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Post release evaluation of *Rodolia cardinalis* (Coleoptera: Coccinellidae) for control of *Icerya purchasi* (Hemiptera: Monophlebidae) in the Galápagos Islands

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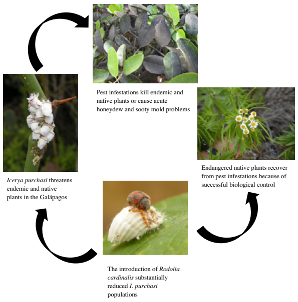
HIGHLIGHTS

- *Rodolia cardinalis* is a specific predator of *Icerya purchasi*.
- This predator was released on the Galápagos Islands for biological control of *I. purchasi*.
- Pest populations have been reduced substantially by *R. cardinalis*.
- Non-target impacts were not observed.
- It is concluded that the first biological control program in the Galápagos was successful.

GRAPHICAL ABSTRACT



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ABSTRACT

Post-release field evaluations (2009–2011) of the impacts of *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) released in the Galápagos Islands in 2002 for the classical biological control of *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) indicated that substantial (~60–98% reduction in *I. purchasi* densities) and persistent suppression of this pest has occurred. Most endemic and native plants surveyed appear to no longer have heavy *I. purchasi* infestations nor disfiguring honeydew contamination. However, pest suppression by *R. cardinalis* was less successful on some plant species, such as the native sand dune-inhabiting *Scaevola plumieri* (L.) Vahl. on which substantial, but fluctuating *I. purchasi* densities remain. In urban areas, invasive ant species tending *I. purchasi* colonies likely interfered with biological control. In 22 h of field cage observations of *R. cardinalis* foraging on native plants infested with various combinations of five non-target arthropod species and *I. purchasi*, no attacks on non-target prey occurred. In field cages, all observed attacks and feeding activity were on *I. purchasi*. Captures of *R. cardinalis* were significantly higher on yellow sticky traps placed in plants infested with *I. purchasi* compared to plants that were not hosts of *I. purchasi*. We conclude that the first biological control project in the Galápagos Islands with *R. cardinalis* against *I. purchasi* has been very successful and *R. cardinalis* is highly unlikely to affect non-target species following its establishment and spread in the archipelago.

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1. Introduction

The Galápagos archipelago (Ecuador) is famous for the evolutionary and ecological insights that scientists have gained studying the unique flora and fauna of these islands. Regrettably, this biota is under threat, and invasive species, either intentionally or accidentally introduced by humans, are the principal stress responsible for the decline of habitat quality and endemic species populations. Every year, cargo boats and commercial flights transport humans, freight, and luggage sufficient to support >170,000 visitors and a resident population of >30,000 people (Gardener and Grenier, 2011). Introductions of exotic insects have increased exponentially as a direct result of tourism and population growth (human settlement growing at ~6% per year). At least 23% of the known Galápagos insect fauna are introduced (Causton et al., 2006). Among the most damaging of these invasive insects is the cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae: Iceryini) (Causton et al., 2006). This hemipteran was first recorded in the Galápagos Islands in 1982, and by 1996 had spread to 15 of the 18 largest islands in this archipelago (Calderón-Alvarez et al., 2012).

I. purchasi is a cosmopolitan plant pest native to Australia and possibly New Zealand that feeds on >200 plant species (Caltagirone and Doutt, 1989; Causton, 2001). Dense scale populations damage plant health, and honey dew excreted by *I. purchasi* stimulates growth of sooty mold on leaves and stems, disfiguring plants. Because honey dew is a rich carbohydrate source, it is highly attractive to invasive ant species (e.g., *Camponotus conspicuus zonatus* Emery, *Solenopsis geminata* (Fabricius), and *Monomorium floricola* (Jerdon) (all Hymenoptera: Formicidae) in the Galápagos, which tend *I. purchasi* colonies to harvest the sugar (Hoddle, 2011). In the Galápagos Islands, *I. purchasi* infests 80 native or endemic plants, 19 of which are on the IUCN Red List of Threatened Species, of which ten are classified as Endangered or Critically Endangered (Calderón-Alvarez et al., 2012). Furthermore, the debilitating effects of *I. purchasi* on some rare plants indirectly harms populations of native vertebrates and invertebrates that depend on those plants for food or shelter (Roque-Albelo, 2003; Causton et al., 2006). In 1996, the Charles Darwin Foundation and the Galápagos National Park Service formed a Technical Advisory Committee to address the *I. purchasi* invasion, and classical biological control with a predatory coccinellid, *Rodolia cardinalis* (Mulsant), was proposed as one potential control option (Causton et al., 2004).

Over the last 120 years, *R. cardinalis* has successfully suppressed *I. purchasi* populations in many countries (Caltagirone and Doutt, 1989). *R. cardinalis* is a specialist predator with a very restricted prey range that is probably limited to the family Monophlebidae, and possibly to the tribe Iceryini (Caltagirone and Doutt, 1989). Strong prey fidelity by *R. cardinalis* has two major consequences: (1) high safety because non-target species are unsuitable to the predators as food sources and (2) high target suppression because all feeding and reproduction occur on the target pest (Hoddle, 2004). Another reason for the success of *R. cardinalis*, is that coccidophagous cocinellids have developmental rates that are equal to or greater than those of their prey (Dixon et al., 1997). This allows coccidophages such as *R. cardinalis* to successfully reduce prey abundance (Dixon et al., 1997).

Following a review period, the Technical Advisory Committee concluded that control of *I. purchasi* in the Galápagos Islands with pesticides was not feasible, and that biological control with *R. cardinalis* offered the best prospect for permanent and widespread suppression of *I. purchasi* across all affected habitats. In 1999, *R. cardinalis* was imported from Australia into quarantine at the Charles Darwin Research Station, Puerto Ayora, Santa Cruz, to undergo safety testing to evaluate the threat, if any, this natural enemy might pose to non-target species, especially native or endemic insects (Causton et al., 2004) and native finches that might prey on this natural enemy (Lincango et al., 2011). Analysis of quarantine host specificity trials and bird feeding studies, coupled with published studies on the use of *R. cardinalis* for *I. purchasi* control in other countries, supported the conclusion that this natural enemy would not present a significant threat to non-target species and that the first biological control program in the Galápagos should be initiated (Causton et al., 2004; Lincango et al., 2011). Between 2002 and 2005, 2206 adult *R. cardinalis* were released on 10 different islands. Populations readily established and dispersed unassisted to additional islands. All habitats infested with *I. purchasi* were infiltrated (e.g., natural, urban, and agricultural zones), and rapid suppression of high density *I. purchasi* populations was observed, sometimes within ~3 months of the arrival of *R. cardinalis* (Calderón-Alvarez et al., 2012).

In October 2009, the *Icerya–Rodolia* biological control project in the Galápagos was considered sufficiently mature for a comprehensive post-release review, and a two year evaluation project was initiated. The project had two major objectives: (1) to monitor the population phenology of *I. purchasi* and retrospectively assess the impact of *R. cardinalis* on *I. purchasi* in natural and urban areas on two different islands (Santa Cruz and San Cristóbal), and (2) to investigate under field-like conditions the hypothesis derived from host specificity studies in quarantine that in the Galápagos *R. cardinalis* has a prey range restricted to *I. purchasi*. The results of these studies are reported here.

2. Materials and methods

2.1. Population phenology of I. purchasi and R. cardinalis 2009–2011

I. purchasi and *R. cardinalis* counts were taken on two islands for a two (Santa Cruz) or one (San Cristóbal) year period during 2009–2011. Timed counts were made monthly at study sites on a variety of native or introduced host plants.

2.1.1. Surveys on Santa Cruz

On Santa Cruz, populations of *I. purchasi* and *R. cardinalis* were sampled monthly at four relatively undisturbed wilderness sites for 26 months, from October 2009 to November 2011. All plant species sampled were excellent hosts for *I. purchasi* and had supported dense pest populations before the release of *R. cardinalis* (Causton, 2001; Calderón-Alvarez et al., 2012). Two study sites, Tortuga Bay and Playa Estación, were located in the salt tolerant littoral zone (Peck, 2001). At both sites, we sampled one host plant: *Scaevola plumieri* (Goodenaceae) (Tortuga Bay) and *Laguncularia racemosa* (L.) C.F. Gaertn (Combretaceae) (Playa Estación), with one-minute timed counts being made on each of 40 randomly selected plants each month. Plants at these two sites existed as near monocultures.

