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## High host specificity in *Encarsia diaspidicola* (Hymenoptera: Aphelinidae), a biological control candidate against the white peach scale in Hawaii

Gabor Neumann<sup>a,\*</sup>, Peter A. Follett<sup>a</sup>, Robert G. Hollingsworth<sup>a</sup>, Jesse H. de León<sup>b</sup>

<sup>a</sup> USDA-ARS, U.S. Pacific Basin, P.O. Box 4459, Hilo, HI 96720, USA

<sup>b</sup> USDA-ARS, Kika de la Garza Agricultural Research Center, Weslaco, TX, USA

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## ABSTRACT

Pre-introductory host specificity tests were performed with *Encarsia diaspidicola*, a biological control candidate against the invasive white peach scale, *Pseudaulacaspis pentagona*. False oleander scale, *P. cockerelli*, coconut scale, *Aspidiotus destructor*, cycad scale, *Aulacaspis yasumatsui*, greenhouse whitefly, *Trialeurodes vaporariorum*, green scale, *Coccus viridis*, and long-tailed mealybug, *Pseudococcus longispinus* were tested in quarantine using traditional no-choice tests and examined for wasp emergence. The Hawaiian endemic palm scale, *Colobopyga pritchardiae* was also tested using no-choice tests and evaluated using species-specific molecular markers. All tests used unexposed non-target cohorts and no-choice exposure of white peach scale to the parasitoid as controls. None of the non-target exotic species yielded wasp emergence, and exposure to wasps had no effect on the mortality of the non-target species examined. Molecular tests with the endemic palm scale showed no evidence of parasitism by *E. diaspidicola*. These results strongly support that *E. diaspidicola* has a narrow host range and that its release in Hawaii will have negligible risk of non-target effects.

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### 1. Introduction

White peach scale, *Pseudaulacaspis pentagona* Targioni-Tozzetti (Hemiptera: Diaspididae), is one of the most polyphagous armored scale insects in the world (Miller and Davidson, 2005), but so far in Hawaii it is only known as a crop pest in papaya, *Carica papaya* L. Infestation can rapidly increase to levels where large areas of the trunks of papaya trees are completely covered by white peach scales. Overcrowding causes scales to spread up the trunk, and in heavily infested trees scales move up onto fruit, preferring to settle in the calyx and peduncle regions. White peach scale on the fruit is a quarantine problem. Infested fruit shipments may be rejected in California, and Japan, a very important market for Hawaii papayas, has zero tolerance for white peach scale. Infested fruits may be brushed to physically remove scales at considerable cost. In papayas grown for the Japan market, fields may be abandoned after 5% of the trees are infested. White peach scale can also decrease plant vigor and yield. Control with available chemical insecticides is not effective (Follett, 2000). The advancing infestation, which is at outbreak levels in some areas of the Big Island (Island of Hawaii), where the largest Hawaii papaya orchards are located, and the inefficiency of chemical control necessitated the development of a biological control program.

Female white peach scales deposit all their eggs ( $\approx 100$ –150 total/female) in about a week. Eggs hatch in 3–4 days and the young scales (“crawlers”) settle on the host plants within 2 days after hatching. Crawlers do not actively disperse far from the point of hatching but can be spread by the wind. Once crawlers settle they remain attached to the host plant throughout their lives. Two subsequent molts requiring about three weeks time produces adult females. Females form a slightly oval waxy cover (scale) during development. Second instar males form an oblong cover and after three molts emerge as adults 19–22 days later. Adult males are winged and immediately start inseminating females. Oviposition by females begins 14–16 days after mating. A generation is completed in 36–40 days at 25 °C (Miller and Davidson, 2005).

*Encarsia diaspidicola* Silvestri (Hymenoptera: Aphelinidae) is a thelytokous solitary endoparasite. The adult females deposit eggs singly in immature stages of white peach scale. The development time of the parasitoid at approximately 23 °C is 30–35 days. In the scientific literature, *E. diaspidicola* is reported from white peach scale (Huang and Polaszek, 1998) and *Quadraspidiotus perniciosus* Comstock, San Jose scale (Peck, 1963). The Universal Chalcidoidea Database (Noyes, 2003) lists other primary hosts but those records may have been based on misidentifications. Later workers (Heraty et al., 2007) only list white peach scale and San Jose scale as primary hosts with a single reference to San Jose scale (Peck, 1963). As there have been no reports since 1963 of *E. diaspidicola* attacking San Jose scale, the Peck record may be questionable.

