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Monitoring the effects of *Rodolia cardinalis* on *Icerya purchasi* populations on the Galapagos Islands

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Abstract In January 2002, the first biological control program was implemented on the Galapagos Islands with the release of the Australian coccinellid *Rodolia cardinalis* Mulsant to control the invasive cottony cushion scale, *Icerya purchasi* Maskell. This was the first time that Galapagos authorities had approved the introduction of a biological control agent to this iconic archipelago and, because of this precedent, it was important to monitor and evaluate its behaviour soon after its introduction. Surveys were carried out after the release of *R. cardinalis* in 2002 to confirm establishment on Santa Cruz Island. In 2009, seven years post-release, a broader survey was done to document spread throughout the archipelago. Directly after the release of *R. cardinalis* in 2002, a predator exclusion study and field observations were carried out on scale insect populations on white mangrove (*Laguncularia racemosa* [L.] Gaertn. F.) on Santa Cruz Island to document impact. In less than three months after *R. cardinalis* was released in 2002, populations of *I. purchasi* on white mangrove that were exposed to the predator in the exclusion experiment, or were monitored in the field, had declined by 99–100%. Results suggest that *R. cardinalis* played a key role in this decline, possibly in combination with high rainfall. *Rodolia cardinalis* dispersed quickly after its release and by 2009 was found in a wide variety of habitats on seven of the eight islands surveyed that had records of *I. purchasi*. Two of these were self-introductions. Further monitoring is recommended to determine whether this biological control agent has successfully reduced scale insect numbers on other valued plant species.

Keywords Biological control · Invasive species · Natural enemy · Coccinellidae · Monophlebidae · Evaluation · Galapagos Islands

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Introduction

Impact studies following the release of a biological control agent are a necessary step for classical

biological control programs (Gurr and Wratten 2000; Lynch et al. 2001; Stanley and Julien 1998). These types of studies are important because they evaluate the efficacy of the agent in controlling the target pest and validate the predictions made in the pre-release screening trials concerning non-target impacts (Barton et al. 2007; Blossey 1999; Dudley and Kazmer 2005). Post-introduction evaluations also provide important feedback for biological control practitioners for improving techniques to assess the impact of released biological control agents. Additionally, positive outcomes that are observed from post-introduction evaluations can help improve the reputation of classical biological control, thereby promoting consideration of its use for invasive species suppression by decision makers and the community as a whole.

Post-release evaluations are critical when classical biological control is used against an environmental pest in ecosystems with endemic flora and fauna that have small populations because impacts, both potentially beneficial and negative, need to be determined to assess program success. Post-release information was politically and administratively important in the conduct of the first government-sanctioned biological control program carried out in the Galapagos Islands in 2002, the release of *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) to control the invasive cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) (Causton et al. 2006; Causton 2009). Reporting on the initial results of the biological control program was not only required by scientists and Galapagos National Park managers, some of whom were hesitant about using biological control in Galapagos, but of interest to the public, many of whom were aware of this project. Extensive public awareness campaigns were carried out before the program and these continued once it had started, with many members of the community being actively involved in releasing adult *R. cardinalis* and monitoring its establishment. Rapid and positive results were expected, and this was anticipated as likely to quell any concerns that might arise that natural enemy introductions could cause more environmental problems. These efforts also provided an opportunity for the Galapagos National Park Service (GNPS) to recognize the efforts of the community helpers who helped mitigate the impacts of this invasive species.

The flora of the Galapagos was seriously affected by the invasion of *I. purchasi*. This scale insect was first reported in the archipelago in 1982 and by 1996 it had spread to 15 of the 18 larger islands. By 2002, it was known that *I. purchasi* was feeding on 80 plant species on the islands: 31 endemic, 31 native but not endemic, and 18 introduced (Causton 2001; Causton et al. 2006). Plant mortality caused by cottony cushion scale caused significant population declines and changes in the International Union for Conservation of Nature (IUCN) threat status for some threatened, endemic species such as *Darwiniothamnus tenuifolius* (Hook, f.) on Alcedo volcano on the island of Isabela (Causton 2001). Furthermore, three endemic moths that are specialist feeders on *D. tenuifolious* disappeared from the area where host plant populations were most severely damaged by *I. purchasi* (Roque-Albelo 2003). Coastal mangrove ecosystems were also damaged by *I. purchasi,* in particular the white mangrove, *Laguncularia racemosa* (L.) Gaertn. F. Studies in Galapagos with young, potted white mangrove plants demonstrated that feeding by *I. purchasi* significantly reduced branch production and growth, as well as reducing root growth (Causton 2001). Mangroves occur on the coastlines of most islands in the Galapagos and are considered a key engineering species providing food and refuge for marine and littoral species. The white mangrove is also an important nesting area for the critically endangered mangrove finch, *Camarhynchus heliobates* Gould, a species with only about 110 individuals restricted to small pockets of mangroves on the western coast of Isabela Island (Fessl et al. 2010).