The other two sites were in the lowland arid zone (Peck, 2001): El Barranco close to the Charles Darwin Research Station (CDRS) campus and the Tortuga Bay pathway. At the El Barranco site, three tree species – *Acacia insulae-iacobi* Riley (Mimosaceae) ($n = 10$), *Acacia macracantha* Humb. & Bonpl. ex Willd (Mimosaceae) ($n = 18$), and *Parkinsonia aculeata* L. (Caesalpiniaceae) ($n = 18$) – were located, tagged, and examined for one minute periods repeatedly at monthly intervals. These tagged trees were the same as those used for previous *I. purchasi* surveys prior to the release of *R. cardinalis* (Causton, pers comm.). At the Tortuga Bay pathway site, a cobbled walking path 2490 m in length was used as a transect, and plants immediately adjacent to the path were randomly selected for inspection at ~10–20 m intervals for the first ~1000 m of the walkway. Five plant species were sampled: *A. insulae-iacobi* (*n* = ~10–15/per sample date), *A. macracantha* (*n* = ~20–25), *Piscidia carthagenensis* Jacq. (Fabaceae) (*n* = ~3–6), *Rhynchosia minima* (L.) DC (Fabaceae) (*n* = ~6–8), and *Waltheria ovata* Cav. (Sterculiaceae) (*n* = ~6–8). The number of sampled plants for each species varied each week depending on the starting position on the pathway, the distance between samples, and relative abundance of these plants immediately adjacent to the pathway at the selected sampling position. The plants selected were examined for one minute periods.

2.1.2. Surveys on San Cristóbal

On San Cristóbal, we sampled two study sites, both in the lowland arid zone (Peck, 2001), over a 13 month period (December 2009–December 2010). The first site was an urban area, Puerto Baquerizo Moreno, and the second was a wilderness area, Cerro Colorado. In Puerto Baquerizo Moreno, four host plant species were monitored: *Cajanus cajun* (L.) Millsp. (Fabaceae) (*n* = 10), *Phyllanthus acidus* (L.) Skeels (Phyllanthaceae) (*n* = 2), *Citrus sinensis* (L.) Osbeck (Rutaceae) (*n* = 3), and *W. ovata* (*n* = 6). On Cerro Colorado, two host plant species were monitored: *W. ovata* (*n* = 20) and *R. minima* (*n* = 4).

2.1.3. Data collection and analysis for surveys

On each sampled plant, at all four sites for all dates, we counted the number of adult *I. purchasi* and *R. cardinalis* (eggs, larvae, pupae, pupal exuviae, and adults) during a one minute visual search of the sample plant. The average number of adult *I. purchasi* and the total number of *R. cardinalis* (eggs, larvae, pupae and adults combined) were calculated per minute per host plant for each site for each month. The monthly percentage of plants infested with *I. purchasi* or *R. cardinalis* was also determined.

2.2. Comparison of *I. purchasi* population counts before and after the release of *R. cardinalis*

Before the 2002 release of *R. cardinalis*, adult *I. purchasi* were counted at three of the sites mentioned above, on the same plant species. At the El Barranco site, *A. macracantha* (*n* = 15) and *P. aculeata* (*n* = 15) were surveyed monthly from July 1998 to July 1999; at Playa Estación, one survey of 24 white mangrove (*L. racemosa*) trees was made in January 2002, immediately before the first release of *R. cardinalis*. Along the Tortuga Bay pathway, 15 *A. macracantha* were surveyed monthly from June 1998 to July 1999. From these data, the average number of adult *I. purchasi* per one minute of visual survey was calculated. These surveys provided estimates of the density of *I. purchasi* before the release of *R. cardinalis* and were used for comparison to post-release scale densities collected in this study.

2.2.1. Data analysis for pre vs. post release counts of scale

"Insect days" provide an estimate of the intensity and duration of pest pressure on plants (Ruppel, 1983). To determine the impact of *R. cardinalis* on densities of adult *I. purchasi*, mean cumulative insect days were calculated for *I. purchasi* densities for each sampling period for pre-*Rodolia* and post-*Rodolia* surveys using Ruppel's (1983) integrative technique. Insect day estimates for each monthly sampling interval were calculated for each host plant and site combination and the average cumulative insect day load was calculated and compared between plants within sites across the two sampling intervals (pre and post-*Rodolia* periods) using *t*-tests at the 0.05 level of significance in SAS (SAS, 2008) and 95% confidence intervals were prepared. Data were log transformed prior to analyses and back transformed data are presented here.

2.3. Observations of adult *R. cardinalis* foraging behavior

Prey fidelity for adult *R. cardinalis* under conditions representative of field situations was assessed by observing individual foraging beetles on potted plants in a walk-in field cage (1.84 m × 1.84 m × 1.84 m; mesh lumite; mesh count 2.88 × 2.44 cm^2) (Bioquip Products, Rancho Dominguez CA) set up on the CDRS campus. The cage was stocked with a total of 15 plants representing six different plant species infested, variously, with up to five potential prey species. The host plants and associated prey exposed to *R. cardinalis* were (1) *A. macracantha* (*n* = 2 plants in the cage) infested with *I. purchasi*; (2) *Chamaesyce viminea* Hook. f. (Euphorbiaceae) (*n* = 3) infested with *Ceroplastes rusci* (L.) (Hemiptera: Coccideae) and *I. purchasi*; (3) *Gossypium darwini* Watt (Malvaceae) (*n* = 3) infested with *Aphis* sp. (Hemiptera: Aphididae), *Coccus viridis* (Green) (Hemiptera: Coccidae), *Paracoccus solani* Ezzat and McConnell (Hemiptera: Pseudococcidae), and *I. purchasi*; (4) *L. racemosa* (*n* = 2) infested with *Aphis* sp. and *C. viridis*; (5) *P. aculeata* (*n* = 2) infested with *I. purchasi*; and (6) *W. ovata* (*n* = 3) infested with *Tetranychus* sp. (Acari: Tetranychidae) and *I. purchasi*.

To observe predator foraging, we collected adult *R. cardinalis* from the field and then held them in the laboratory, first for 48 h on honey only, and then under starvation conditions for 24 h. Beetles were then placed one at a time in the prey-stocked field cage and their activities observed. A total of 43 *R. cardinalis* of undetermined age, sex, and prior prey exposure experience were prepared in this manner and used for observations over the period 18 October 2009–25 November 2009 inclusive.

2.3.1. Data collection and analysis for *R. cardinalis* foraging

Within the field cage mentioned above, individual adult beetles were released onto randomly selected plants and observed continuously for ~20–60 min (being either the total time the beetle was tracked before being lost, or a maximum of 60 min if not lost). Every minute the behavior of the beetle was recorded in one of seven different categories: walking (searching the plant); resting (beetle was motionless); flying (beetle flew from plant to some other point in the cage); eating (beetle consumed prey or honeydew droplets); grooming (beetle engaged in self-cleaning activities); inspecting prey (beetle inspected prey or prey parts); and ovipositing (beetle deposited eggs on prey). Behavior frequency, by category, across all observed beetles, was calculated. The number and life stage of all prey that were attacked and consumed was summed over all observations.

2.4. Assessing the strength of the association of *R. cardinalis* with *I. purchasi* in the field

To measure where *R. cardinalis* adults foraged under field conditions, sticky traps were placed in two types of habitat on the CDRS campus: (1) plants infested with *I. purchasi* and (2) un-infested plants that were not host plants for the scale. At fifteen locations for each of these two habitat types, one yellow sticky card (8 cm × 13 cm) (Gempler's Madison WI) was hung and checked daily. All 15 cards in the "with *I. purchasi*" habitat were hung in *A. macracantha*, and cards in the "without *I. purchasi* habitat" were hung in three non-hosts: 8 in *Lycium minimum* Hitchc. (Solanaceae), 3 in *Scutia spicata* Weberb. (Rhamnaceae), and 4 in *Avicennia germinans* (L.) L. (Verbenaceae).

2.4.1. Data collection and analysis

Cards were inspected daily and replaced for 11 consecutive days (20–30 November 2009), and the numbers of *R. cardinalis* and non-*Rodolia* coccinellids were recorded for each of the two habitat types. A two-way contingency analysis was conducted on total *R. cardinalis* and total other coccinellids captured by habitat type. Pair-wise Kruskal–Wallis tests were performed as means separation tests. All analyses were performed at the 0.05 level of significance using SAS (SAS, 2008).

2.5. Surveys of two endemic plants on Isla Isabela for *I. purchasi* infestation

Visual surveys of two endemic and endangered plants, *Darwiniothamnus tenuifolius* (Hook.f.) Harling and *Scalesia* sp. (both Asteraceae), both of which had been reported as good hosts for *I. purchasi* (Calderón-Alvarez et al., 2012), were inspected on Isla Isabela near Volcán Sierra Negra in November 2009. For *D. tenuifolius* and *Scalesia* sp., 33 and 37 plants, respectively, were inspected for 2–3 min for *I. purchasi* and *R. cardinalis*. The numbers of *I. purchasi* and *R. cardinalis* were recorded per plant.

