity to spread a bacterial disease of citrus (Anon 2010).

D. citri is notorious for its ability to acquire and vector a fastidious phloem-limited bacterium, *Candidatus Liberibacter asiaticus* Jagoueix, Bové, and Garnier (α-Proteobacteria) (Jagoueix et al. 1994), the causative agent of a lethal and incurable citrus disease known as huanglongbing (HLB) or citrus greening (Halbert and Manjunath 2004). The *D. citri*–HLB combination has been particularly destructive in many parts of the world where citrus is produced commercially (Bové 2006). Impacts have been especially great in Brazil (HLB was first detected in 2004 in the world's largest producer of orange juice (Hodges and Spreen 2012) and Florida (first detection of HLB was in 2005 (Hall and Hentz 2011), where economic losses to growers attributable to *D. citri*-vectored HLB have been estimated at >US$1.7 billion, which translates to ≈16% reduction in grower revenues (Hodges and Spreen 2012). In March 2012, HLB was detected for the first time in a single residential citrus tree in Los Angeles County, CA (Leavitt 2012).

[1] Department of Entomology, University of California, Riverside, CA 92521.
[2] Center for Invasive Species Research, University of California, Riverside, CA 92521.
[3] Corresponding author, e-mail: mark.hoddle@ucr.edu.

Classical biological control of *D. citri* has focused primarily on the use of parasitoids that attack the nymphs of this pest. Two parasitoids, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam, and Agarwal) (Hymenoptera: Encyrtidae), have received the most research attention (Halbert and Manjunath 2004). *T. radiata* has been used in several different countries for classical biological control of *D. citri* with mixed results. On Réunion Island in the Indian Ocean, *T. radiata* has been credited with providing excellent control of *D. citri*, which led to improved citrus production (Aubert et al. 1996). Conversely, studies assessing the efficacy of *T. radiata* against *D. citri* on larger continental landmasses, in areas such as Florida, indicate that impacts have been modest at best, with parasitism levels often <10% on average, in commercial citrus production areas (Michaud 2004, Qureshi and Stansly 2009). The developmental and reproductive biology and host stage preferences of *T. radiata* are well understood (Gómez-Torres et al. 2012).

The initial reaction to *D. citri* finds in urban areas in California was the commencement of pesticide treatments of infested trees in summer 2011 and to establishment of chemically-treated buffer zones around find sites to control this pest (California Citrus Pest and Disease Prevention Program [CPDPP] 2011). Because *D. citri* infestations in urban areas in Los Angeles were highly dispersed and often of high densities, the difficulty of finding and treating every infested citrus tree in private gardens with pesticides as part of a *D. citri* containment and eradication program by the CDFA (CPDPP 2011, Grafton-Cardwell et al. 2011, Hill 2012) was extraordinarily challenging. It became apparent that this strategy was very costly and unsustainable; with some sites in need of repeat applications at the same time new infestations were being discovered and some homeowners prevented treatment of infested trees.

The U.S. Census Bureau "Selected Housing Characteristics" summary data for 2006–2010 indicated the presence of 3,425,736 housing units in Los Angeles County, of which 2,216,170 (or 65%) consisted of 4 housing units or less (http://factfinder2.census.gov/bkmk/table/1.0/en/ACS/10_5YR/DP04/0500000US06037), a size that typically affords sufficient land for occupants to cultivate gardens, of which citrus is a popular component. Informal surveys in *D. citri*-infested neighborhoods in Los Angeles County (666 properties surveyed in four cities) indicated that 36 ± 2% (SE) of properties had at least one citrus plant (M.S.H. unpublished data). By October 2012, 46,941 residences or 6% of properties likely to have citrus in Los Angeles County had been treated with pesticides at a cost of US$4,702,435 (S. McCarthy CDFA personal communication 4 October 2012), or ≈US$100 per residence.

An alternative to pesticides for controlling *D. citri* in southern California, especially urban areas, is classical biological control. Preliminary studies that examined >1,000 *D. citri* nymphs failed to detect parasitism, suggesting that *D. citri* was benefiting, to some degree, from a dearth of natural enemies in Los Angeles (M.S.H. unpublished data). The natural enemy of choice for use in a classical biological control program in California is *T. radiata.* However, most importantly *T. radiata* sourced from the Punjab of Pakistan was of primary interest because CLIMEX modeling (http://www.climatemodel.com/climex.htm) indicated that this part of the natural range of *T. radiata* had ≈70% climate match with major citrus production areas in California's Central Valley (M.S.H. unpublished data). Climate matching is considered important for classical biological control because imported natural enemies should theoretically be preadapted to the prevailing climatic conditions in the receiving environment (Van Driesche et al. 2008). However, before *T. radiata* from Pakistan could be released in California, U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS) required host specificity testing to assess the risk this parasitoid posed to nontarget psyllid (NTP) species in California.

Assessing host ranges of natural enemies, in particular predators and parasitoids, is a nascent research area in the classical biological of arthropod pests (Van Driesche 2004), and this field is growing in importance as greater emphasis is placed on evaluating the safety of imported exotic natural enemies for suppression of invasive pests (Babendreier et al. 2006). The challenges of selecting nontarget species for testing and designing and conducting host range tests in quarantine are complex. There are practical impediments pertaining to limited understanding of the taxonomy, biology, and rearing of candidate species, and how best to select a subset of native species for testing when there are potentially hundreds or thousands of candidate nontarget species that could be evaluated (Van Driesche 2004). This latter issue is significant because a well-developed theoretical framework does not exist for developing criteria that could guide the selection of nontarget species, especially parasitoids, whose biology and behavior may not be constrained by phylogenic relationships between the target and close relatives in the intended receiving range (Van Driesche 2004).

Methods for testing the host range of entomophagous natural enemies vary (Van Driesche and Murray 2004, van Lenteren et al. 2006) but they always employ laboratory-based manipulative experimentation to assess attack rates in the presence or absence of target and nontarget species. The results of these experiments require the application of statistical tests so that conclusions about attack rates can be reached with a high level of confidence (Hoffmeister et al. 2006). Work reported here presents the results from mandated host specificity testing of *T. radiata* in quarantine before its release and establishment in southern California for the classical biological control of *D. citri.* Three different types of host range evaluation tests, sequential no-choice tests, static long exposure no-choice tests, and static choice tests, were conducted in quarantine with *D. citri* (target) and seven NTP species. The results of these host range assessment studies are part of this growing body of work in classical

arthropod biological control (Bigler et al. 2006, Andreassen et al. 2009, Murray et al. 2010, Mason et al. 2011, Vieira et al. 2011).

Materials and Methods

Source of *D. citri* for Colonies in Quarantine at University of California Riverside (UCR). HLB-free adult *D. citri* (≈100) were shipped from U.S. Department of Agriculture–Animal and Plant Health Inspection Service – Plant Protection and Quarantine-Center for Plant Health Science and Technology (USA–APHIS–PPQ–CPHST) Mission Laboratory, Edinburg, TX, to the UCR Quarantine and Insectary Facility (USDA–APHIS Facility Number 93) under USDA–APHIS Permit Number P526-09-03526, (S and R 10-04). These HLB-free *D. citri* were used to establish colonies at UCR on *Citrus volkameriana* Tenore & Pasquale in accordance with quarantine protocols set forth by USDA–APHIS and CDFA.