A review by a multi-agency team of the effects of the *I. purchasi* invasion and the available control options concluded that classical biological control using *R. cardinalis* was the only option available for effectively reducing the impact of *I. purchasi* in the Galapagos Islands. This natural enemy was chosen because it had controlled *I. purchasi* in a wide range of climatic conditions in many countries and the majority of available evidence suggested that it also had a very restricted prey range indicating it probably would not threaten native and endemic insects in the Galapagos Islands (Causton et al. 2004). Extensive trials were conducted by scientists at the Charles Darwin Research Station (CDRS) to confirm the impact of the target pest, *I. purchasi*, on native plants and on the safety of *R. cardinalis* to native invertebrates and vertebrates (Causton 2003; Causton et al. 2004; Lincango et al. 2011). After reviewing a risk analysis produced by CDRS, permission was granted by the

GNPS for the release of *R. cardinalis* from quarantine. Priority areas for introductions were identified based on the location of threatened and ecologically important plant species. Here we report on the establishment and impact of *R. cardinalis* soon after its release in 2002 and again in 2009, seven years after the initiation of releases of *R. cardinalis.* The studies in 2002 were conducted on Santa Cruz Island and focused primarily on the effect of *R. cardinalis* on suppressing *I. purchasi* on two heavily infested stands of white mangrove. Data from 2009 reported here concern further distributional records for *R. cardinalis* in urban, agricultural, and protected areas throughout the archipelago.

Methods

Between January 2002 and January 2003, 1709 *R. cardinalis* adults, 27 pupae, and five larvae were released on Fernandina, Floreana, Genovesa, Isabela, Marchena, Pinta, Pinzon, Rabida, San Cristobal, Santa Cruz, and Santiago Islands (Table 1). Additional releases (total 497 adults) were made on Isabela, Marchena, Pinta, Santa Cruz, and San Cristobal in 2003–2005 (Table 1). This was either because *R. cardinalis* was not thought to have established (e.g., Marchena and Pinta) or because members of the public requested additional beetles (Isabela, Santa Cruz, and San Cristobal). All beetles originated from a colony that had been maintained at a quarantine facility at CDRS on Santa Cruz Island since 1999. The source population for this colony was obtained from a laboratory colony at CSIRO, Brisbane, Australia.

At the study sites on Santa Cruz Island, 380 adult beetles were released between 25 and 31 January 2002 (Fig. 1a). A total of 100 adults were liberated on infested white mangroves along the coast (at Hotel Galapagos and GNPS headquarters) and 80 were deployed on a heavily infested mango tree in the downtown Puerto Ayora (Fig. 1a). In addition to this, 200 *R. cardinalis* adults were released on white mangrove stands at Punta Estrada, across the bay and one mile from Puerto Ayora (Fig. 1a).

Establishment and spread of *R. cardinalis*

Ten weeks following the release of *R. cardinalis,* all known host plants of *I. purchasi* along the perimeter of the town of Puerto Ayora were surveyed for *R. cardinalis* (Fig. 1a). The presence of any developmental

Table 1 Islands and locations where *R. cardinalis* was released in 2002–2005 with number of releases and number of beetles released

Island[a]	Location	Date	No. releases	No. adult *R. cardinalis*
Fernandina	Volcano rim	2002	1	15 (+11 pupae)
Floreana	Urban zone	2002	1	80
Genovesa	Prince Philip steps	2003	1	22
Isabela	Urban zone	2002, 2003	2	205
	Agricultural zone	2003	1	12
	Cerro Verde	2002	1	20
	Tagus Cove	2002	1	40 (+16 pupae)
	Alcedo Volcano	2002	2	300
Marchena	Playa Negra	2002, 2003, 2005	3	311
Pinta	Cabo Chalmers	2002, 2003	2	60
Pinzon	Pozo de las tortugas	2002	2	80
Rabida	Laguna de los Lobos	2002	1	40
San Cristobal	Urban zone	2002, 2004	2	232
	Cerro Colorado	2002, 2004	2	55
Santa Cruz	Urban zone	2002–2004	7	674 (+5 larvae)
Santiago	Zona D	2002	1	60
Total		2002–2005	30	2206 (+32 immatures)