\* Corresponding author.

E-mail addresses: [gabor.neumann@ars.usda.gov](mailto:gabor.neumann@ars.usda.gov), [gn28@cornell.edu](mailto:gn28@cornell.edu) (G. Neumann).

Several biological control programs have targeted white peach scale worldwide (Collins and Whitcomb, 1975; Waterhouse and Norris, 1987; Liebrechts et al., 1989). *Encarsia berleseii* Howard (Hymenoptera: Aphelinidae) and *E. diaspidicola* were released in Samoa as biological control agents to control white peach scale on passion fruit, *Passiflora edulis* L. Over time, *E. diaspidicola* displaced *E. berleseii* in Samoa and lowered white peach scale population numbers significantly (Liebrechts et al., 1989). Because of its successful establishment and control of white peach scale populations in Samoa, which has a similar climate to Hawaii, *E. diaspidicola* was selected as the preferred biological agent and was imported into Hawaii from Samoa in 2006. A colony was established at the USDA Forestry Service Quarantine Facility in Hawaii Volcanoes National Park.

Parasitoid wasps in the genus *Encarsia* Foerster mostly attack whiteflies (Aleyrodidae) and armored scale insects (Diaspididae) (Huang and Polaszek, 1998), with four species recorded from aphids in the family Hormaphididae (Evans et al., 1995). Females of most *Encarsia* species develop as primary internal parasitoids of diaspidid armored scales or whiteflies, but the males of the same species develop as parasitoids of female parasitoid larvae or pupae, often of their own species (Viggiani, 1984; Hunter and Woolley, 2001). In the case of *E. porteri*, females develop as primary parasitoids of whiteflies and males are obligate parasitoids of lepidopteran eggs (Hunter et al., 1996).

The Hawaiian insect fauna has a high rate of endemism, and biological control programs must show a high degree of host specificity for candidate agents and minimal risk to non-target native species before permission to release will be granted. *E. diaspidicola* is known to attack only diaspidid scales and there are no native diaspidid scale insects in Hawaii, making it a promising candidate. Nevertheless, host specificity testing was required by state and federal regulatory agencies to demonstrate minimal risk of non-target effects. The native Hawaiian insect fauna includes three species of scale insects in the family Halimococcidae or pupillaral palm scales; these halimococcids were at one time classified as diaspidid scales (Beardsley, 1963). One of the species, *Colobopyga pritchardiae*, an endemic palm scale found only on *Pritchardia* palms and recently recorded for the first time on the island of Hawaii (Neumann et al., 2007), was included in host testing as a representative of the Hawaiian Halimococcidae. *Encarsia diaspidicola* was also tested for its ability to parasitize several invasive, economically important diaspidid scales and close relatives: the false oleander scale, *P. cockerelli* Cooley (Diaspididae), the coconut scale, *Aspidiotus destructor* Signoret (Diaspididae), the cycad scale, *Aulacaspis yasumatsui* Takagi (Diaspididae), the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Aleyrodidae), the green scale, *Coccus viridis* Green (Coccidae), and the long-tailed mealybug, *Pseudococcus longispinus* Targioni-Tozzetti (Pseudococcidae).

## 2. Materials and methods

### 2.1. Insect colonies and rearing

*Encarsia diaspidicola* was originally collected in Samoa from white peach scale on passion fruit (*P. edulis* L.) and was imported to Volcanoes National Park Quarantine Facility in Hawaii.

White peach scale crawlers were collected in papaya, *C. papaya* L., orchards from the trunks of infested papaya trees to start a colony in quarantine, and thereafter propagated on butternut squash, *Cucurbita moschata* Duchesne ex Lam. For *E. diaspidicola* rearing, a squash infested with approximately 500 settled white peach scale crawlers was placed into a 1-gallon wide-mouth transparent plastic jar (diameter: 14 cm, height: 25 cm, opening diameter: 10 cm). Small butternut squashes were selected for rearing so that they

could fit whole through the mouth of the jar. When the scales were 14 days old (second instar), 30–40 *E. diaspidicola* were introduced to the jar. The wasps were provided with honey solution in a small, sealed plastic container with a wick made out of filter paper. The wasps were left with the hosts for their entire life span.