3. Results

3.1. Population phenology of *I. purchasi* and *R. cardinalis* on Santa Cruz

3.1.1. Tortuga Bay

Populations of *I. purchasi* and *R. cardinalis* on sea grape (*S. plumieri*) in the sand dune habitat fluctuated by 1–2 orders of magnitude for about 21 months of the 26 month study (Fig. 1A and B). Peaks of *I. purchasi* density were followed in five instances by a subsequent upsurge in the density of *R. cardinalis*, both in terms of numbers counted (Fig. 1A) and percentage plants infested

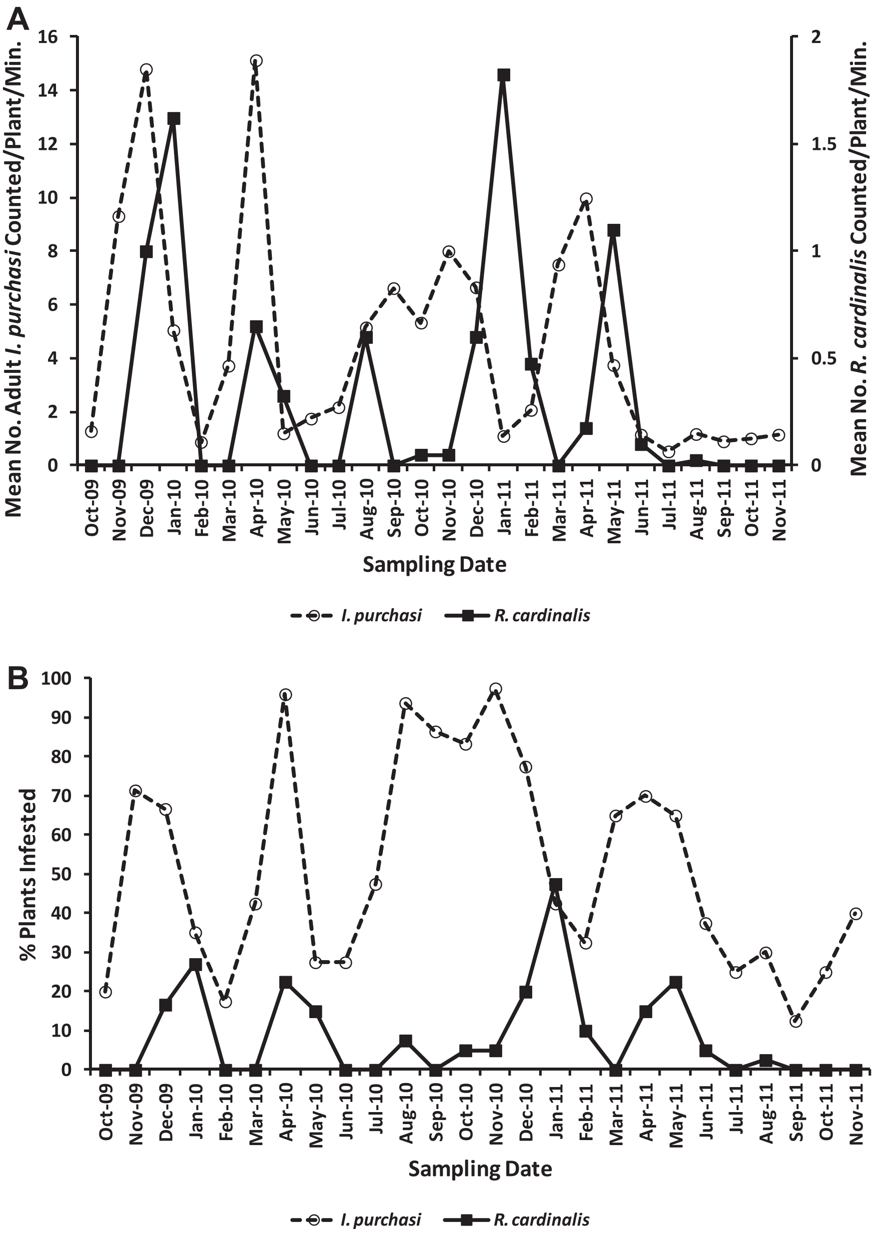

Fig. 1. Phenology of *Icerya purchasi* and *Rodolia cardinalis* on *Scaevola plumieri* (A) and the percentage of surveyed plants infested with *I. purchasi* and *R. cardinalis* (B) at Tortuga Bay, Santa Cruz.

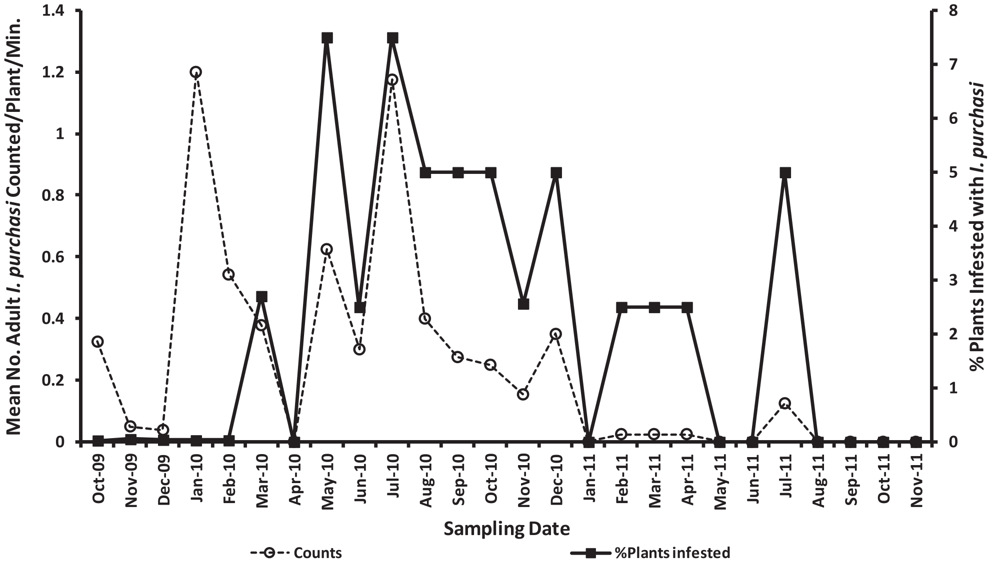

Fig. 2. Mean number of *Icerya purchasi* counted per one minute of searching and percentage of *Laguncularia racemosa* infested with *I. purchasi* at Playa Estación, Santa Cruz.

(Fig. 1B), with peaks being about 4 months apart. In contrast, from July 2011 to November 2011 (when surveys ceased), densities of *I. purchasi* showed little variation (~1 adult per plant) (Fig. 1A), while previous dynamics would have predicted a strong upsurge of scale density in August or September, which did not happen. At these low prey densities, *R. cardinalis* was not detectable (Fig. 1A). In October 2011, the percentage of *S. plumieri* infested with *I. purchasi* began to increase (Fig. 1B) possibly indicating the beginning of an increase in pest population growth presumably resulting from relaxed predation pressure by *R. cardinalis*. Despite *I. purchasi* being common on this host (about 50% of plants being infested on most dates), *S. plumieri* plants at the site were generally in good health.

3.1.2. Playa Estación

Densities of *I. purchasi* were extremely low on white mangrove (*L. racemosa*) for the full 26 months of this survey, and scales were generally so rare that 1 min searches ususally did not detect *I. purchasi*. Average densities of *I. purchasi* never exceeded 1.5 adults per minute, and the percentage of plants infested did not exceed 8% (Fig. 2). *R. cardinalis* was detected just twice during surveys at this site, two eggs were found on adult *I. purchasi*, once in December 2009 and again in February 2011.

3.1.3. El Barranco

I. purchasi was present at this site every month for the full 26 months of the survey (Fig. 3A and B). The number of adult *I. purchasi* per minute averaged over all three plant species monitored never exceeded five per plant (Fig. 3A), but infestations were consistently greater on *A. macracantha*, reaching ~ 9 adults per plant in July 2011 (Fig 3A). *Acacia insulae-iacobi* and *P. aculeata* appeared less infested by *I. purchasi*. The detection of *R. cardinalis* at this site was sporadic and when detected, its numbers were typically low (Fig. 3B). However, higher *R. cardinalis* densities on some sample dates were associated with high scale density, especially in July 2011, when a rapid increase in *R. cardinalis* density correlated with a period when 50% of sampled trees were infested with *I. purchasi*, before declining, along with a sharp decrease in the percentage of trees infested with adult *I. purchasi* (Fig. 3B).