[a] Islands in bold have human settlements

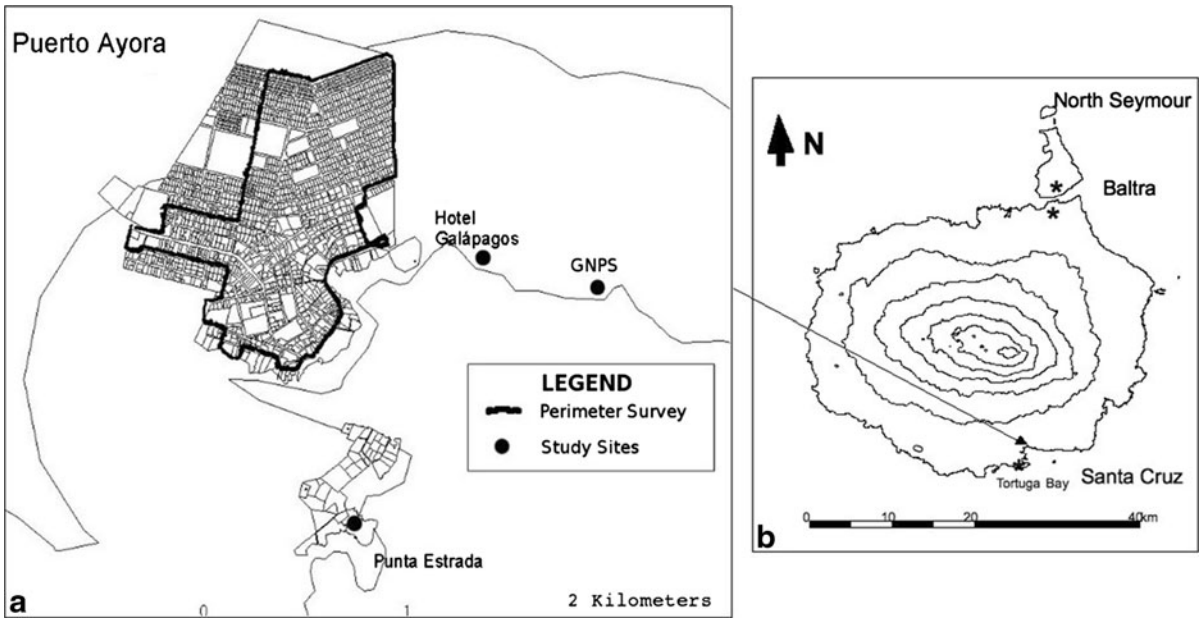

Fig. 1 **a** *Rodolia cardinalis* release and study sites on Santa Cruz island: i) Punta Estrada (exclusion cages) and ii) Puerto Ayora (unmanipulated field studies); **b** locations on Santa Cruz and Baltra islands where *R. cardinalis* was recorded less than five months post-release

stage of *R. cardinalis* was noted and the location of each plant was recorded with a handheld GPS. Between 2002–2009, known host plants of *I. purchasi* (Table 2) were surveyed for *R. cardinalis* at additional locations on Santa Cruz and on other islands in the archipelago.

In 2009, a two year monitoring program was initiated and between October and December 2009, Baltra, Champion, Española, Fernandina, Floreana, Isabela, Marchena, San Cristobal, and Santa Cruz Islands were surveyed for the presence of *I. purchasi* and *R. cardinalis* via visual observations of native plants or with yellow sticky traps hung in bushes and trees. Sticky traps were placed in National Park areas on each island as well as in urban and agricultural areas on the inhabited islands (Baltra, Floreana, Isabela, San Cristobal, Santa Cruz). Up to thirty traps were placed on each island and traps were deployed for one–two weeks to trap adult *R. cardinalis.*

Effect of *R. cardinalis* on population numbers of *I. purchasi*

Two studies were conducted between January and April 2002 at *R. cardinalis* release sites on Santa Cruz Island: 1) exclusion cages were used to measure the effects of the predator at Punta Estrada and 2) unmanipulated populations of *I. purchasi* and *R. cardinalis* were monitored on marked branches of heavily infested stands of *L. racemosa* along the coast at Puerto Ayora (Hotel Galapagos to GNPS) (Fig. 1a).