Coconut scale was also reared on butternut squash using the same methods as for white peach scale. False oleander scale was reared on *Nerium oleander* L. plants. When crawlers emerged, they were brushed from the leaves onto a fresh oleander plant. For experimental purposes, plants small enough to fit in the 1-gallon plastic jars, planted in 10-cm upper-rim diameter plastic pots, were used. Cycad scale was reared on potted *Cycas* sp. When crawlers emerged, they were brushed onto a fresh plant. For experimental purposes, a cycad frond was chosen on the plant and a 1-gallon plastic jar was used to enclose the frond as follows: the jar lid was cut from the side along the radius of the lid to the center. A small hole was drilled in the center just large enough to accommodate the stem of the frond. The stem was fitted into the hole in the center of the lid by sliding it through the cut from the side of the lid into the center. The cut, as well as the hole in the center were sealed with glue and the lid was left undisturbed until the glue set. Once the glue set, the jar could be screwed onto the lid so that the frond with the insects on it was enclosed inside the jar. Greenhouse whitefly was reared on tomato plants potted in above-mentioned pots. Whiteflies were allowed to self-transfer from one plant to another. For experimental purposes, small potted plants were used that fit into the 1-gallon jars. Green scale was reared on *Gardenia* sp.; immature insects were transferred by removing infested leaves from a host plant and securing the leaves on the target host plant with paper clips. For experimental purposes, potted plants were used and pruned so that they could fit inside the 1-gallon plastic jars. Long-tailed mealybug was reared on sprouted potatoes; the insects were transferred by brush to fresh potatoes as needed.

Attempts to rear *C. pritchardiae* in the laboratory were not successful. Therefore, *C. pritchardiae* specimens were collected from mature *Pritchardia* palms from the Waiakea Forest Reserve near Hilo, Hawaii. Fruiting branches on the palms were inspected for the presence of *C. pritchardiae* and if present, the entire fruiting branches were cut from the palms. The cuttings were transferred into water bottles and transported into the USDA Forestry Service Quarantine Facility in Hawaii Volcanoes National Park for testing immediately after collection.

All insect colonies were maintained in the USDA Forestry Service Quarantine Facility in Hawaii Volcanoes National Park at 20–23 °C.

### 2.2. Limited time exposure no-choice tests

Coconut scale, false oleander scale, and cycad scale were tested using limited time exposure no-choice tests. The test arena was the 1-gallon plastic jar with the host plant material for the given insect to be tested. In each replication, 100 second instar insects were tested, and 10 replications for each species were carried out over time. Insects were exposed to 20 *E. diaspidicola* for a period of 72 h. Before the experiments, the wasps were collected as soon as possible after emergence and were kept for 1 day without hosts and with honey solution provided. Hence, the wasps used were <2 days old. For each replication, a positive control was set up to ensure that the batch of parasitoids used for the test was of good quality capable of parasitism (Van Driesche and Murray, 2004). The positive controls consisted of 100 white peach scales exposed to the parasitoids the same way as the experimental insects. A negative control was also set up in each case, where the experimental insects were not exposed to wasps. This was done to determine whether the exposure to wasps had any effect on insect mortality

(e.g., mortality due to probing) even if successful parasitism had not occurred. After exposure to wasps, host plant material was transferred to another container after careful inspection to make sure that no wasps remained with the test insects. The containers were monitored daily for 25 days after the end of the exposure period, for wasp emergence. Three days after the first emerging wasps were noted in the positive controls (white peach scale exposed to *E. diaspidicola*), all the containers were carefully inspected for live wasps. If live wasps were noted, the inspection was repeated the next day. If no live wasps were found, the inner surface of the containers and the surface of the squashes were gently but thoroughly swept with a soft brush onto an A4-size white sheet of paper. The swept material was then carefully inspected for dead wasps and the number of wasps was recorded. Containers of test insects were processed the same way. This procedure was carried out 3 days later. The mortality of tested insects was also recorded immediately after emergence counts were finished. Differences in mortality between treatment groups (exposed scale insects) and negative controls (unexposed scale insects) were analyzed using 2-sample *t*-tests (JMP 7.0.1, SAS Institute, 2007).