Host Plant Production for *D. citri* Colonies in Quarantine and Rearing. Pesticide-free *C. volkameriana* seedlings, ≈1–2 yr of age, received from Willits and Newcomb Inc. (Arvin, CA), or from the CDFA Rearing Facility, Mt. Rubidoux, Riverside, CA, were used as host plants for *D. citri* colonies maintained in quarantine. Successful *D. citri* rearing depended on producing new flush on plants on a regular and predictable cycle. Production of flush growth is critical, as female *D. citri* lay eggs in the axils of very young leaves (i.e., feather flush; Hall et al. 2008). Consequently, well rooted plants in 10-cm-diameter pots were cut back to ≈10–15 cm above soil level (UCR soil blend UC-3 formulated for citrus was used) to encourage development of lateral branches. All buds developing from the lower trunk were removed, leaving three to four branches arising from the upper quarter of the main stem. These plants bearing the desired branching pattern were then pruned regularly to induce new flush on lateral branches. To develop a continuous supply of flushing plants for *D. citri* oviposition, sets of preselected plants were pruned on a twice-a-week schedule to ensure adequate numbers of plants with appropriate flush growth would be ready to be cycled into *D. citri* rearing cages (see below for caging details). Repeated regular pruning also kept plants to an appropriate size that would fit into *D. citri* rearing cages in quarantine. Pruned plants produced new flush that was appropriate for *D. citri* egg laying ≈10–12 d after pruning in greenhouses maintained at 27 ± 2°C, 50 ± 6% relative humidity (RH), and natural day lengths. Plants were watered daily and fertilized with granular slow release fertilizer Osmocote smart release 19:6:12 (The Scotts Company LLC, Marysville, OH) as needed, usually once every 3 mo. Plants exhibiting young feather-flush suitable for oviposition were transferred at regular intervals from greenhouses to quarantine for *D. citri* inoculation.

In quarantine, *D. citri* colonies were maintained under a double caging system, that is a cage within a cage, in accordance with CDFA permit requirements. The smaller internal cage contained *D. citri* and its host plant. The larger external cage provided additional protection to minimize the likelihood of *D. citri* escaping from the internal cage. Internal cages were made by gluing two (15 by 15 by 15 cm) highly transparent 'U' shaped acrylic risers (SW Plastics F2191) to form a rectangular (15 by 15 by30 cm L by W by H) box. The rear side of the box was covered with ultrafine white no-see-um fabric (Skeeta Inc., Bradenton, FL), whereas the front side was fitted with a no-see-um fabric sleeve to allow access to cage contents. Two to four acrylic cages were contained within the larger external cage, a Bugdorm 2,400 (MegaView Science, Taiwan), that acted as the required second cage in the double-cage set up.

Potted *C. volkameriana* plants with multiple branches exhibiting feather-flush were removed from greenhouses for presentation to *D. citri* adults contained within double cages in quarantine. The top of the pot and the soil surface were covered with a piece of nylon stocking that was tied snuggly around the plant stem. This stocking cover helped minimize emergence of soil-borne fungus gnats (*Bradysia* sp.) into cages. Mature leaves on *C. volkameriana* were removed to force *D. citri* females to find young flush on which to oviposit.

A maximum of six cages (two each on Monday, Wednesday, and Friday) with *C. volkameriana* bearing feather-flush were setup each week for *D. citri* oviposition in quarantine. *D. citri* adults (≈15–20) were introduced into cages and allowed to oviposit for 2–3 d before being transferred to a new cage. Adult *D. citri* mortality was compensated for by adding new adults from other cages to keep the stocking rate constant at 15–20 adults per cage for the 2–3 d oviposition period. Plants were watered three times a week.

All *D. citri* rearing and colony maintenance in quarantine was conducted at 29°C, 40% RH, and a photoperiod of 14:10 (L:D) h (light sources used were Sylvania Fluorescent Octron 4100K, Osram Sylvania Inc., Danvers, MA).

NTP Species for Host Specificity Testing. California has a rich native psyllid fauna, with 164 species in 37 genera and four families (Percy et al. 2012). Because of this large psyllid fauna in California, only a subset of these species could be tested for their suitability as hosts to *T. radiata.* Consequently, multiple criteria were used for the selection of seven representative NTPs for host specificity testing and follows a "safety first" approach to assess aspects of potential direct impacts for a new entomophagous biological control agent (Kuhlmann et al. 2006). The five selection criteria, selected test species, and geographic origin of test species for use in *T. radiata* host specificity tests in quarantine are presented in Table 1. The nymphs of all NTP species were free-living and did not inhabit protective structures (i.e., galls or lerps). Colonies of five of these native test species were started from adults collected in the field, or from existing laboratory colonies, and this circumvented contamination issues with native parasitoids that attack nymphs. *Euphyllura olivina* (Costa) (Liviidae: Euphyllurinae) and *Arytainilla spartiophylla* (Foerster) (Psyllidae: Psylli-

Table 1. The five selection criteria and selected species of NTP used for host specificity testing of *T. radiata* in quarantine at UCR

Selection criteria	Selected species	Host plant species used in testing	Source of test psyllids
Target pest species	*D. citri* (Liviidae: Euphyllurinae)	*M. paniculata* (seedlings)	USDA–APHIS-PPQ-CPHST Mission Laboratory, Edinburg, TX
Close phylogenetic relatedness to *D. citri*	*D. fremontiae* (Liviidae: Liviinae) (native)[a]	*F. californicum* (bouquet[b])	Wrightwood, San Bernardino Co., CA
	E. olivina (Costa) (Liviidae: Euphyllurinae) (invasive pest)[c]	*Olea europaea* L. (seedlings)	Temecula, Riverside Co., CA
Native host plant related to citrus (Sapindales)	*C. californica* Schwarz (Calophyidae: Calophyinae) (native)[a]	*R. ovata* (bouquet[b])	UCR botanical garden, Riverside Co., CA
High probability of occurrence in native vegetation surrounding citrus groves	*H. texana* Crawford (Psyllidae: Ciriacreminae) (native)[a]	*P. glandulosa* (seedlings)	UCR botanical garden, Riverside Co., CA
	Heteropsylla sp. (Psyllidae: Ciriacreminae) (native)[a]	*A. farnesiana* (seedlings)	UCR botanical garden, Riverside Co., CA
Native pest psyllid	*Bactericera cockerelli* (Šulc) (Triozidae: no sub-family classification is available for Triozidae) (native)[d]	*C. annuum* (seedlings)	Trumble Laboratory, Dept. of Entomology, UCR
Beneficial psyllid attacking a noxious weed	*A. spartiophylla* (Foerster) (Psyllidae: Psyllinae) (self-introduced exotic)[e]	*Cytisus scoparius* L. (bouquet[b])	El Dorado Co., CA