3.1.4. Tortuga Bay pathway

Mean monthly densities of *I. purchasi* were very low across four of the five host plant species surveyed, often being less than four adults per minute of observation (Fig. 4A). On the fifth species, *R. minima*, densities of *I. purchasi* from April 2010 to July 2010 reached 18 adults per minute of observation (Fig. 4A). The abrupt decline of *I. purchasi* on *R. minima* in August 2010 was preceded by a dramatic and short lived spike of *R. cardinalis* in July 2010 (Fig 4B). A similar but less pronounced outbreak of *I. purchasi* was observed on *W. ovata* in June 2011 together with an increase in *R. cardinalis* in the same month, followed by a gradual decline in predators during July and August 2011 (Fig. 4B) that coincided with declining *I. purchasi* densities on *W. ovata* (Fig. 4A).

3.2. Population phenology of I. purchasi and R. cardinalis on San Cristóbal

3.2.1. Puerto Baquerizo Moreno

Populations of *I. purchasi* on the four species of plants surveyed were detectable year round in the urban areas of Puerto Baquerizo Moreno. Infestation severity varied greatly among host plants, with *C. cajun* and *P. acidus* having the highest scale densities (Fig. 5A). Populations on all host plants declined to low levels after September 2010 (Fig. 5A) but only one year's worth of survey data were collected at this site. *R. cardinalis* populations increased greatly once *I. purchasi* infested more than 50% of surveyed plants (Fig. 5B).

3.2.2. Cerro Colorado

Rhynchosia minima and *W. ovata*, the only two plants surveyed at this location, were infested with low densities of adult *I. purchasi* year round on Cerro Colorado (usually less than 4 adult scales per minute of observation) (Fig. 6A). *R. cardinalis* was not detected at the study site for 5 months, March–July 2010, after which the predator's numbers increased sharply in August 2010 once the percentage of plants surveyed infested with *I. purchasi* exceeded 50% (Fig. 6B).

3.3. I. purchasi population counts before and after the release of R. cardinalis

At the El Barranco site, population densities of *I. purchasi* infesting *A. macracantha* post-release of *R. cardinalis* (July 2010–July 2011) exceeded pre-release *R. cardinalis* (over July 1998–July 1999) densities for ~50% of sampled months when compared to the corresponding sampling periods over July 2010–July 2011

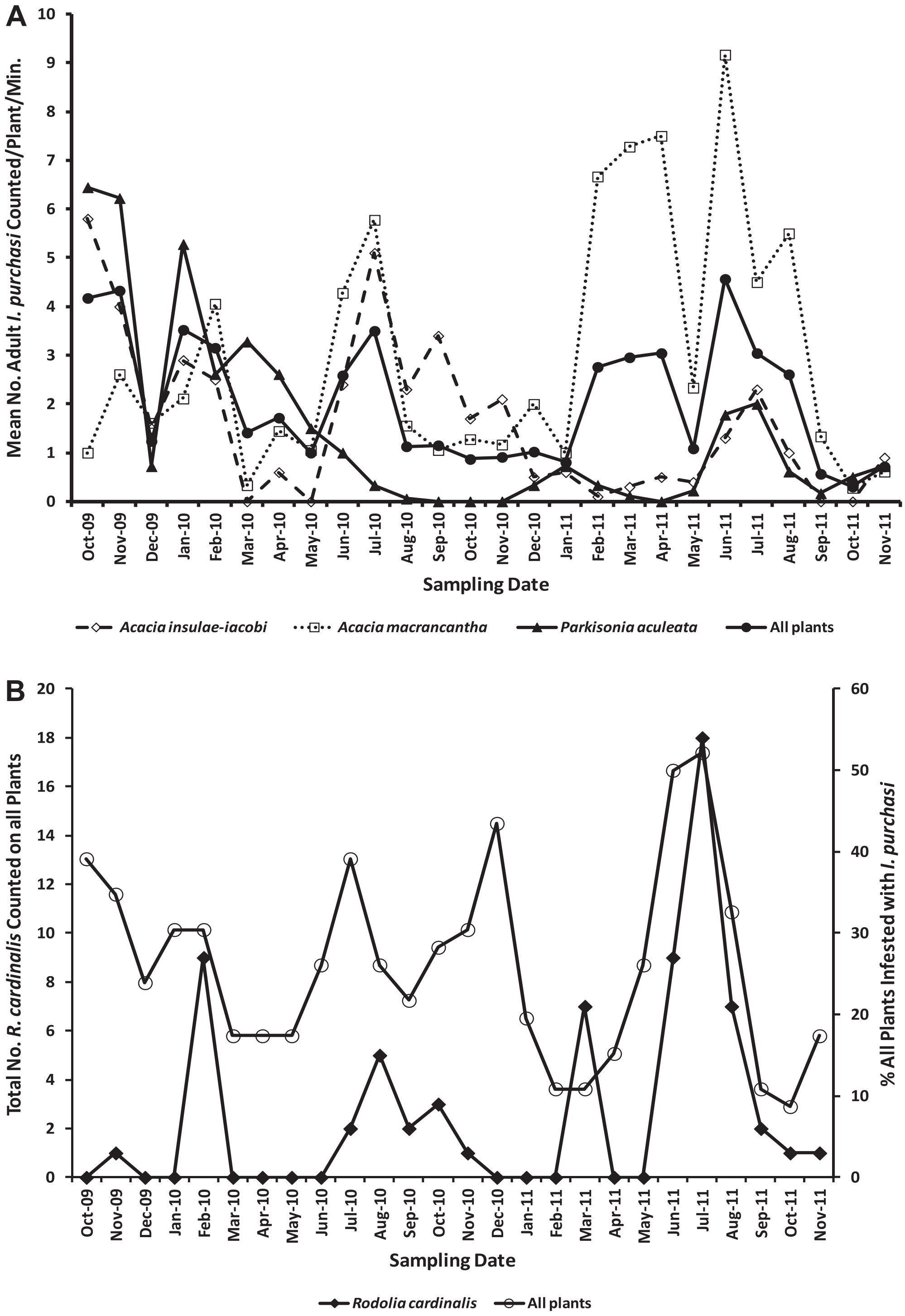

Fig. 3. Phenology of *Icerya purchasi* on three species of host plants (A) and the total number of *Rodolia cardinalis* counted and percentage of plants infested with *I. purchasi* (B) at El Barranco, Santa Cruz.

(Fig. 7A). Comparison of mean cumulative insect days for adult *I. purchasi* on *A. macracantha* pre and post-release *R. cardinalis* at El Barranco was not significant ($t = -0.39$, $df = 31$, $p = 0.70$) (Fig. 7B) showing no effective control by *R. cardinalis* on this plant at this site over the time intervals of interest. Additionally, mean numbers of *I. purchasi* on *P. aculeata* were consistently higher before the release of *R. cardinalis* (Fig. 7C), and cumulative mean insect days were significantly lower (~90% [95% C.I. indicated 53–98% reduction in *I. purchasi* densities]) for *P. aculeata* after the release of *R. cardinalis* ($t = -2.96$, $df = 31$, $p < 0.005$) (Fig. 7D) at El Barranco.

At the Tortuga Bay pathway, *I. purchasi* densities were typically higher on *A. macracantha* at this site before *R. cardinalis* was released (Fig. 7E). This was reflected in mean cumulative insect days which were significantly lower (~55% reduction [95% C.I. indicated a 38–85% reduction in *I. purchasi* densities]) ($t = -2.29$, $df = 33$, $p = 0.02$) after the release of *R. cardinalis* (Fig. 7F.).

At the Playa Estación site, there was a dramatic reduction (~98%) in the average number of adult *I. purchasi* counted on *L. racemosa* between pre and post-release of *R. cardinalis* population counts (Fig. 8).