### 2.3. Full-time exposure no-choice tests

All insect species involved with the exception of *C. pritchardiae* were tested using extended time exposure no-choice tests. This method was similar to the limited time exposure tests with three differences: (1) the parasitoids were not separated from the experimental insects after 72 h, but were left with them for their entire life span; (2) three age groups, first and second instars and adults were exposed to the wasps, with the exception of greenhouse whitefly, and (3) inspection for wasp emergence was continued in treatment groups (exposed experimental insects) for 10 days after no more live wasps were found in positive controls. The numbers of insects tested were 200 individuals/age group, 50 individuals/age group, and 100 individuals/age group in the case of the diaspids, the coccid and the pseudococcid test species, respectively. In the case of greenhouse whitefly, only third instar insects were tested, 50 individuals/replication and the scale insects were exposed to 10 instead of 20 *E. diaspidicola*. A positive control group of white peach scale (white peach scale exposed to *E. diaspidicola*) was set up in each replication and the number of emerging wasps was recorded; scale mortality was not assessed.

### 2.4. *Colobopyga pritchardiae* no-choice tests

*Colobopyga pritchardiae* specimens were exposed to *E. diaspidicola* using no-choice tests. Fruiting branches with clusters of *C. pritchardiae* were cut into pieces  $\approx$ 20-cm length. The cuts were made so that the clusters of scale insects were located <2 cm from one end of the cutting. The number of scale insects was adjusted to 20 per replication by removing excess insects from the cluster using a dissecting pin. The proximal end of the cutting was then placed in water in a 50-ml glass flask. A test arena enclosing the 20 insects was constructed from a plastic cylindrical vial (diameter: 2 cm, height: 5 cm). The lid was removed from the top of the vial and discarded. A hole (diameter: 1 cm) was drilled into the bottom of the vial. The vial was then placed over the cluster of insects with the drilled hole being approximately 1 cm from the end of the cutting. The other end of the vial was secured to the cutting using modeling clay. This way the arena enclosed the cluster of scale insects on the cutting and the hole on the upper end could be used as a portal to introduce *E. diaspidicola* wasps. Newly emerged (<1 day old) *E. diaspidicola* wasps collected from the colony were kept for 1 day without hosts in 1-gallon clear plastic jars and provided with honey solution. To start this test five wasps were transferred into the test arena, and the portal was closed with

a small piece of sponge. After a 48-h exposure period, the arena was removed from the fruiting branch cutting, and the cutting with the exposed scale insects was held for 72 h at 20–23 °C. The scale insects were then carefully removed from the cutting and were transferred into >95% ethanol. Scales in the ethanol were then frozen at –20 °C for 5–7 days before shipment to Weslaco, TX for molecular analysis. Molecular analysis was needed to show the presence of parasitoid eggs inside the scale insects because the scales would not survive long enough for the parasitoids to emerge if they were indeed parasitized.

White peach scales were exposed to *E. diaspidicola* as positive controls. An area of white peach scale-infested butternut squash was randomly selected. The scale cluster size was adjusted to approximately 2.5 cm in diameter by wiping off insects from the squash leaving only the chosen cluster. The number of individuals in the cluster was adjusted to 20 by removing excess numbers using a dissecting needle. The plastic vial arena described above for *C. pritchardiae* was then placed over the cluster and secured on the squash using modeling clay. The rest of the exposure procedure was done using the same methods as with *C. pritchardiae*. White peach scale individuals not exposed to *E. diaspidicola* (negative controls) were set up similarly.

Each of the five, no-choice test replications consisted of four groups, with 20 individual insects in each group: (1) adult *C. pritchardiae* exposed to *E. diaspidicola*, (2) sub-adult (body not yet heavily sclerotized, exact age unknown) *C. pritchardiae* exposed to *E. diaspidicola*, (3) second instar white peach scale exposed to *E. diaspidicola* as positive control, and (4) second instar white peach scale not exposed to *E. diaspidicola*. An additional 20 *C. pritchardiae* individuals not exposed to *E. diaspidicola* were also analyzed as negative controls.