[a] Field collected adults used to start colonies.
[b] Bouquets of cut stems were used in experiments because of the extreme difficulty of producing small rooted seedlings that could sustain psyllid nymphs for the duration of the exp.
[c] Field collected nymphs transferred to olive seedlings in quarantine.
[d] Colony initiated from a preexisting laboratory culture.
[e] Field collected nymphs of this univoltine species were used because of overwintering diapause requirements that could not be easily replicated in the laboratory. *A. spartiophylla* nymphs were transferred to broom seedlings in quarantine.

nae), both adventive species, were field collected as nymphs for use in experiments because they lack a known parasitoid fauna in California (Table 1). All test psyllids were moved to UCR under the appropriate CDFA-issued permits. Due to high host plant fidelity, each native psyllid species had to be reared on its native host plant. Potted *Rhus ovata* Watson and *Fremontodendron californicum* Coville seedlings were acquired from a local southern California native plant nursery (Mockingbird Nurseries, Riverside, CA). *Acacia farnesiana* (L.) and *Prosopsis glandulosa* Torrey were grown under green house conditions from seeds collected from the UCR botanical garden. Egg plant (*Solanum melongena* L.) seedlings were grown using a commercial variety "Long Purple" (Botanical Interests Inc., Broomfield, CO). Olive and Scotch broom plants were reared by CDFA and supplied to UCR on an as-needed basis for experiments. All host plants were maintained in greenhouses at UCR and provided necessary fertilization and water. Plants were pruned when necessary to maintain their size to fit into cages and to encourage feather-flush for psyllid oviposition.

NTP colonies on their respective host plants were individually maintained in the laboratory in Bugdorms (model 2120, MegaView Science, Taiwan). Native host plants with young flush were removed from greenhouses, transported to the laboratory, placed in Bugdorms, and inoculated with 20–30 native adult psyllids specific to that test plant species. New cages with plants were inoculated with psyllids produced in the laboratory as necessary to maintain colonies. Usually, NTP species (the exception here is *Bactericera Cockerelli* Sulc) laid their eggs on shoot tips and very young leaves. First- and second-instar nymphs preferentially fed on these young tissues. As nymphs matured, they moved downward onto thicker stems where they completed development. In contrast, the native pestiferous *B. cockerelli* oviposited on the leaf lamina, most commonly along the leaf margin, and nymphs fed along leaf veins.

Test Plant Preparation and Psyllid Transferal to Test Plants. Seeds of *Murraya Paniculata* (L.), *P. glandulosa*, *A. farnesiana*, and *Capsicum annuum* L. were sown in seed germination flats containing soil (UCR soil blend UC-3) and organic matter and watered and fertilized as needed. When seedlings developed 2–3 true leaves, they were transplanted in Ray Leach Cone-tainers (SC7 Stubby, 3.8 cm in diameter and 114-ml capacity, Stuewe and Sons Inc., Portland, OR). Leaves on transplanted seedlings were removed leaving only the youngest leaves at the plant's apex. The soil surface was covered with a white piece of circular foam with netting to reduce fungus gnat emergence and to more easily facilitate the recovery of test insects (i.e., psyllids or *T. radiata*) that fell from plants for record keeping and fate determination (i.e., alive or dead; Fig. 1). For bouquets of plant material used for psyllid presentation to *T. radiata*, the cone-tainers were replaced with 10.8-cm aqua-pics (Syndicate Sales Inc., Kokomo, IN). Clear plastic vials, 148-ml capacity (Thornton Plastics Co., Salt Lake City, UT), with three 12-mm-diameter holes (two on opposite sides, one on the bottom) covered with ultra-fine organza mesh, were inverted to fit on the vial lid that

Fig. 1. The series of management activities for the preparation of test plants in cone-tainers for host specificity testing trials. From left to right: 1) untrimmed seedling growing in a cone-tainer, 2) Plant trimmed to remove excess foliage, 3) Soil surface covered with netted foam to prevent emergence of fungus gnats, 4) Cone-tainer passed through the lid to which an inverted vial will be secured, and 5) attached inverted ventilated vial that restrains psyllids and test parasitoid to the test plant. (Online figure in color.)

was firmly secured around the cone-tainer (Fig. 1, no. 5). This ventilated inverted vial enclosed the test plant infested with test psyllids and confined *T. radiata* with the psyllid species of interest.

D. citri nymphs developing on *C. volkameriana* reached the fifth instar (a highly preferred life stage for attack by *T. radiata* [Skelley and Hoy 2004, Gómez-Torres et al. 2012]) 10–12 d postoviposition in quarantine at 29 ± 1°C and 40 ± 5% RH. NTP species were reared at a lower temperature 25 ± 1°C and 40 ± 5% because this improved survivorship rates, and NTPs typically took 3–4 wk to reach the fifth instar, the developmental stage appropriate for testing.

Five fifth-instar nymphs were used for trials with female *T. radiata.* To set up exactly five nymphs per test plant, fifth-instar nymphs on their respective host plants were gently nudged with a fine hair brush to encourage them to walk. Once mobile, nymphs were lifted off the plant with the brush and transferred onto the test plant. All nymph transfers were carried out in the morning on the test day and left for ≈2 hr to settle, feed, and acclimate. This fifth-instar nymph transfer process had a very high success rate of > 95%.

Source of *T. radiata* used in Tests and Host Specificity Test Methods. Adult *T. radiata* sourced from the Punjab of Pakistan were shipped to the Insectary and Quarantine Facility at UCR under permit (USDA–APHIS permit number: P526P-09-02585) at weekly intervals from the Division of Plant Industries, Gainesville, FL, over the period 24 March 2010 to 19 October 2011. Adult parasitoids were allowed to mate and feed on honey for 24 h before use in experiments. At time of testing, a single, mated, naïve (i.e., no previous exposure to psyllids) female *T. radiata* ≈48 h of age was isolated in a 0.6-ml microcentrifuge tube and then introduced into the inverted ventilated vials. 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 vial. Each *T. radiata* female was used only once for choice and no-choice tests. In sequential no-choice tests, the same female was tested four consecutive times.

Sequential No-Choice Tests. No-choice tests followed one of two different sequential exposure programs. In this design test, the first block of *T. radiata* were exposed to a NTP for a specified exposure period and then immediately to *D. citri* for a second exposure of the same duration (Table 2). In the second block of no-choice sequential testing, *T. radiata* females were first exposed to *D. citri* before being moved to NTP. This process for both test blocks of *T. radiata* was repeated on Day 2 (Table 2). The rationale for this exposure sequence was to determine if the attack rates by *T. radiata* on *D. citri* and NTP was influenced by first exposure experience of naïve females.

Static Long Exposure No-Choice Tests. In this design, *T. radiata* females were set up with either *D. citri* or NTP and left for 24 h before being removed (Table 2). This experimental design was implemented to determine attack rates on NTP when *T. radiata* was repeatedly encountering NTP and was intended to mimic conditions in nontarget habitat should *T. radiata* migrate into areas where ACP would be absent but

Table 2. Treatment summary for exposure tests of female *T. radiata* to *D. citri* and NTP species

Treatments		Day 1		Night	Day 2	
		4 h	4 h		4 h	4 h
T1	Sequential (*D. citri* first)	[*D. citri*]	▸[NTP]	Rest	▸[*D. citri*]	▸[NTP]
T2	Sequential (NTP first)	[NTP]	▸[*D. citri*]	Rest	▸[NTP]	▸[*D. citri*]
T3	Static choice test	[*D. citri* + NTP]				
T4	Control	[*D. citri*]/[NTP] No parasitoid exposure to measure natural nymph mortality under prevailing experimental conditions				
T5	Static prolonged no choice exposure	[*D. citri*] or [NTP] (24 h)				

▸, *T. radiata* movement to new psyllid hosts

Rest, containment of test female *T. radiata* in a ventilated 2-ml O-ring vial with honey and no psyllid exposure for ≈16 h.

other psyllid species would be present and encounterable for significant time periods.