Predator exclusion studies

Two experimental conditions were established: (1) controls that were *I. purchasi*-infested branches enclosed in sleeve cages to prevent access by *R. cardinalis* (following Luck et al. 1998; Prasad 1989; van Driesche and Bellows 1996) and (2) treatment branches that were continuously exposed to foraging *R. cardinalis*. A preliminary study was conducted to determine the minimum sample size required to estimate scale density. To do that, mangrove plants were observed from a distance of 1 m through a transparent plastic sheet that was divided into numbered squares. Branches falling into 21 randomly chosen squares in the viewing sheet were identified and the adult females of *I. purchasi* on each chosen branch were counted. Data was plotted and an average sample number curve (ASN) (Southwood 1978) used to determine the minimum sample size, which was determined to be ten branches (i.e., ten replicates). To compensate

Table 2 Known host plants of *I. purchasi* in Galapagos (Lincango et al. 2010 and additional, unpublished records) indicating species where *R. cardinalis* was recovered between 2002–2009 (*recovered at ten weeks after the release of *R. cardinalis* on Santa Cruz Island)

Family	Species (Origin, IUCN status)[a]	*R. cardinalis* present
Acanthaceae	*Avicennia germinans* (N)	+*
	Blechum pyramidatum (N)	
Amaranthaceae	*Alternanthera echinocephala* (N)	
Anacardiaceae	*Mangifera indica* (I)	+*
Annonaceae	*Annona cherimola* (I)	+
Apiaceae	*Cyclospermum leptophyllum* (N?)	
Apocynaceae	*Nerium oleander* (I)	
	Vallesia glabra (N)	
Arecaceae	*Cocos nucifera* (I)	
Asteraceae	*Brickellia diffusa* (N)	
	Darwiniothamnus lancifolius (E, EN)	
	Darwiniothamnus tenuifolius (E, EN)	+
	Gamochaeta purpurea (N?)	
	Lecocarpus darwinii (E, EN)	
	Lecocarpus pinnatifidus (E, CR)	
	Macraea laricifolia (E, LC)	+
	Porophyllum ruderale (I)	
	Scalesia aspera (E, VU)	
	Scalesia atractyloides (E, CR)	
	Scalesia baurii (E, VU)	
	Scalesia cordata (E, EN)	+
	Scalesia divisa (E, EN)	
	Scalesia gordilloi (E, CR)	
	Scalesia helleri (E, VU)	
	Scalesia pedunculata (E)	
Bombacaceae	*Matisia cordata* (I)	
Boraginaceae	*Cordia leucophlyctis* (E)	
	Cordia lutea (N)	+*
	Heliotropium angiospermum (N)	
	Tournefortia psilostachya (N)	
	Tournefortia rufo-sericea (E, VU)	
Brassicaceae	*Brassica oleracea* (I)	
Burseraceae	*Bursera graveolens* (N)	+
Cactaceae	*Jasminocereus thouarsii* (E, VU)	+
Caesalpinaceae	*Bauhinia monandra* (I)	+*
	Parkinsonia aculeata (N)	+*
	Senna obtusifolia (I)	
	Senna occidentalis (N)	
	Senna pistaciifolia (N)	+
Celastraceae	*Maytenus octogona* (N)	
Combretaceae	*Conocarpus erectus* (N)	
	Laguncularia racemosa (N)	+*
Convolvulaceae	*Ipomoea habeliana* (E, LC)	
	Ipomoea nil (I)	
	Ipomoea pes-caprae (N)	
	Merremia aegyptia (N)	
Cyperaceae	*Cyperus anderssonii* (E, LC)	
Euphorbiaceae	*Acalypha abingdonii* (E, VU)	
	Acalypha parvula (E, LC)	
	Acalypha wigginsii (E, CR)	
	Chamaesyce amplexicaulis (E, LC)	
	Chamaesyce punctulata (E, LC)	
	Chamaesyce viminea (E, LC)	+
	Croton scouleri (E, LC)	+
	Euphorbia cyathophora (I)	
	Phyllanthus acidus (I)	
	Phyllanthus caroliniensis (N)	
	Ricinus communis (I)	+*
Fabaceae	*Cajanus cajan* (I)	+
	Canavalia maritima (N)	
	Centrolobium paraense (I)	
	Crotalaria incana (N)	
	Desmanthus virgatus (N)	
	Desmodium incanum (N?)	+
	Phaseolus mollis (E, NT)	
	Phaseolus vulgaris (I)	
	Piscidia carthagenensis (N)	+*
	Rhynchosia minima (N)	+
	Stylosanthes sympodiales (N)	
	Vigna luteola (N)	+
Goodeniaceae	***Scaevola plumieri*** (N)	+*
Lamiaceae	*Hyptis pectinata* (I)	
	Mentha piperita (I)	
	Ocimum basilicum (I)	
Malvaceae	*Bastardia viscosa* (N)	
	Gossypium darwinii (E, LC)	+
	Gossypium klotzschianum (E, NT)	
	Hibiscus rosa-sinensis (I)	+
	Hibiscus tiliaceus (N)	
Mimosaceae	***Acacia insulae-iacobi*** (N)	
	Acacia macracantha (N)	+
	Acacia nilotica (I)	+
	Acacia rorudiana (E?)	+
	Inga edulis (I)	+
	Neptunia plena (N)	
	Prosopis juliflora (N)	+
Moraceae	*Ficus* sp. (I)	+*
Myrtaceae	*Psidium guajava* (I)	+