*Colobopyga pritchardiae* parasitism was evaluated using genomic DNA isolation and screening with *E. diaspidicola*-specific molecular markers (Edia-F/R). A rapid crude DNA extraction procedure was utilized as described in previous work (de León et al., 2006; de León and Morgan, 2007). In the final step, the supernatant was transferred to fresh microfuge tubes and stored at –20 °C. 28S primers at an annealing temperature of 65 °C (forward: 5'-CCTG-TTGAGCTTGACTCTAGTCTGGC-3' and reverse: 5'-AAGAGCCGACATCGAAGGATC-3') (Werren et al., 1995) with 1.0  $\mu$ l of stock DNA, 1.5 mM MgCl<sub>2</sub>, were utilized to confirm the presence of genomic DNA. This assay was also used as an internal control to test for the presence of PCR inhibitors or failures (Pooler et al., 1997; Vega et al., 1993; de León et al., 2006; Fournier et al., 2008). The *E. diaspidicola*-specific (Edia-F/R) molecular markers were developed toward the cytochrome oxidase subunit I gene. The development, testing, and specific assay conditions of these markers are described in de León et al. (accepted for publication). Parasitism by *E. diaspidicola* was assessed by the presence of the *E. diaspidicola* genetic material in analyzed scale insects using the *E. diaspidicola*-specific molecular marker.

## 3. Results

### 3.1. No-choice tests with exotic species

#### 3.1.1. Limited time exposure tests

In the limited time exposure no-choice tests, no *E. diaspidicola* emerged from any of the three non-target diaspidid species tested while the positive controls with white peach scale yielded *E. diaspidicola* emergence in all cases between  $21.2 \pm 17$  and  $25.8 \pm 1.7$  wasps/100 exposed white peach scales (Table 1). Experimental insect mortality in the limited time exposure tests was observed only in coconut scale ( $15.1 \pm 1.7$ ) but this was not significantly different from the mortality in the unexposed negative control group ( $14.5 \pm 1.5\%$ ) ( $t = 0.2644$ ,  $df = 18$ ,  $P = 0.7945$ ).

**Table 1**  
*E. diaspidicola* adult emergence and mortality in three diaspidid scales in no-choice, limited time exposure experiments. The scale insects were exposed to 20 wasps for 72 h. White peach scale was used as positive controls. Mortalities with same letters were not significantly different (where mortality was not observed in treatment and control, differences were not analyzed).

Species	No. of insects/replication	No. of replications	Mean ± SE No. of wasps emerged	Mortality in treatment	Mortality in control	Mean ± SE No. of wasps emerged in positive control
False oleander scale	100	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	22.00 ± 2.7
Coconut scale	100	10	0.0 ± 0.0	15.1 ± 1.7a	14.5 ± 1.5a	21.2 ± 1.7
Cycad scale	100	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	25.80 ± 1.7

**Table 2**  
*E. diaspidicola* adult emergence from Hemiptera hosts in no-choice, full-time exposure experiments. The scale insects were exposed to 20 wasps for the entire life span of the wasps except in the case of greenhouse whitefly where only 10 wasps were used. White peach scale was used as positive controls.

Species	No. of insects/replication	No. of replications	Mean ± SE No. of wasps emerged	Mean ± SE No. of wasps emerged in positive control
False oleander scale	600	6	0.0 ± 0.0	38.5 ± 4.7
Coconut scale	600	6	0.0 ± 0.0	35.8 ± 3.4
Cycad scale	600	6	0.0 ± 0.0	34.7 ± 5.2
Greenhouse whitefly	50	5	0.0 ± 0.0	22.4 ± 2.0
Green scale	150	5	0.0 ± 0.0	37.6 ± 2.1
Long-tailed mealybug	300	5	0.0 ± 0.0	39.6 ± 3.3

**Table 3**  
 No-choice exposure of *C. pritchardiae* to *E. diaspidicola*. Parasitism was determined by species-specific genetic markers. Positive amplification indicates successful parasitism. Exposure of white peach scale to *E. diaspidicola* was used as a positive control; unexposed white peach scale was the negative control.