Static Choice Tests. Choice tests were conducted whereby *T. radiata* was exposed to *D. citri* and NTP simultaneously on their respective host plants and allowed to forage and choose among psyllid species for attack for 4 h (Table 2).

Execution of *T. radiata* Host Specificity Tests in Quarantine. A single mated adult female *T. radiata* (≈2–3 d of age) was introduced into a test cage for a 4-h exposure period in sequential no-choice (T1, T2) and choice tests (T3) or for 24 h in prolonged exposure (T5) no-choice tests (Table 2). Naturally occurring mortality of psyllids under prevailing experimental conditions was assessed with control treatments (T4), where test psyllids were set up in an identical manner but were not exposed to *T. radiata.* Experiments were set up as complete blocks that were repeated over time for 10 replications for each test species and exposure scenario. All experiments were conducted in quarantine at 27°C, 40% RH, and a photoperiod of 14:10 (L:D) h.

Sequential Tests (T1 and T2). Female *T. radiata* were first introduced into either a *D. citri* cage (T1) or NTP cage (T2). After a 4-h exposure period, the same female was transferred to a new NTP cage (T1) or *D. citri* cage (T2) that had had no previous *T. radiata* exposure for a further 4 h. At the end of this 8-h exposure period, females were removed from cages in a ventilated 2-ml O-ring vial provisioned with a droplet of honey and rested overnight for 16 h until the next day. The same test sequences were repeated for these female *T. radiata* the following day (Table 2).

Choice Tests (T3). Ventilated inverted vials were removed from cone-tainers to expose test psyllids on their respective host plants. Cone-tainers were then placed inside a screened cage (15 by 15 by 20 cm) and then a test *T. radiata* female was introduced. After the 4 h exposure time, *T. radiata* was removed from the test arena and ventilated vials were replaced over each individual test plant to contain experimental psyllids (Table 2).

Control (T4). One set of five *D. citri* or five NTP on their respective host plants in cone-tainers were set up and maintained in a manner identical to that for psyllids exposed to *T. radiata.* Control psyllids of each test species were not exposed to *T. radiata.* Control psyllids provided estimates of naturally-occurring mortality that was because of the process of setting up psyllids on seedlings in containers and the subsequent maintenance in quarantine under prevailing standardized rearing conditions to determine developmental fate (i.e., death from unknown causes or development to adult psyllids; Table 2).

Prolonged No-Choice Exposure Test (T5). Female *T. radiata* were exposed to *D. citri* and NTP nymphs of a single species on their respective host plants for 24 h. After the 24-h exposure period, parasitoids were removed and nymphs were held to determine developmental fate (Table 2).

Data Recording and Statistical Analyses. All test cages were observed twice to record psyllid developmental outcomes. The first observation was 4–6 d postexposure to *T. radiata* and the second observation was 10–12 d postexposure to parasitoids. Records were taken of the number of adult psyllids that successfully emerged, psyllid nymph mortality, and number of *T. radiata* that emerged from hosts for each treatment.

Psyllid nymph mortality analyses were run using the PROC FREQ in SAS (SAS Institute Inc. 2008, SAS Institute, Cary, NC). Fisher's exact test (0.05 level of significance) was used to test the null hypothesis that psyllid nymph mortality in control treatments (i.e., mortality from unknown causes) was independent of nymph mortality in parasitoid exposure treatments (i.e., mortality from unknown causes, host feeding, and parasitism) within each of the NTP treatments or *D. citri* exposures. Fisher's exact test was adjusted using the sequential Bonferroni method to control for the experiment-wise error rate to determine significance. For each experiment, *P* values were first ordered from smallest to largest then compared using α/n, $\alpha/n-1$, ... , α (where n = number of experimental treatment comparisons) to determine if significant differences existed. Out of seven tested NTP species, *T. radiata* only parasitized *B. cockerelli,* the pestiferous native potato psyllid (see Results). To determine if *T. radiata* exhibited a host preference between *D. citri* and *B. cockerelli,* nymph mortality within each treatment pair (i.e., *D. citri* and *B. cockerelli*) were compared using Fisher's exact test. This test was also used to compare nymph mortality within each species (*D. citri* or *B. cockerelli*) and whether initial exposure of *T. radiata* (either to *D. citri* or *B. cockerelli*) had any effect on total nymph mortality. Fisher exact test for these analyses was adjusted using the sequential Bonferroni method to control the experiment-wise error rate. For each experiment, *P* values were first ordered from smallest to

Table 3. Suitability of *D. citri* and NTP as hosts for *T. radiata*

Treatment	Total nymphs exposed	Parasitism (%)	Total mortality (%)	*P* value	Total nymphs exposed	Parasitism (%)	Total mortality (%)	*P* value
	D. citri				*E. olivina*			
Sequential *D. citri* first	95	41	84	<0.01*	100	0	4	0.30
Sequential NTP first	100	27	81	<0.01[a]	100	0	4	0.30
Choice test	50	2	52	<0.01[a]	50	0	2	1.00
Prolonged exposure	51	20	95	<0.01[a]	50	0	6	0.24
Control (no parasitoid)	50	0	20	–	50	0	0	–
	D. citri				*D. fremontiae*			
Sequential *D. citri* first	98	34	83	<0.01[a]	100	0	41	0.37
Sequential NTP first	100	28	85	<0.01[a]	100	0	34	0.86
Choice test	50	4	58	<0.01[a]	50	0	42	0.41
Prolonged exposure	50	16	76	<0.01[a]	50	0	48	0.15
Control (no parasitoid)	50	0	24	–	50	0	32	–
	D. citri				*C. californica*			
Sequential *D. citri* first	99	34	87	<0.01[a]	98	0	46	0.13
Sequential NTP first	100	38	84	<0.01[a]	93	0	42	0.26
Choice test	50	18	70	0.02	45	0	44	0.27
Prolonged exposure	50	24	96	<0.01[a]	50	0	50	0.09
Control (no parasitoid)	50	0	46	–	42	0	31	–
	D. citri				*H. texana*			
Sequential *D. citri* first	100	16	71	<0.01[a]	100	0	18	0.82
Sequential NTP first	100	29	85	<0.01[a]	100	0	29	0.11
Choice test	50	10	78	<0.01[a]	49	0	14	1.00
Prolonged exposure	49	6	92	<0.01[a]	50	0	16	1.00
Control (no parasitoid)	50	0	34	–	50	0	16	–
	D. citri				*Heteropsylla* sp.			
Sequential *D. citri* first	100	20	75	<0.01[a]	100	0	4	0.30
Sequential NTP first	95	17	72	<0.01[a]	100	0	9	0.03
Choice test	42	7	43	0.02	49	0	6	0.12
Prolonged exposure	39	8	80	<0.01[a]	40	0	5	0.19
Control (no parasitoid)	50	0	20	–	50	0	0	–
	D. citri				*A. spartiophylla*			
Sequential *D. citri* first	100	24	73	<0.01[a]	100	0	34	0.85
Sequential NTP first	100	29	73	<0.01[a]	95	0	42	0.28
Choice test	50	14	68	<0.01[a]	45	0	31	1.00
Prolonged exposure	50	14	82	<0.01[a]	40	0	48	0.19
Control (no parasitoid)	50	0	18	–	50	0	32	–
	D. citri				*B. cockerelli*			
Sequential *D. citri* first	100	27	83	<0.01[a]	100	5	44	<0.01[a]
Sequential NTP first	100	29	88	<0.01[a]	98	6	46	<0.01[a]
Choice test	47	0	64	<0.01[a]	50	2	26	0.47
Prolonged exposure	50	18	100	<0.01[a]	50	2	58	<0.01[a]
Control (no parasitoid)	50	0	34	–	50	0	18	–