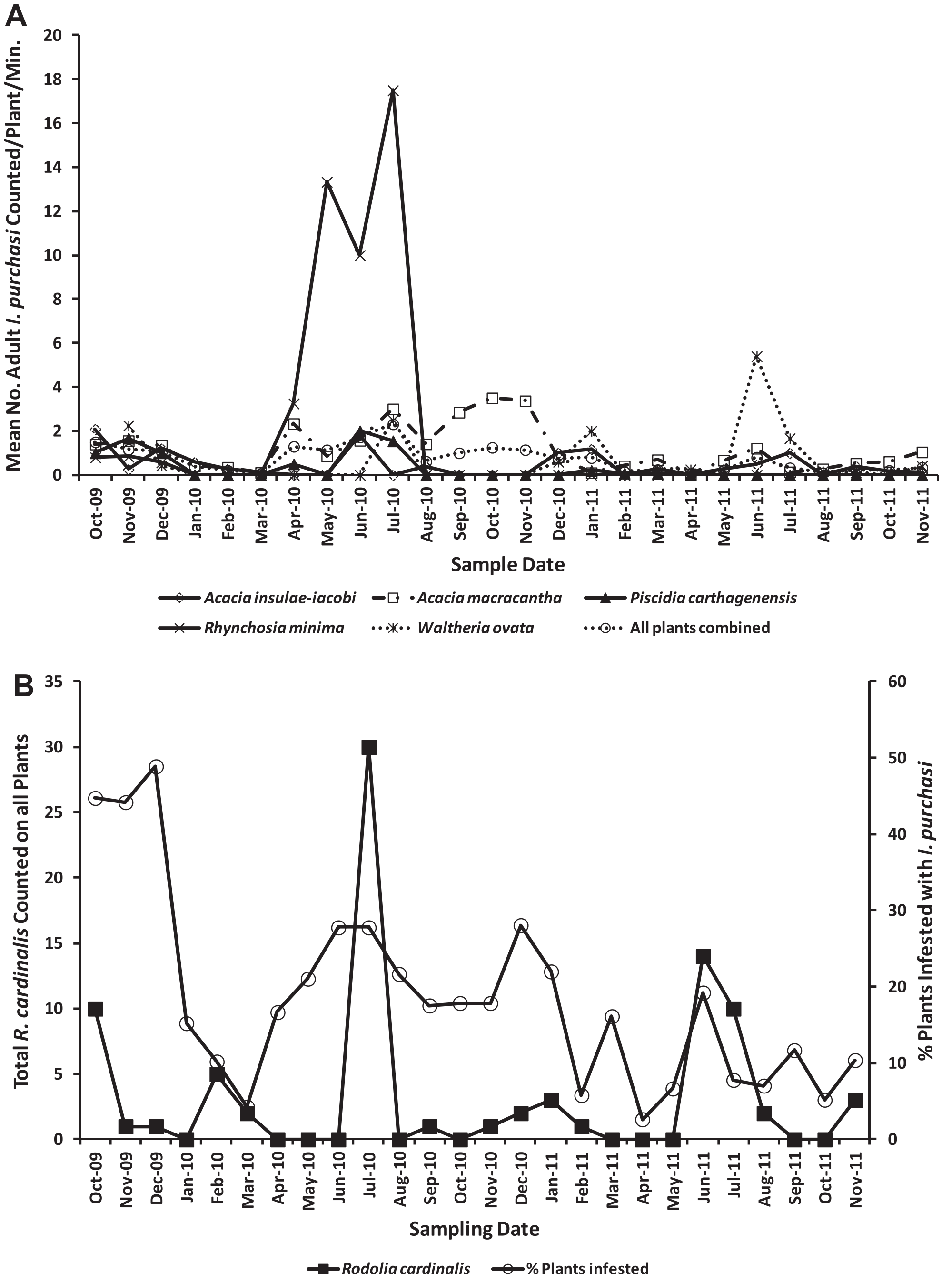

Fig. 4. Mean number of *Icerya purchasi* counted on five different host plants (A) and the total number of *Rodolia cardinalis* counted and percentage of sampled plants infested with *I. purchasi* (B) along the Tortuga Bay pathway, Santa Cruz.

3.4. *R. cardinalis* foraging behavior

3.4.1. Field cage observations

The foraging of 43 *R. cardinalis* adults was observed in the walk-in field cage. Of these individuals, 12 were discarded as “unresponsive” as they either sat on host plants refusing to move, despite prodding, or repeatedly flew to walls or the roof of the cage and did not forage on plants infested with either *I. purchasi* or non-target arthropods. The 31 beetles that actively foraged were observed for an average of 43.1 min (±2.12 min [SE]; range: 17–62 min), for a total of 22.3 h of observation. The dominant observed behavior was walking, which accounted for 43% of recorded events (Fig. 9).

A total of 351 encounters with potential prey were observed; 166 with *I. purchasi* and 185 with non-target prey. Encounters with *I. purchasi* resulted in 53 attacks (32% rate) and/or feeding events (9 on crawlers, 13 on first instars, 14 on second instars, 7 on third instars, and 10 on adults). Of the 185 non-target prey encounters, 27 occurred with *Aphis* sp., 31 with the scale *C. rusci*, 82 with the green scale *C. viridis*, 37 with the mealybug *P. solani*, and 8 with spider mites (*Tetranychus* sp.). None of these non-target encounters by *R. cardinalis* resulted in attacks or feeding activity. In every instance, the non-target species was ignored, often it was walked over or around, and no inspection-type behaviors were observed. Two incidences of what was presumed to be honeydew feeding were observed when one adult *R. cardinalis* appeared to be eating

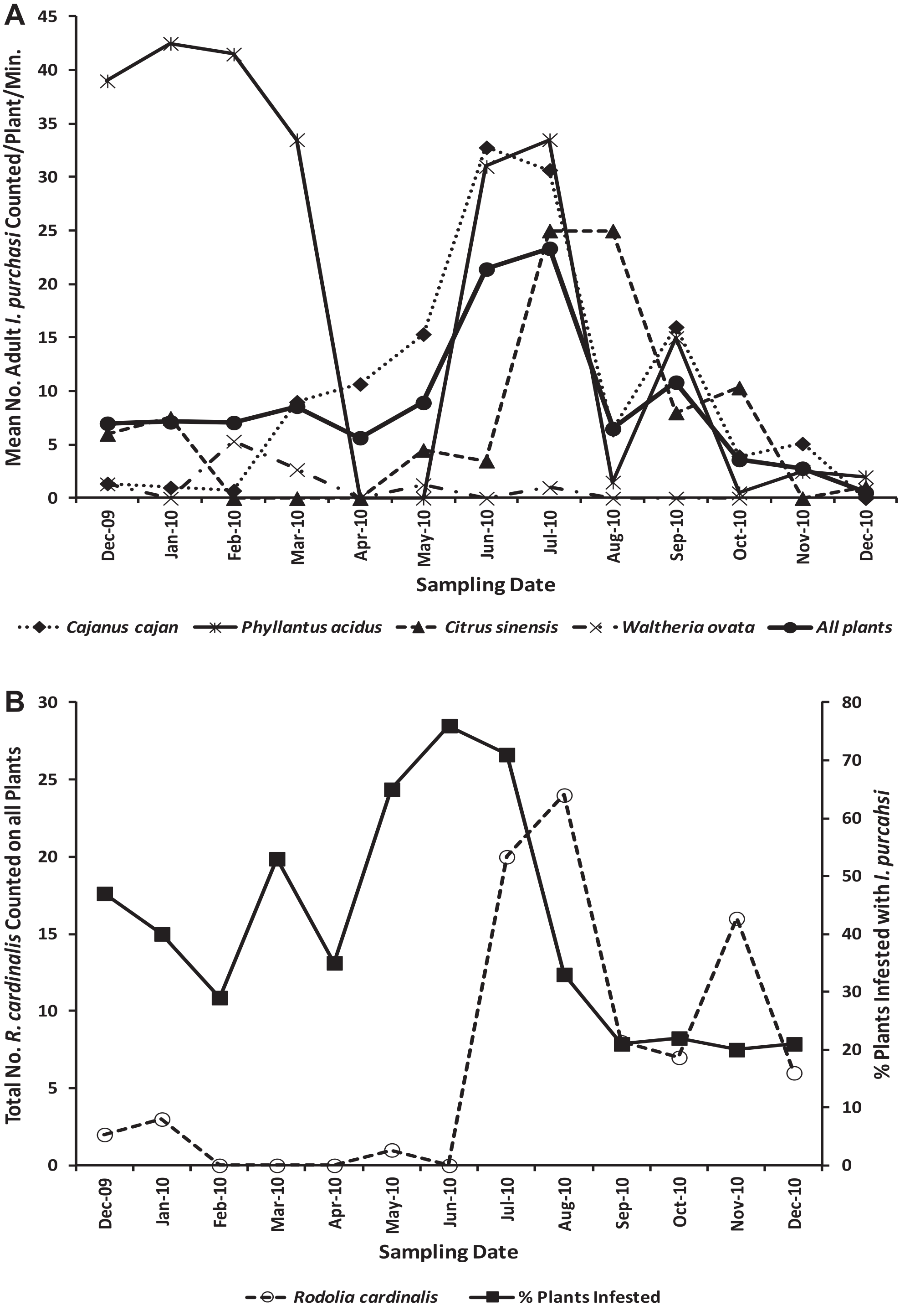

Fig. 5. Mean number of *Icerya purchasi* counted on four different host plants (A) and the total number of *Rodolia cardinalis* counted and percentage of sampled plants infested with *I. purchasi* (B) in Puerto Baquerizo Moreno, San Cristóbal.

partially-dried *Aphis* sp. honeydew on the upper surface of a *G. darwinii* leaf.