Table 2 continued

Family	Species (Origin, IUCN status)[a]	*R. cardinalis* present
Nyctaginaceae	*Commicarpus tuberosus* (N)	
	Cryptocarpus pyriformis (N)	
	Pisonia floribunda (E, LC)	
Passifloraceae	*Passiflora quadrangularis* (I)	+*
Plumbaginaceae	***Plumbago scandens*** (N)	
Polygalaceae	*Polygala galapageia* (E, VU)	
Portulacaceae	***Calandrinia galapagosa*** (E, CR)	+
	Portulaca oleracea (N?)	+
Punicaceae	*Punica granatum* (I)	
Rhizophoraceae	*Rhizophora mangle* (N)	+
Rosaceae	*Rosa* sp. (I)	
Rubiaceae	*Borreria ericaefolia* (E)	
	Chiococca alba (N)	
	Psychotria rufipes (E, VU)	
Rutaceae	*Citrus aurantiifolia* (I)	+
	Citrus sinensis (I)	+
Scrophulariaceae	*Russelia equisetiformis* (I)	+
	Scoparia dulcis (N)	
Sterculiaceae	*Waltheria ovata* (N)	+
Ulmaceae	*Trema micrantha* (N?)	
Verbenaceae	*Clerodendrum molle* (E, VU)	+
	Lantana camara (I)	
	Lantana peduncularis (E, LC)	
	Tectona grandis (I)	

[a] *E* endemic, *N* native, *I* introduced; IUCN status: *CR* critically endangered, *EN* endangered, *VU* vulnerable, *NT* near threatened, *LC* least concern, Species in bold mortality attributed to *I. purchasi*

for any unexpected damage or branch mortality that might occur during the experiment, we sampled 15 branches/treatment. Thirty branches were randomly selected using the methodology described for the preliminary study and randomly assigned to the experimental treatments. Only branches with more than ten *I. purchasi* were selected. During the experiment, five cages were damaged and these were eliminated from the experiment leaving ten caged branches, and 15 uncaged branches.

The first 50 cm of each branch, the part of the branch where most scale insects are typically located (Prasad 1992), was marked. Cylindrical cages (50 cm in diameter and 80 cm in length) were used to cover the control branches. The wire cylinders were covered with muslin, which was supported by wire rings at each end of the cage. Wire supports prevented contact between the muslin and the branch, which was intended to increase air circulation and reduce honeydew contamination and excessive moisture (van Driesche and Bellows 1996). Sleeve cages were closed and sealed on the trunk side to prevent the entry or exit of any insects. Neighbouring branches were moved away from the cages to prevent branches acting as bridges for natural enemies and other insects thus minimizing the likelihood of their unwanted entry. In addition, sticky traps were also placed around cages to further reduce the possibility of invasion of control cages by *R. cardinalis* or other predators.