P values generated using Fisher's exact test at the 0.05 level of significance with Bonferroni adjustments.
[a] Fisher's exact test with Bonferroni adjusted experiment-wise error rate was significant.

largest then compared using α/n, $\alpha/n-1$, ... , α (where n = number of experimental treatment comparisons) to determine if significant differences existed.

Results

***D. citri* Mortality.** Mortality of *D. citri* nymphs due to undetermined causes in control cages averaged 28%, while 72% successfully emerged as adult psyllids. There was no *T. radiata* emergence in *D. citri* control treatments indicating no accidental contamination had occurred. *D. citri* nymph mortality significantly increased when exposed to *T. radiata*, irrespective of the method of exposure (choice or no-choice) or duration of exposure (4 h vs. 24 h; all $P \leq 0.02$; Table 3). Across all trials and exposure scenarios parasitism of *D. citri* by *T. radiata* averaged 20 ± 2%) and parasitism was observed in 100% of exposure events (Table 3). The extent of nymph mortality (undetermined mortality of nymphs and parasitism) was highest in the prolonged exposure treatment, and ranged from 76 to 100%. Nymph mortality in sequential tests ranged from 71 to 88%, and in choice tests *D. citri* mortality ranged from 43 to 78% (Fig. 2A–G).

Non-Target Psyllid Mortality. *B. cockerelli* was the only NTP species that was parasitized by *T. radiata.* In total, 13 adult *T. radiata* emerged from 298 nymphs (= 4.4% level of parasitism and parasitism was observed across all exposure trials [2–6%] [Table 3]) exposed to *T. radiata* during these tests (Table 3; Fig. 2A). Total nymph mortality of *B. cockerelli* under no-choice conditions of exposure to *T. radiata* was significantly higher (44 to 58%) than total nymph mortality in the control cages (18%; $P \leq 0.01$). However, *B. cockerelli* mortality under choice conditions (26%)

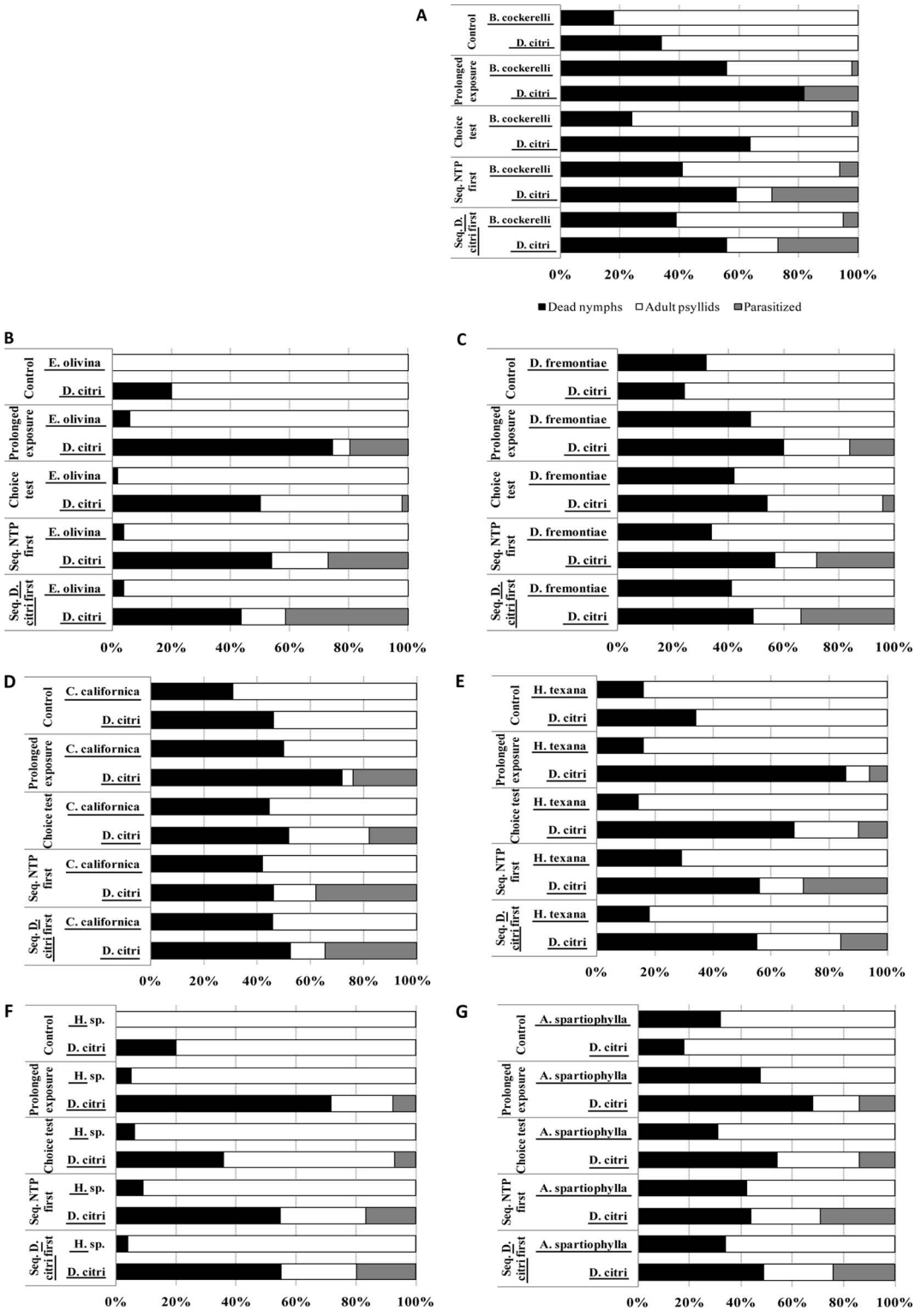

Fig. 2. Comparison of mortality rates for *D. citri* nymphs and NTP species exposed to *T. radiata* under different host preference testing regimens. *T. radiata* testing against: (A) *B. cockerelli*, (B) *E. olivina*, (C) *D. fremontiae*, (D) *C. californica*, (E) *H. texana*, (F) *Heteropsylla* sp., and (G) *A. spartiophylla*. Note, Seq = no choice sequence test.