3.4.2. *R. cardinalis* captures on host trees infested with *I. purchasi*-infested vs. non-host plants

A total of 302 coccinellids were captured during the 11 days that sticky cards were deployed. Of these 302 captures, 228 were *R. cardinalis* (10 were captured in non-*Icerya* habitat, while 218 were captured in plants infested with *I. purchasi*) and 74 were other species of unidentified coccinellids (61 caught in non-*Icerya* habitat and 13 trapped in *Icerya*-infested plants). A significant difference was found between the number of *R. cardinalis* and other species of coccinellid trapped by habitat type ($\chi^2 = 57.73$, $df = 1, 56$, $P < 0.001$). The mean number of *R. cardinalis* trapped per card was significantly greater ($\chi^2 = 16.96$, $df = 1$, $P < 0.001$) in plants infested with *I. purchasi* (Fig. 10) and 96% of all captured *R. cardinalis* were trapped in this habitat. *R. cardinalis* captures in non-*Icerya* infested trees were similar to captures of other coccinellids in *Icerya*-infested trees ($\chi^2 = 0.42$, $df = 1$, $P = 0.52$). The mean numbers of other coccinellids were similar on *Icerya*-infested host plants and non- host plants ($\chi^2 = 1.17$, $df = 1$, $P = 0.28$) (Fig. 10). When all coccinellid captures are considered, 72% of trapped beetles were

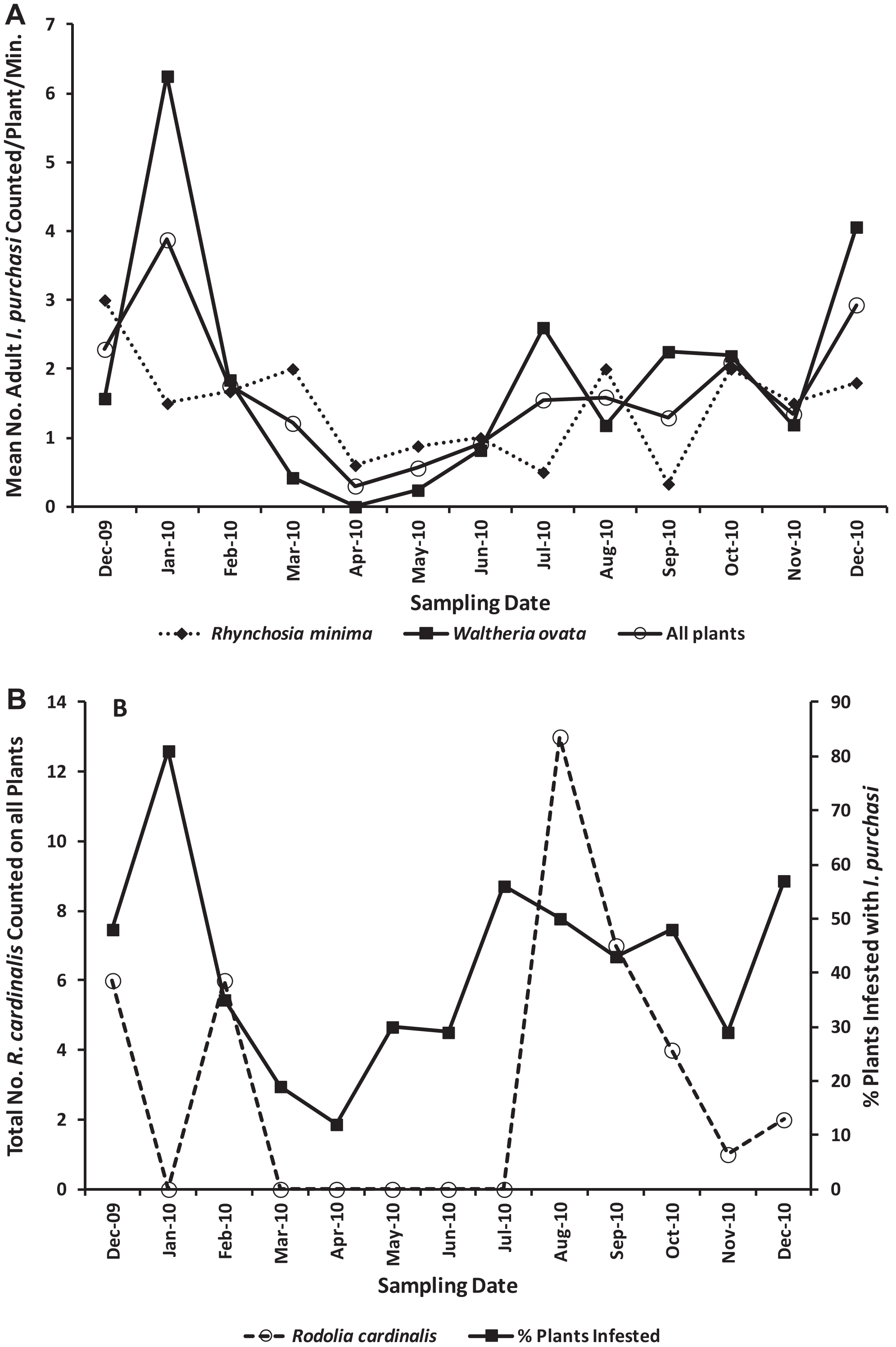

Fig. 6. Mean number of *Icerya purchasi* counted on two different host plants (A) and the total number of *Rodolia cardinalis* counted and percentage of sampled plants infested with *I. purchasi* (B) on Cerro Colorado, San Cristóbal.

R. cardinalis in *Icerya*-infested plants. On non-*Icerya*-host plants, *R. cardinalis* accounted for just 3% of total coccinellids caught.

3.5. Surveys of two endemic plants on Isla Isabela for I. purchasi infestation

Visual surveys of *D. tenuifolius* and *Scalesia* sp. revealed that 100% of the 37 *Scalesia* sp. inspected were free of *I. purchasi* even though very low density *I. purchasi* populations were sometimes found on neighboring *Acacia* spp. Only one *D. tenuifolius* from 33 plants inspected had a low infestation of *I. purchasi* ($n = 6$ adults counted) and this plant had a *R. minima* vine infested with *I. purchasi* entwined around the trunk.

4. Discussion

The major motivation for the classical biological control program against *I. purchasi* with *R. cardinalis* was the urgent need