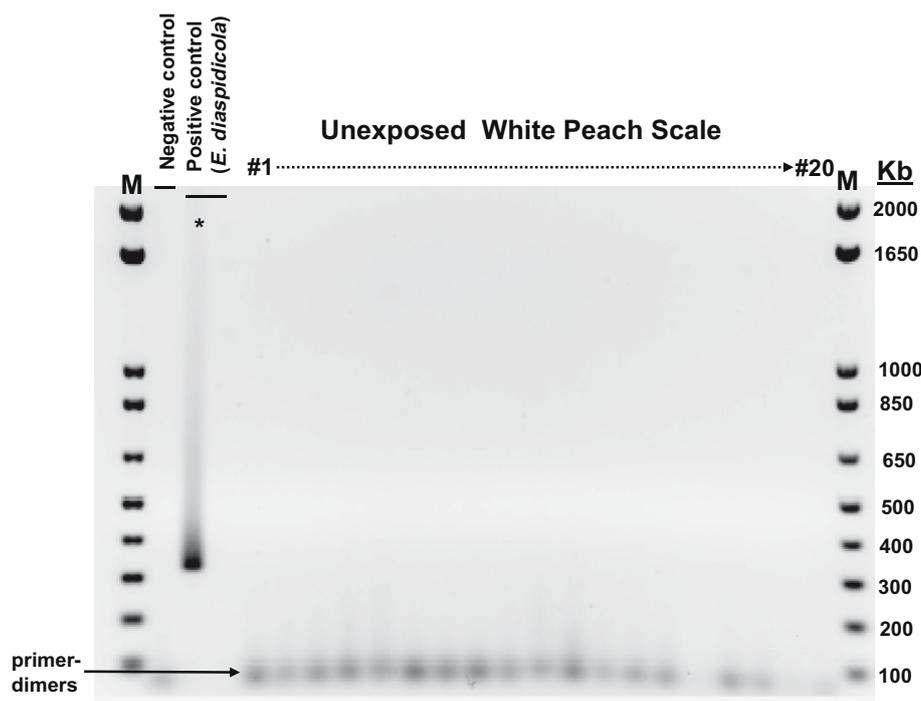
Species exposed	Total no. of insects tested	Total no. of replications	Mean ± SE % showing amplification
Sub-adult <i>C. pritchardiae</i>	100	5	0.0 ± 0.0
Adult <i>C. pritchardiae</i>	100	5	0.0 ± 0.0
White peach scale positive control	100	5	55.0 ± 4.0
White peach scale negative control	100	5	0.0 ± 0.0

3.1.2. Full-time exposure tests

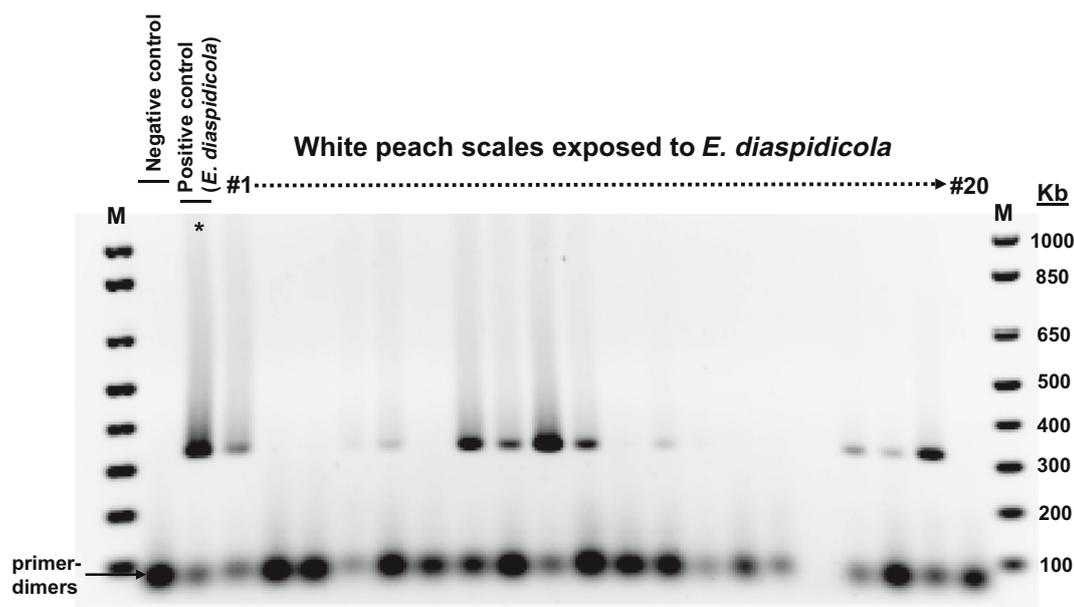
In the full-time exposure no-choice tests, no *E. diaspidicola* emerged from any of the non-target tested species. The positive controls with white peach scale yielded *E. diaspidicola* emergence in all cases. Between 22.4 ± 2.0 and 39.6 ± 3.3 wasps emerged per exposed white peach scale cluster (Table 2).