Table 4. Comparison of nymph mortality and *T. radiata* emergence rates between *D. citri* and *B. cockerelli* under different exposure methods

Treatments	Psyllid species	Total nymphs exposed	Parasitism (%)	Total mortality (%)	*P* value
Sequential *D. citri* first	*D. citri*	100	27	83	<0.01[a]
	B. cockerelli	100	5	44	
Sequential *B. cockerelli* first	*D. citri*	100	29	88	<0.01[a]
	B. cockerelli	98	6	46	
Choice test	*D. citri*	47	0	64	<0.01[a]
	B. cockerelli	50	2	26	
Prolonged exposure	*D. citri*	50	18	100	<0.01[a]
	B. cockerelli	50	2	58	
Control	*D. citri*	50	0	34	0.11
	B. cockerelli	50	0	18	

[a] Fisher's exact test with Bonferroni adjusted experiment-wise error rate was significant.

was not significantly different from the control mortality rates (18%; $P = 0.47$; Table 3). In comparison, control *D. citri* mortality was 34%, which was significantly lower than mortality rates observed in the *T. radiata* exposure treatments, where it ranged from 64 (choice test) to 100% (prolonged exposure; Table 3). Total *D. citri* mortality in the control cages (34%) was not significantly different from total *B. cockerelli* nymph mortality (18%) in the control cages ($P = 0.11$; Table 3). Irrespective of the parasitoid exposure method or duration of exposure, *D. citri* mortality in each treatment was significantly higher (64 to 100%) than *B. cockerelli* mortality (26 to 58%; all $P \leq 0.01$; Table 4).

In sequential tests, whether *T. radiata* was first exposed to *D. citri* or *B. cockerelli* had no significant effect on the rate of either *D. citri* (88 vs. 83%; $P = 0.42$) or *B. cockerelli* mortality rates (46 vs. 44%; $P = 0.78$).

E. olivina, an invasive pest that is phylogenetically closest to *D. citri* among tested psyllid species. *E. olivina* mortality was low (≤6%) in all *T. radiata* exposure treatments and this did not differ significantly from control mortality (Table 3; Fig. 2B). *D. citri* control mortality was 20%, which was significantly lower than nymph mortality observed in choice tests and prolonged exposure experiments (Table 3).

Diclidophlebia fremontiae (Klyver) was the native California psyllid most closely related to *D. citri* among tested species. *D. fremontiae* nymph mortality in control treatments was 32%, which was not significantly different from mortality recorded for the *T. radiata* exposure treatments (Table 3). For *D. citri*, control mortality at 24% was significantly lower than that observed for nymphs in the *T. radiata* exposed treatments, which ranged from 58 to 85% (Table 3; Fig. 2C).

Calophya californica Schwarz nymph mortality in control cages (31%) was not significantly different from nymph mortality recorded for *T. radiata* exposure treatments. Control *D. citri* nymph mortality was 46%, which was significantly lower than the ACP nymph mortality in the *T. radiata* exposure treatments which ranged from 70 (choice test) to 96% (prolonged exposure; Table 3; Fig. 2D).

Heteropsylla texana Crawford control mortality (16%) was not significantly different when compared with mortality rates recorded for nymphs exposed to *T. radiata*. *D. citri* mortality in control treatments was 34%, which was significantly lower than *D. citri* nymph mortality in the *T. radiata* exposure treatments, which ranged from 71 (sequential tests) to 92% (prolonged exposure; Table 3; Fig. 2E).

Heteropsylla sp. nymph mortality in control cages (0%) was not significantly different from mortality observed in the *T. radiata* exposure treatments, except for the sequential test, where *Heteropsylla* sp. nymphs were exposed to *T. radiata* first. In this instance, nymph mortality was 9%, which differed significantly from control mortality (Table 3). The biological significance of this result is uncertain. Control mortality for *D. citri* nymphs was 20%, which was significantly lower than nymph mortality in the *T. radiata* exposure treatments which ranged from 43 (choice test) to 80% (prolonged exposure; Table 3; Fig. 2F).

A. spartiophylla nymph mortality in control treatments (32%) was not significantly different from nymph mortality in the *T. radiata* exposure treatments. Control *D. citri* nymph mortality was 18%, which was significantly lower than *D. citri* nymph mortality in the *T. radiata* exposure treatments, where it ranged from 68 (choice test) to 82% (prolonged exposure; Table 3; Fig. 2G).

Discussion

While a parasitoid's host range includes all potential species that can be parasitized, host specificity refers to the relative degree of use by the natural enemy for each species used (Van Driesche and Murray 2004). In this study, *T. radiata* exhibited a narrow host range under sequential no-choice, static no-choice, and choice exposure scenarios, attacking two species of test psyllids, *D. citri* (target) and *B. cockerelli* (nontarget), when offered seven NTP species; a test range that included five native species and two self-introduced species representing five psyllid families. A high degree of host specificity was observed for *T. radiata*, with ≈20% parasitism recorded for *D. citri* versus <5% for *B. cockerelli*.

Because *D. citri* (Liviidae) is in a different family to *B. cockerelli* (Triozidae), we postulate that parasitism of *B. cockerelli* nymphs was not the result of phylo-

genetic relatedness, but may be due to either experimental conditions in quarantine or the size of *B. cockerelli* nymphs, which are similar to *D. citri.* Low-levels of parasitism by *T. radiata* when exposed to *B. cockerelli* in small exposure arenas in quarantine may have resulted from experimental conditions that promoted parasitism, which may be unlikely to occur in the field. The artificiality of host range assessments conducted in small test arenas may exaggerate host range estimates if it alters the behavior of natural enemies (van Lenteren et al. 2006). Host size, in addition to taxonomic affinity, has been demonstrated to be an important host selection factor for other parasitoid species subjected to host range assessments under standardized laboratory conditions that removed environmental cues that are normally relevant to host selection (Andreassen et al. 2009).

The pestiferous olive psyllid, *E. olivina* (Liviidae), and the native fremontia psyllid, *D. fremontiae* (Liviidae), were two species tested that were most closely related to *D. citri* (Liviidae). Neither species was attacked by *T. radiata.* This suggests that family and tribe level classifications for nontarget species in the receiving range may not be generally useful for predicting host vulnerability to this parasitoid. This result questions the usefulness of selecting the most closely related nontarget species for evaluating the host specificity of *T. radiata,* and suggests that nymph size and general morphology may be more important indicators of potential hosts. Consequently, in California, *T. radiata* may attack NTP species that either have similar sizes, morphology, and life history traits to *D. citri,* and this risk of attack may be more likely when species coexist in a common habitat. For example, solanaceous host plants that support *B. cockerelli* co-occur with *D. citri*-infested citrus in urban gardens in southern California, which could provide opportunities for *T. radiata* to attack *B. cockerelli.* This possibility should be examined experimentally to determine whether *T. radiata* will attack *B. cockerelli* under field conditions.