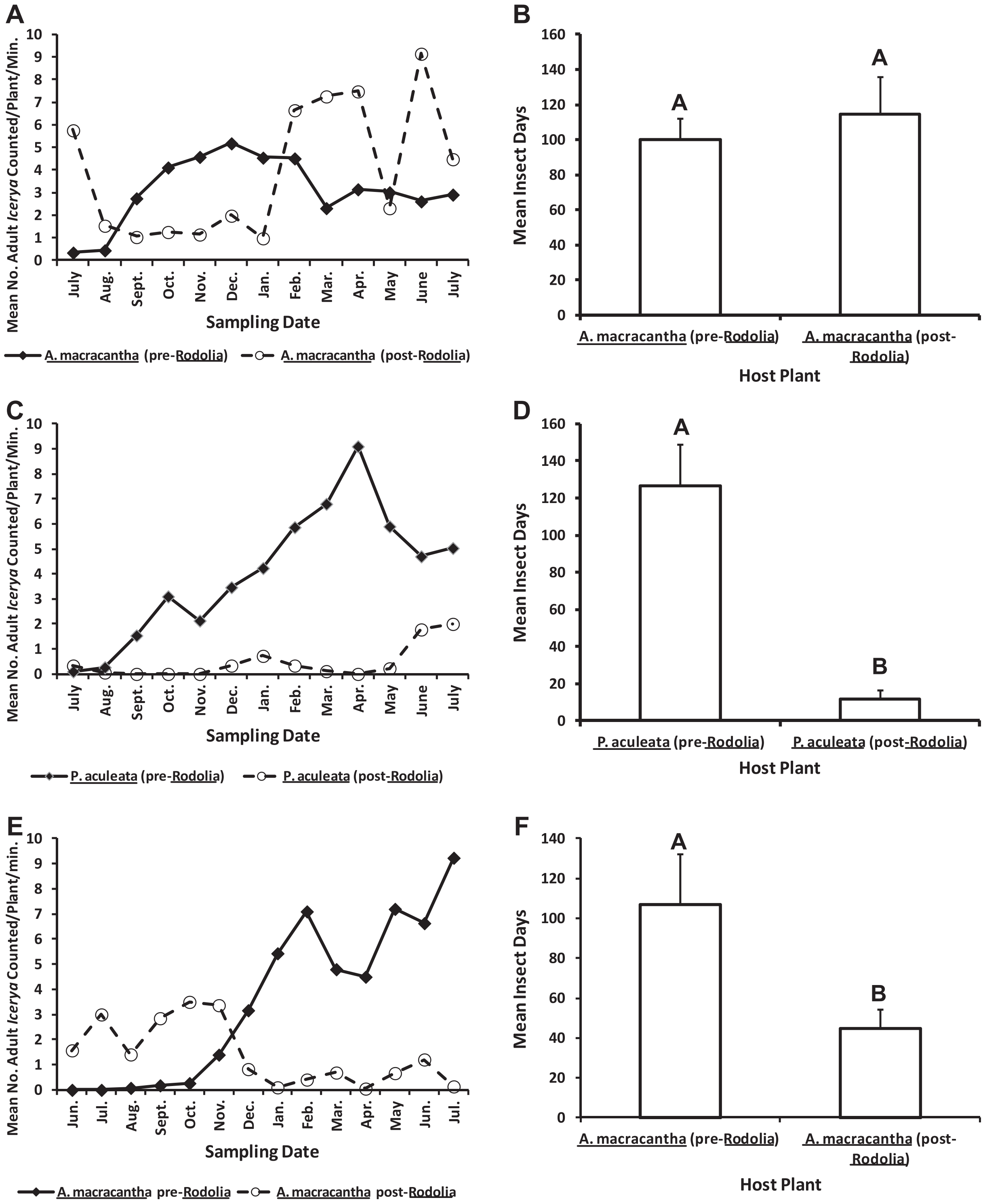

Fig. 7. Mean number of adult *Icerya purchasi* pre (1998–1999) and post-release (2009–2010) of *Rodolia cardinalis* counted per minute on *Acacia macracantha* (A) and *Parkinsonia aculeata* (C) at El Barranco, and *A. macracantha* along the Tortuga Bay pathway (E). Mean cumulative insect days (±SE) were compared to determine if the monthly densities of *I. purchasi* on *A. macracantha* (B) and *P. aculeata* (D), were significantly different pre and post-release of *R. cardinalis* at El Barranco and on *A. macracantha* at the Tortuga Pathway (F). Different letters above bars in B, D, and F indicate significant statistical differences at the 0.05 level.

to reduce mortality and degradation of endemic and native plants on the Galápagos and to protect the habitat and associated fauna supported by this flora (Causton et al., 2004; Calderón-Alvarez et al., 2012). Three significant objectives were addressed here as part of the post-release impact studies conducted over 2009–2011: (1) demonstrating continued population suppression of *I. purchasi* through use of monthly population surveys for 2 yrs on Santa Cruz and 1 year on San Cristóbal, (2) retroactively analyzing differences between adult *I. purchasi* counts before the release of *R. cardinalis* (July 1998–July 1999) for El Barranco and Tortuga Bay pathway or comparing data from Jan. 2002 at Playa Estación and counts from these post-release surveys (2009–2010) over the same

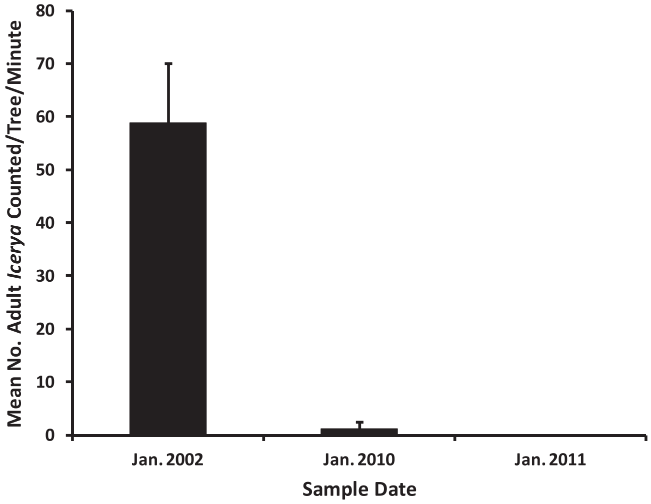

Fig. 8. Mean number (±SE) of adult *Icerya purchasi* counted per minute on *Laguncularia racemosa* at Playa Estación before (January 2002) and after (January 2010 and 2011) the release of *Rodolia cardinalis*.

monthly intervals, and (3) documenting prey fidelity by *R. cardinalis* (behavioral observations inside a walk-in cage, and sticky card deployment in *Icerya* and non-*Icerya* host plants).

This post-release monitoring study was of special importance because this biological control program was a flagship project for the Galápagos, being the first classical biological control project in this World Heritage site. Additionally, post-release information was not only scientifically important, but also prudent from a political and administrative viewpoint as documentation of positive outcomes would assure National Park representatives and associated publics of the safety of the approach, which could facilitate other potential classical biological control projects, against additional invasive species of conservation importance (Van Driesche et al., 2010).

Studies initiated in 2002, soon after the release of *R. cardinalis*, demonstrated significant impact on *I. purchasi* infesting *L. racemosa*, a mangrove that forms dense littoral stands that provide coastal protection and habitat for marine species and endangered finches (Calderón-Alvarez et al., 2012; Lincango et al., 2011). Before the establishment of *R. cardinalis* on Santa Cruz, *I. purchasi* populations on *L. racemosa* were extraordinarily high, averaging >100 adult females per sampled branch (Calderón-Alvarez et al., 2012), a density ~100 times greater than that observed during the two years surveys were conducted at Playa Estación, the same general area on Santa Cruz that the Calderón-Alvarez et al. (2012) study examined. Following the completion of the 85 day study by Calderón-Alvarez et al. (2012) on the impact of *R. cardinalis* on *I. purchasi* infesting *L. racemosa*, pest populations had dropped to near zero on *L. racemosa* at Playa Estación, a density similar to that observed during the 2009–2011 surveys presented here.

Substantial reductions (~90%) of *I. purchasi* also occurred on *P. aculeata* at the El Barranco site following the release of *R. cardinalis* in 2002 and very low densities of *I. purchasi* were observed on *A. insulae-iacobi* as well during this study. However, scale infestations were persistently more common and had higher average densities on *A. macracantha* at El Barranco. Mean cumulative insect days calculated from pre and post-release density data for *I. purchasi* on *A. macracantha* at El Barranco suggested that no significant reduction in pest density occurred on this host plant and site due to *R. cardinalis*. The higher abundance of *I. purchasi* on *A. macracantha* was also observed along the Tortuga Bay pathway, but to a lesser extent, and at this site *I. purchasi* densities were significantly lower (~60%) following the release of *R. cardinalis*. It is possible that differences in vegetation diversity and density, and moisture availability (all being greater at the Tortuga Bay pathway) may have been important site differences affecting the strength of *I. purchasi* suppression by *R. cardinalis*. Interestingly, *Acacia* spp. also appear to be very good host plants for *I. purchasi* in its native Australia (Prasad, 1989, 1990).

Despite the suppressive activity of *R. cardinalis* in many areas of the Galápagos, we found *I. purchasi* densities to be high on some species of plants at certain times of the year. For example, in urban areas on San Cristóbal, on *C. cajun* (pigeon pea), *P. acidus* (gooseberry tree), and to a lesser extent *C. sinensis* (sweet orange) there were relatively high densities of *I. purchasi* and these pest colonies were always heavily tended by invasive ant species (e.g., *C. conspicuus zonatus*, *S. geminata*, and *M. floricola* [data not shown]). Hemipteran tending by ants is generally recognized as having a detrimental impact on natural enemy activity (Van Driesche et al., 2008), including coccinellids (Hodek and Evans, 2012). In this instance, ants could have negatively affected biological control by

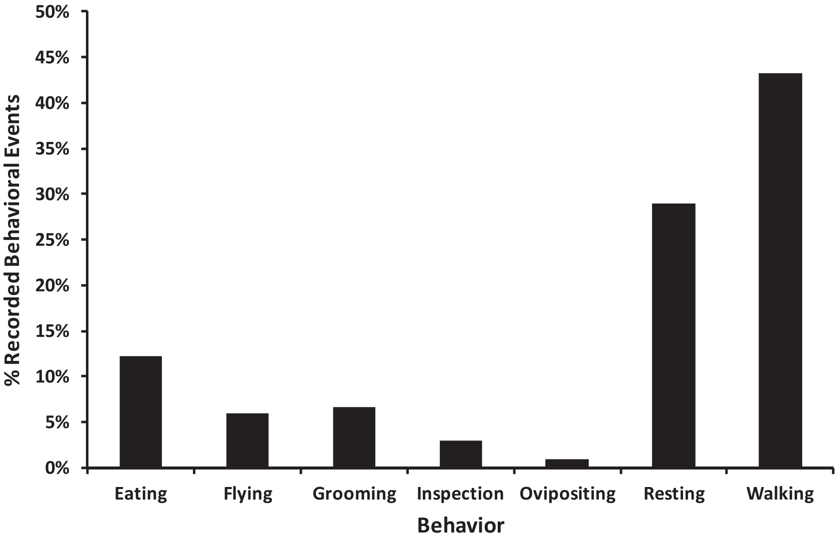

Fig. 9. Percentage of behavioral events recorded for *Rodolia cardinalis* freely foraging inside a walk-in field cage on five different species of host plants (*n* = 15 plants in the cage) infested with five different species of non-target arthropod prey, and the target pest *Icerya purchasi*.