Scale density was measured on each branch on six relatively equally spaced dates between 22 January 2002 and 19 April 2002. The first count was made three days before the release of *R. cardinalis* in the immediate vicinity of the experimental set up. The second count was made one month later, and subsequent counts were taken every 14 or 15 days. Scale insect life stages (instars 1, 2, 3, and adult females) were recorded separately during counts. Scale insects on branches were counted from the furthest growing tip, working backwards to the trunk end of the branch along a 50 cm section. *Rodolia cardinalis* adults, pupae and larvae were also recorded. The presence of pupal exuvia and eggs was noted, but these were not included in the calculations of population density because eggs are hard to detect without dissection (they are hidden under or inside *I. purchasi*) and pupal exuviae remain attached to plants for an extended period of time. On each sampling date, caged branches were checked for the presence of any unwanted insects or spiders, and these were removed if found.

Impact of R. cardinalis under unmanipulated field conditions

White mangrove trees along the coast of Puerto Ayora from Hotel Galapagos to the GNPS dock were used in this study (Fig. 1a). The methodology for selecting the branches was similar to the predator exclusion experiment described above. Twenty-four branches were randomly selected, and the first 50 cm of the branch was marked for monitoring the density of *I. purchasi* in the presence of the released population of *R. cardinalis*. Adult females of *I. purchasi* and adults, pupae and larvae of *R. cardinalis* were counted on each branch at six relatively equally spaced times between 15 January 2002 and 18 April 2002. The first counts were taken ten days before the release of *R. cardinalis* in the neighbouring area. The second

count was made five weeks later, with subsequent counts being made every 12–15 days.

Data analysis

For the predator exclusion studies, means and standard deviations were calculated for each developmental stage of *I. purchasi* and for all life stages combined for each experimental treatment at each time point. There were no missing observations. Mixed models (Littell et al. 2006) were used to compare differences between experimental treatments, including time effects and treatment × time interactions. The model for the mean total count, Y_{ijtk}, of branch j at time t on occasion k under experimental condition i was the following:

$$Y_{ijtk} = \mu + \alpha_i + \tau_t + \alpha\tau_{it} + B_{ij} + E_{ijtk}$$

where i represents treatment, j branch, t time (day), and k occasion of measurement.

In this model, $\mu + \alpha_i$ represents the average per branch for each scale insect life stage or for all life stages combined over the time point for each experimental treatment, i, $\tau_i + \alpha\tau_{it}$ represents the deviation from the average at time t under experimental treatment i, and B_{ij} represents the deviation for the jth randomly selected branch under experimental treatment i, where we assume that $E(B_{ij}) = 0$ and $\text{var}(B_{ij}) = \sigma^2$. The final term, E_{ijtk}, represents the residual variability including random replication error for the kth replication (where $k = 1$) of the count on the same branch, and at the same time and under the same experimental treatment, and variation due to possible heterogeneity of fixed effects between branches where $E(E_{ijtk}) = 0$ and $\text{var}(E_{ijtk}) = \sigma_e^2$. This model assumes that branches are randomly assigned to treatments, with σ^2 representing the variance in the average (over time) branch effect. The model includes fixed effects representing a main effect for each treatment (an average treatment effect), α_i; a time effect, τ_t; and a treatment × time interaction, $\alpha\tau_{it}$. Random effects include the branch effect, and the residual error.

We also represented parameters for the means of each life stage and all life stages combined over time by slope over intercept. We fit a linear regression model to time (using day from introduction of *R. cardinalis*) as a time scale, and allowed slopes and intercepts to vary between treatments using the no cage treatment as a reference group and the change in slope (increment) as the estimate for the closed cage treatment. Data more than 56 days after the release of *R. cardinalis* were excluded from this analysis because of low scale insect density on uncaged branches.

Results

Establishment and spread of *R. cardinalis*

Surveys demonstrated that *R. cardinalis* quickly became established at release sites in Puerto Ayora, Santa Cruz Island. Ten weeks after it was first released, *R. cardinalis* was present on 82% ($n = 60$) of plants infested with *I. purchasi* that were surveyed along the perimeter of Puerto Ayora. Immature and adult *R. cardinalis* were recorded on 11 plant species from ten families (Table 2). Furthermore, *R. cardinalis* spread quickly after its release to other parts of Santa Cruz Island. Eight weeks after release (March 2002), larvae and pupae were found on *Scaevola plumieri* (L.) plants along the coast at Tortuga Bay, three km from the release site (Fig. 1b). Twenty-two weeks after it was released in Puerto Ayora, *R. cardinalis* was detected on the north side of Santa Cruz Island (45 km). At the same time, adult *R. cardinalis* were also found on Baltra Island, which is separated from the northern coast of Santa Cruz by an ocean channel approximately 200 m wide (Fig. 1b). A 2003 survey found that *R. cardinalis* had dispersed naturally to North Seymour Island, north of Baltra (Fig. 1b).