Potential nontarget impacts, should they occur outside of the quarantine laboratory, are difficult to predict. If *T. radiata* attacks NTP species in California, especially native species in native habitats, impacts may be negligible due to possible inferior host quality (demonstrated here for *B. cockerelli* <5% parasitism), and competition from speciose resident guilds of specialist native parasitoids (see Percy et al. 2012 for summary of information on California psyllid parasitoids). Additional uncertainty pertains to the infiltration capacity of *T. radiata,* and its ability to disperse in sufficient numbers widely and deeply enough from urban and commercial citrus to penetrate native ecosystems, especially areas dominated by chaparral (a mixture of native xeric-adapted plants that host native California psyllids) that may border commercial production areas. We are unaware of any records documenting host use other than *D. citri* by *T. radiata* in the United States (e.g., Florida [released in 1999 {Qureshi et al. 2009} and Texas] [self-introduced {De Léon and Sétamou 2010}]), the Caribbean, Mexico, or Brazil where *T. radiata* is widespread (De Léon and Sétamou 2010). This lack of rearing data for *T. radiata* from other psyllid species may simply be due to insufficient survey work.

Hopper (2001) suggests that understanding host range in the source region is one way to help design studies to predict potential host range of a natural enemy before introduction in a new area. However, the host range of *T. radiata* in its presumed native range (the Indian subcontinent [Waterston 1922] and southeast Asia [Qureshi et al. 2009]) is not well documented, but could include at least one other species in addition to *D. citri* (*Euphalarus citri* listed by Noyes [2012] is a synonym of *D. citri*), *Psylla hyalina* Mathur (Hemiptera: Psyllidae: Psyllinae) in India (Peter et al. 1990, Noyes 2012). Records of *Trioza erytreae* (Del Guerci) (Hemiptera: Triozidae) and *Trioza* sp. in South Africa as hosts for *T. radiata* are questionable, and likely a misidentification of a congeneric species (McDaniel and Moran 1972). This can be a common problem when developing nontarget species lists for safety testing from literature reviews and museum records (Hoddle 2004, Sands and Van Driesche 2004). Surveys of psyllids for parasitoids in the home range of *T. radiata,* which at a minimum focus on other species of *Diaphorina,* could greatly increase our understanding of host breadth for this parasitoid. For example, there are at least two other species of *Diaphorina* in Pakistan, *Diaphorina gymnosporiae* Mathur and *Diaphorina aegyptiaca* Puton (Hodkinson 1986); both species are sympatric with *D. citri* in the Pakistan Punjab, and could be unknown hosts for *T. radiata.* Similar surveys for nontarget impacts by *T. radiata* could be conducted in countries where this parasitoid has been introduced, intentionally or not, for biological control of *D. citri.*

A 60-page Environment Assessment Report based on the results of the studies presented here was submitted for review by USDA–APHIS on 15 November 2011. On 7 December 2011, USDA–APHIS issued a permit (P526P-11-04159) authorizing the release of *T. radiata* from quarantine for biological control of *D. citri,* and on 20 December 2011 the first releases of *T. radiata* sourced from the Punjab of Pakistan were made (Hoddle 2011). By November 2013, > 180,000 *T. radiata* had been released at >350 different sites spanning six counties (Imperial, Los Angeles, Orange, Riverside, San Bernardino, and San Diego), mainly in urban areas, and recovery and spread from release sites has been documented (Hoddle 2012a,b). Studies are currently underway to assess the impact *T. radiata* is having on *D. citri* populations in urban areas.

Acknowledgments

This work was supported in part by the California Department of Food and Agriculture Specialty Crops Grant SCB09056 and Citrus Research Board grant 5500-183 awarded to MSH. We thank Ben Rangel and David Morgan, CDFA Mt. Rubidoux for assistance with plant production. Kris Godfrey (CDFA and University of California Davis) supplied *A. spartiophylla* for testing. Ruth Amrich, University of California Riverside, assisted with various laboratory ac-

tivities and collected *E. olivina* for testing. Karen Huaying Xu, Department of Statistics, University of California Riverside, helped with data analyses. We thank the anonymous reviewers and Julio Bernal for comments that greatly improved this manuscript.

References Cited

Andreassen, L. D., U. Kuhlmann, P. G. Mason, and N. J. Holliday. 2009. Host range testing of a prospective classical biological control agent against cabbage maggot, *Delia radicum*, in Canada. Biol. Control 48: 210–220.

Anon. 2010. Citrus research board aggressively responds to unprecedented threat. Citrograph 1: 12–14.

Atkins, B. 2010. Asian citrus psyllid and quarantines. Citrograph 1: 7.

Aubert, B., M. Grisoni, M. Villemin, and G. Rossolin. 1996. A case study of hunglongbing (greening) control in Réunion, pp. 276–278. *In* J. V. da Graça, P. Moreno, and R. K. Yokomi (eds.), Proceedings of the 13th Conference of the International Organization of Virologists (IOCV). University of California, Riverside, CA.

Babendreier, D., F. Bigler, and U. Kuhlmann. 2006. Current status and constraints in the assessment of non-target effects, pp. 1–14. *In* F. Bigler, D. Babendreier, and U. Kuhlmann (eds.), Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment. CABI Publishing, Wallingford, Oxon, United Kingdom.

Bigler, F., D. Babendreier, and U. Kuhlmann (eds). 2006. Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment. CABI Publishing, Wallingford, Oxon, United Kingdom.

Bové, J. M. 2006. Hunglongbing: a destructive newly emerging, century-old disease of citrus. J. Plant Pathol. 88: 7–37.

California Citrus Pest and Disease Prevention Program. 2011. California Citrus Pest and Disease Update. 1: 2. (http://www.cdfa.ca.gov/citruscommittee/docs/newsletters/NewsletterDec2011.pdf) (accessed 10 July 2013)

De Léon, J. H., and M. Sétamou. 2010. Molecular evidence suggests that populations of the Asian citrus psyllid parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) from Texas, Florida, and Mexico represent a single species. Ann. Entomol. Soc. Am. 103: 100–110.

Gómez-Torres, M. L., D. E. Nava, and J. R. Postali Para. 2012. Life table of *Tamarixia radiata* (Hymenoptera: Eulophidae) on *Diaphorina citri* (Hemiptera: Pysllidae) at different temperatures. J. Econ. Entomol. 105: 338–343.

Grafton-Cardwell, E. 2010. How serious is the threat of Asian citrus psyllid and Huanglongbing? Citrograph 1: 8–10.

Grafton-Cardwell, E., J. Morse, and B. Taylor. 2011. Asian citrus psyllid treatment strategies for California-Arizona. Citrograph 5: 5–10.

Halbert, S. E., and K. L. Manjunath. 2004. Asian citrus psyllid (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87: 330–353.

Hall, D. G., and M. G. Hentz. 2011. Season flight activity by the Asian citrus psyllid in east central Florida. Entomol. Exp. Appl. 139: 75–85.

Hall, D. G., M. G. Hentz, and R. C. Adair. 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. Environ. Entomol. 37: 914–924.

Hill, N. 2012. Update from the citrus pest and disease prevention committee. Citrograph 3: 10.