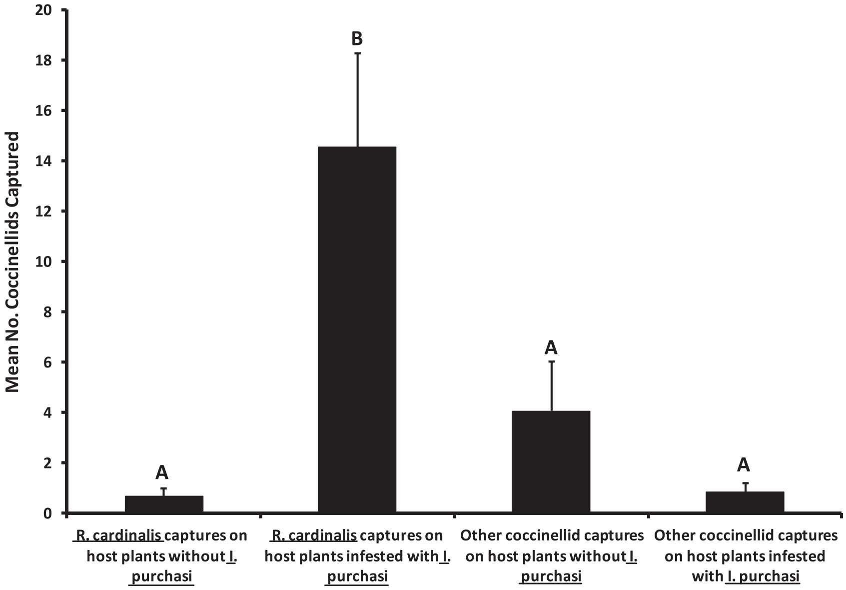

Fig. 10. Mean number of *Rodolia cardinalis* and unidentified species of coccinellids captured on yellow sticky cards deployed on plants either infested with *Icerya purchasi* or on plants lacking infestations of this pest.

R. cardinalis by attacking eggs, larvae, or pupae and harassing foraging adults, which would have created natural enemy free space for *I. purchasi* and allowed populations to flourish. Experiments investigating the effects of ant suppression and *I. purchasi* colony persistence would be instructive in understanding whether or not invasive ants have a detrimental impact on *I. purchasi* biological control by *R. cardinalis* in the Galápagos, especially in urban areas where ant activity tended to be greater.

I. purchasi appeared to outbreak periodically on the native *R. minima* (least snout bean) along the Tortuga Bay pathway (Santa Cruz), while populations of *I. purchasi* were consistently present on this plant at lower densities on Cerro Colorado (San Cristóbal). We suspect that this difference between locations may have been due, in part, to seasonal variation in plant quality, as *R. minima* along the Tortuga Bay pathway periodically ceased growth and became dried out, but remained more consistently in a state of vegetative growth on Cerro Colorado.

The most interesting *Icerya*–*Rodolia* population dynamics from our study were observed at Tortuga Bay in a near monoculture of *S. plumieri*. These plants, growing within the salt spray zone in sand dunes, were persistently infested with moderate-to-high densities of adult *I. purchasi*; plant infestation rates ranged from ~20–90% and plant condition was assessed as healthy. Population peaks of *I. purchasi* were closely followed, however, by subsequent increases in *R. cardinalis* and this pattern was repeated periodically over the 26 month survey period. The system appeared to reach some level of stability over August–November 2011, when *I. purchasi* averaged about one adult per plant and *R. cardinalis* was almost undetectable. This site would be ideal for future studies involving exclusion cages or some other type of manipulation to better understand the top-down mechanisms driving *Icerya*–*Rodolia* population cycles at Tortuga Bay. Alternatively, other factors such as such host plant nutritional quality, proximity of other species of infested host plants (the *S. plumieri* site was ~200–300 m from other native vegetation), and susceptible plant/prey phenotypes (Rocca et al., 2009), or the unsuitability of host plant species for *R. cardinalis* foraging (Prasad, 1992) might have been responsible for observed population cycling and could potentially be examined experimentally.

Behavioral observations in walk-in cages provided very clear data on the feeding preferences of *R. cardinalis* when presented with a choice of five common non-target arthropods (*Aphis* sp., *Ceroplastes rusci*, *Coccus viridis*, *Planococcus solani*, and *Tetranychus* sp.) and the target pest *I. purchasi*. All attacks and feeding events ($n = 53$) by *R. cardinalis* were directed at *I. purchasi*. Non-target prey were encountered 185 times and no attacks or feeding events were observed. These results support similar laboratory observations made by Causton et al. (2004), indicating that *R. cardinalis* does not attack or feed on commonly encountered aphids, coccids, mealybugs, or tetranychid mites, but is highly likely to attack *I. purchasi* if encountered. As opposed to the Causton et al. (2004) study, these prey preferences were observed when *R. cardinalis* was foraging freely on native plants naturally infested with non-target species, a situation similar to what would occur daily in urban, agricultural, and wilderness areas across the Galápagos.

R. cardinalis demonstrated high habitat fidelity, being captured on yellow sticky cards significantly more often on plants infested with *I. purchasi* ($n = 218$ beetles trapped) compared to traps placed in plants that were not hosts for this pest ($n = 10$ beetles trapped). This result strongly suggests that *R. cardinalis* is not foraging or aggregating across habitats randomly. Rather, it would appear that *R. cardinalis* is highly capable of directly finding plants infested with *I. purchasi*, often at very low density (see Prasad (1990) for similar findings). This finding suggests that *R. cardinalis* may use kairomones, perhaps airborne volatiles, to locate prey patches, rather than random searching of habitat and plants as typically assumed to be the case for coccinellids (Hodek and Evans, 2012). Other species of coccinellid were captured less frequently on *I. purchasi*-infested plants ($n = 13$ beetles captured) when compared to non-host plants ($n = 61$). This result, while not statistically different, does suggest that *I. purchasi* infestations are not likely to be frequented any more commonly by non-*Rodolia* species, in fact *Icerya* patches may be less likely to be visited because this hemipteran is not suitable prey for these coccinellids due to their probable aphidophagous nature.

Visual surveys of two endemic and endangered plants, *D. tenuifolius* and *Scalesia* sp. on Isla Isabela near Volcán Sierra Negra in November 2009, 7 years after release of *R. cardinalis* on this island, revealed that 100% of *Scalesia* sp. were free of *I. purchasi*. Only one *D. tenuifolius* (3% of surveyed plants) had a low infestation of *I. purchasi* and this was likely due to an *Icerya*-infested *R. minima* vine that was growing around the trunk of this plant These observations, while incidental, are significant because declines of *D. tenuifolius* on neighboring Volcán Alcedo due to *I. purchasi* infestations had been implicated in the reductions of endemic lepidopteran species (Roque-Albelo, 2003).

5. Conclusion

We conclude that the *I. purchasi* biological control program with *R. cardinalis* in the Galápagos has resulted in substantial suppression (~60–98% depending on host plant and habitat) of *I. purchasi* and that significant non-target impacts are unlikely. Results presented here strongly suggest that *I. purchasi* populations are at relatively low densities, and on average, lower than those observed before 2002 when *R. cardinalis* was first released in the Galápagos. One exception is *I. purchasi* infestations on *A. maracantha* in very dry habitats. Across all study sites in wilderness areas, we observed no instances of plants dying from *I. purchasi* infestations or disfiguring honeydew contamination of leaves. However, some host plants in wilderness areas, such as *R. minima* and *S. plumieri*, and possibly *A. macracantha* periodically suffer heavier *I. purchasi* infestations. The reasons for this are unclear, especially for *S. plumieri* at Tortuga Bay. In areas with ant activity, *I. purchasi* populations may be high because ants probably protect pest colonies from *R. cardinalis*. In these situations honeydew contamination was observed, but host plant mortality was not recorded. Walk-in cage studies and yellow sticky card captures indicate that *R. cardinalis* is not only very prey specific, but it may also preferentially orient to and aggregate in plants infested with *I. purchasi*. In summation, we observe that this biological control program has met its original objective, suppression of *I. purchasi*, and done so safely in the context of a World Heritage conservation site. We also note that control levels may vary by plant species, habitat, and possibly ant tending, all of which lend themselves to future investigation.

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