3.2. No-choice tests with *C. pritchardiae*

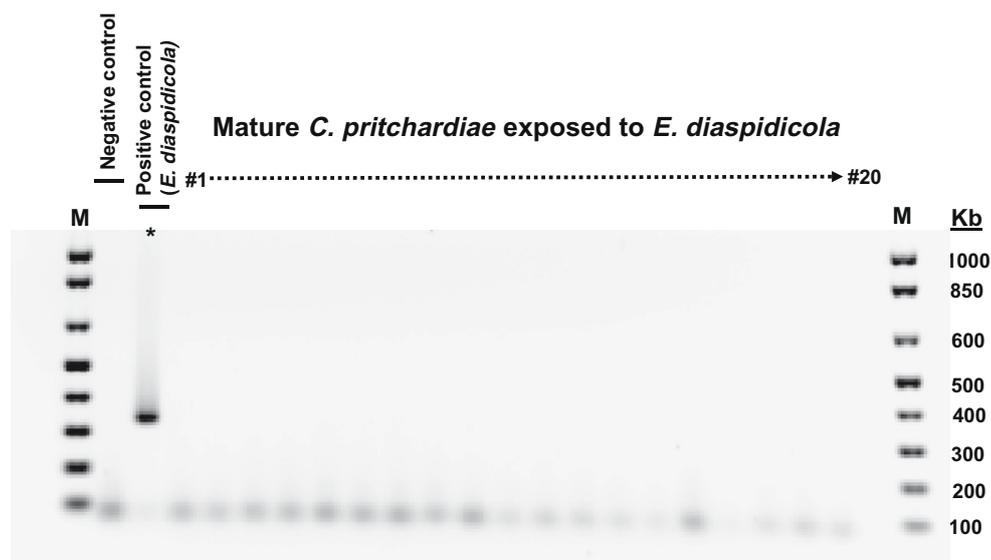
All of the white peach scales not exposed to *E. diaspidicola* showed no amplification when assayed with the Edia-F/R markers, whereas, the control lane with *E. diaspidicola* genomic DNA showed



**Fig. 1.** Representative results of the assessment of parasitism by *E. diaspidicola* using species-specific molecular markers. Unexposed white peach scales (20 individuals) showed no amplification while the positive control (genetic material acquired directly from *E. diaspidicola*) showed amplification.



**Fig. 2.** Representative results of the assessment of parasitism by *E. diaspidicola* using species-specific molecular markers. A total of 11 exposed white peach scales showed amplification (bands) out of 20 individuals exposed in this replication. Amplification indicates parasitism by *E. diaspidicola*.



**Fig. 3.** Representative results of the assessment of parasitism by *E. diaspidicola* using species-specific molecular markers. Exposed mature *C. pritchardiae* showed no amplification which indicates no parasitism by *E. diaspidicola*. The positive control (genetic material acquired directly from *E. diaspidicola*) showed amplification.

positive amplification in all five replications (Table 3). Fig. 1 shows a representative control experiment of unexposed white peach scales showing no amplification. In the positive controls,  $55.0 \pm 4.0\%$  of white peach scales exposed to *E. diaspidicola* tested positive for *E. diaspidicola* genetic material (Fig. 2 shows a representative replication). All immature and mature stages of *C. pritchardiae* exposed to *E. diaspidicola* tested negative (Fig. 3 shows a representative replication for the mature stage of *C. pritchardiae*). All unexposed *C. pritchardiae* also tested negative (data not shown) (Table 3).

#### 4. Discussion

The no-choice tests in this study demonstrated that *E. diaspidicola* was specific to white peach scale. This was expected based on the scientific literature: except for a single, and maybe question-

able report of *E. diaspidicola* from the San Jose scale (Peck, 1963), *E. diaspidicola* is known to attack only white peach scale. However, host testing with *E. diaspidicola* has not been reported in the literature. We tested three, closely related, economically important diaspidid scale insects, including the false oleander scale which is in the same genus as the white peach scale. We also tested greenhouse whitefly, green scale, and long-tailed mealybug as potential hosts for *E. diaspidicola*. None of the tested insect species were hosts for *E. diaspidicola*. Furthermore, exposure to *E. diaspidicola* had no effect on the mortality of these non-target species suggesting that host feeding or probing by *E. diaspidicola* did not occur. The positive controls (white peach scale exposed to *E. diaspidicola*) in all cases yielded wasp emergence confirming that the wasps used in the experiments were capable of parasitizing their hosts, and therefore, the lack of wasp emergence from the tested species

was the result of non-acceptance of the tested insects by *E. diaspidicola*.