Hoddle, M. S. 2004. Analysis of fauna in the receiving area for the purpose of identifying native species that exotic natural enemies may potentially attack, pp. 24–39. *In* R. G. Van Driesche and R. Reardon (eds.), Assessing host ranges for parasitoids and predators used in classical biological control: a guide to best practices. U.S. Dep. Agric.-FS FHTET-2004–03, Morgantown, WV.

Hoddle, M. S. 2011. First release of *Tamarixia radiata* for the biological control of Asian citrus psyllid in California. (http://cisr.ucr.edu/blog/news/first-release-of-tamarixia-radiata-in-california-for-the-biological-control-of-asian-citrus-psyllid/) (accessed 10 July 2013)

Hoddle, M. S. 2012a. Has the Asian citrus psyllid parasitoid, *Tamarixia radiata*, established in California? (http://cisr.ucr.edu/blog/asian-citrus-psyllid-2/has-the-asian-citrus-psyllid-parasitoid-tamarixia-radiata-established-in-california/) (accessed 10 July 2013)

Hoddle, M. S. 2012b. Foreign exploration for natural enemies of Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), in the Punjab of Pakistan for use in a classical biological control program in California USA. Pak. Entomol. 34: 1–5.

Hodges, A. W., and T. H. Spreen. 2012. Economic impacts of citrus greening (HLB) in Florida, 2006/07–2010/11. EDIS document FE903, Food and Resource Economics Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL. (http://edis.ifas.ufl.edu) (accessed 10 July 2013)

Hodkinson, I. D. 1986. The psyllids (Homoptera: Psylloidae) of the oriental zoogeographical region: an annotated checklist. J. Nat. Hist. 20: 299–357.

Hoffmeister, T. S., D. Babendreier, and E. Wajnberg. 2006. Statistical tools to improve the quality of experiments and data analysis for assessing non-target effects, pp. 222–240. *In* F. Bigler, D. Babendreier, and U. Kuhlmann (eds.), Biological control of arthropods using invertebrates: methods for environmental risk assessment. CABI Publishing, Wallingford, United Kingdom.

Hopper, K. R. 2001. Research needs concerning non-target impacts of biological control introductions, pp. 39–56. *In* E. Wajnberg, J. K. Scott, and P. C. Quimby (eds.), Evaluating indirect ecological effects of biological control. CABI Publishing, United Kingdom.

Jagoueix, S., J. M. Bové, and M. Garnier. 1994. The phloem limited bacterium of greening disease of citrus is a member of the alpha-subdivision of the proteobacteria. Int. J. System. Bacteriol. 44: 379–386.

Kuhlmann, U., U. Schaffner, and P. G. Mason. 2006. Selection of non-target species for host specificity tests, pp. 15–37. *In* F. Bigler, D. Babendreier, and U. Kuhlmann (eds.), Biological control of arthropods using invertebrates: methods for environmental risk assessment. CABI Publishing, Wallingford, United Kingdom.

Leavitt, R. 2012. Huanglongbing confirmed in California. Citrograph 3: 8–9.

Mason, P. G., A. B. Broadbent, J. W. Whistlecraft, and D. R. Gillespie. 2011. Interpreting the host range of *Peristenus digoneutis* and *Peristenus relictus* (Hymenoptera: Braconidae) biological control agents of *Lygus* spp. (Hemiptera: Miridae) in North America. Biol. Control 57: 94–102.

McDaniel, J. R., and V. C. Moran. 1972. The parasitoid complex of the citrus psylla *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). Entomophaga 17: 297–317.

Michaud, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Control 29: 260–269.

Murray, T. J., T. M. Withers, and S. Mansfield. 2010. Choice vs. no-choice test interpretation and the role of biology and behavior in parasitoid host specificity tests. Biol. Control 52: 153–159.

Noyes, J. S. 2012. Universal Chalcidoidea Database. (http://www.nhm.ac.uk/chalcidoids) (accessed 10 July 2013).

Peter, C., P. Nagarathinam, and B. V. David. 1990. Note on the natural enemies of *Psylla hyaline* (Homoptera: Psyllidae) on *Albizia lebbeck.* Entomophaga 35: 659–660.

Percy, D. M., A. Rung, and M. S. Hoddle. 2012. An annotated checklist of the psyllids of California (Hemiptera: Psylloidea). Zootaxa 3193: 1–27.

Qureshi, J. A., and P. A. Stansly. 2009. Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus. Biol. Control 50: 129–136.

Qureshi, J. A., M. E. Rogers, D. G. Hall, and P. A. Stansly. 2009. Incidence of invasive *Diaphorina citri* (Hemiptera: Psyllidae) and its introduced parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida citrus. J. Econ. Entomol. 102: 247–256.

SAS Institute. 2008. SAS/STAT® 9.2. User's Guide. SAS Institute, Cary, NC.

Sands, D.P.A., and R. G. Van Driesche. 2004. Using the scientific literature to estimate host range of a biological control agent, pp. 15–23. *In* R. G. Van Driesche and R. Reardon (eds.), Assessing host ranges for parasitoids and predators used in classical biological control: a guide to best practice. U.S. Dep. Agric.-FS FHTET-2004-03, Morgantown, WV.

Skelley, L. H., and M. A. Hoy. 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. Biol. Control 29: 14–23.

Van Driesche, R. G. 2004. Introduction: predicting host ranges of parasitoids and predacious insects – what are the issues?, pp. 1–3. *In* R. G. Van Driesche and R. Reardon (eds.), Assessing host ranges for parasitoids and predators used in classical biological control: a guide to best practice. U.S. Dep. Agric.-FS FHTET-2004-03, Morgantown, WV.

Van Driesche, R. G., and T. J. Murray. 2004. Overview of testing schemes and designs used to estimate host ranges, pp. 68–89. *In* R. G. Van Driesche and R. Reardon (eds.), Assessing host ranges for parasitoids and predators used in classical biological control: a guide to best practice. U.S. Dep. Agric.-FS FHTET-2004-03, Morgantown, WV.

Van Driesche, R., M. Hoddle, and T. Center. 2008. Control of pests and weeds by natural enemies–an introduction to biological control. Blackwell Publishing, Malden, MA.

van Lenteren, J. C., M.J.W. Cock, T. S. Hoffmeister, and D.P.A. Sands. 2006. Host specificity in arthropod biological control, methods for testing and interpretation of data, pp. 38–63. *In* F. Bigler, D. Babendreier, and U. Kuhlmann (eds.), Biological control of arthropods using invertebrates: methods for environmental risk assessment. CABI Publishing, Wallingford, United Kingdom.

Vieira, L. C., T. J. McAvoy, J. Chantos, A. B. Lamb, S. M. Salom, and L. T. Kok. 2011. Host range of *Laricobius osakensis* (Coleoptera: Derodontidae) a new biological control agent of woolly hemlock adelgid (Hemiptera: Adelgidae). Environ. Entomol. 40: 324–332.

Waterston, J. 1922. On the chalcidoid parasites of psyllids (Hemiptera, Homoptera). Bull. Entomol. Res. 13: 41–58.

Received 10 July 2013; accepted 14 December 2013.