A critical non-target species tested was the endemic Hawaiian palm scale, *C. pritchardiae*, because it is a representative of the group of endemic Hawaiian palm scales. This species may garner attention when a decision is to be made whether the release of *E. diaspidicola* to control white peach scale in Hawaii will have minimal non-target potential. Testing *C. pritchardiae* was more challenging than all other species tested because keeping *C. pritchardiae* alive on *Pritchardia* palm plant material for the duration of wasp development and emergence under laboratory conditions was not possible. Attempts were made to detect parasitism by dissection in tested *C. pritchardiae* and exposed white peach scales immediately after exposure but these dissections proved to be labor intensive and unreliable. We used molecular diagnostic markers specific toward *E. diaspidicola* to assess parasitism of *C. pritchardiae* by *E. diaspidicola* (de León et al., accepted for publication). Molecular markers have been used extensively for the purpose of assessing insect parasitism (reviewed in Greenstone (2006)) and predation (reviewed in Symondson (2002)). In our study, amplification assays of sub-adult and adult *C. pritchardiae* exposed to *E. diaspidicola* generated no banding with the molecular markers, demonstrating the absence of *E. diaspidicola* eggs inside the scales which, in turn, suggests that *C. pritchardiae* is not an acceptable host for *E. diaspidicola* even with rigorous exposure. The internal consistency of our technique as shown in all replicates of our positive and negative controls indicates that the technique we employed to assess parasitism by *E. diaspidicola* in *C. pritchardiae* is reliable. Therefore, it appears highly unlikely that *E. diaspidicola* will utilize endemic palm scales of Hawaii as hosts when released.

The biology of *E. diaspidicola* has not been studied extensively and the species-specific molecular markers will be very useful in future, pre-introductory and post-release studies of this parasitoid. The developed molecular markers will make it possible to conveniently assess host sex- and age-preference and parasitism efficiency of *E. diaspidicola*. This information will be crucial for developing release strategies and ensuring successful establishment of *E. diaspidicola*. Also, there have been no studies investigating the interspecific interactions of *E. diaspidicola* and *Arrhenophagus albitibiae* Girault (Hymenoptera: Encyrtidae), a white peach scale parasitoid already present in Hawaii. de León et al. (accepted for publication) developed species-specific molecular markers for this parasitoid as well, and this will enable us to quantify the interactions of *E. diaspidicola* and *A. albitibiae*. The potential competition and superparasitism between the two parasitoids may be lower if differences in host sex- and age-preferences separate the niches of the two parasitoids to some extent. Contemporaneous mortality of insects is typically difficult to study, and molecular markers may also offer a means of quantifying multiple parasitism from different enemies. The molecular markers will allow us to answer some of these questions and understand the interspecific interactions between *E. diaspidicola* and *A. albitibiae*.

*Encarsia diaspidicola* has not been recognized as an effective biological control agent of white peach scale (Clausen et al., 1978), unlike *E. berleseii*, which has been widely used in biological control programs. Greathead (1971) claimed that *E. diaspidicola* was not an effective biological control agent of white peach scale in Europe. However, Sands et al. (1990) suggested that the identification of *E. berleseii* and *E. diaspidicola* may have been confused in biological control programs. Flanders (1960) considered *E. diaspidicola* and *E. berleseii* as synonyms. Based on these facts, Sands et al. (1990) came to the conclusion that some examples of successful biological control of white peach scale attributed to *E. berleseii* may have referred to *E. diaspidicola*, and stated that a taxonomic reassessment of the *Encarsia* species in biological control programs was clearly

warranted. The molecular markers used in this study would be excellent tools to carry out the taxonomic reassessment as long as good quality voucher specimens are available.

In summary, based on the results of our study involving traditional no-choice tests with several scale insects including scale insects very closely related to white peach scale and the molecular studies with the endemic Hawaiian scale insect, we propose that the release of *E. diaspidicola* as a biological control agent against the economically important white peach scale poses minimal risk to non-target species or to the environment.

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