During an archipelago-wide survey in 2009, *R. cardinalis* was found on all of the islands that were surveyed (Baltra, Champion, Fernandina, Floreana, Isabela, Marchena, San Cristobal, Santa Cruz) with the exception of Pinzon, which was only partially surveyed, and Española, where there were no records of *I. purchasi* (Fig. 2). *Rodolia cardinalis* was observed or collected on sticky traps in a wide range of vegetation types, ranging from xeric habitats to humid highlands, and in dry, high altitude zones on the rims of volcanoes (altitudinal range of 0 to ~1200 m elevation), as well as in urban, agricultural, and National Park areas.

Between 2002 and 2009, *R. cardinalis* was recorded on 40 of 112 (36%) of the known host plants of *I. purchasi* (Table 2). Most of these records were

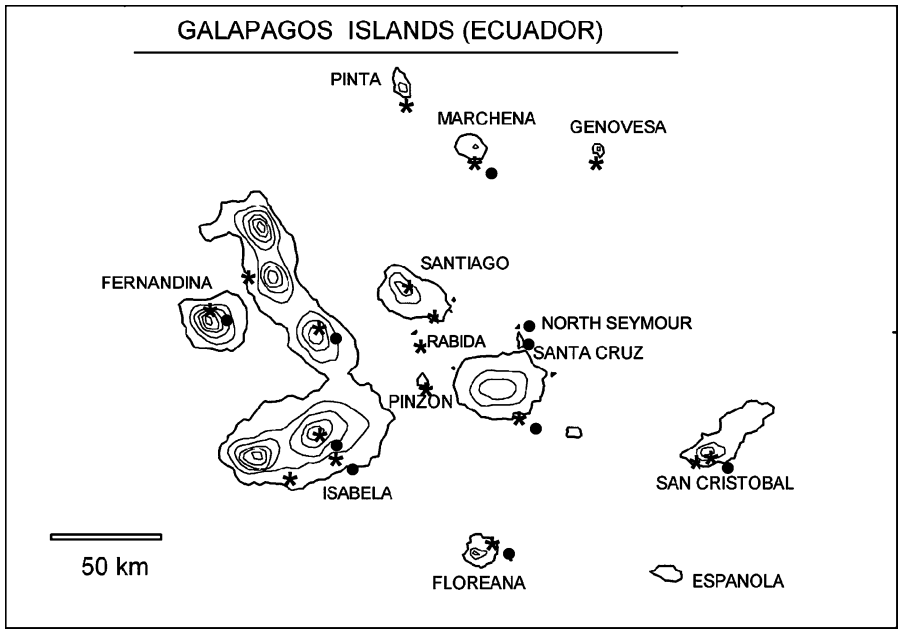

Fig. 2 *Rodolia cardinalis* release sites (asterisk) and islands where the predator has been recovered between 2002 and 2009 (filled circle)

reported by CDRS scientists and GNPS personnel during field trips undertaken for other projects, and not from surveys specifically designed to study the distribution of *R. cardinalis*. Ten of these plant records are endemic plant species and include the threatened species *D. tenuifolius*, *Calandrinia galapagosa* H. St. John, and *Scalesia cordata* A. Stewart. *Rodolia cardinalis* has also been found on 16 native but not endemic species, including all four of the mangrove species native to the Galapagos Islands (*Avicennia germinans* L., *Conocarpus erectus* L., *L. racemosa*, and *Rhizophora mangle* L.), and 14 introduced plant species.

Effect of *R. cardinalis* on population numbers of *I. purchasi*

Predator exclusion studies

Overall, a decrease in the number of *I. purchasi* over time was observed in both experimental treatments. However, populations that were exposed to the predator, *R. cardinalis,* declined at a faster rate than populations that were isolated from the predator in cages (Table 3; Fig. 3).

One month after the release of *R. cardinalis*, population numbers of *I. purchasi* on uncaged branches had declined by 46% from an average of 120.5 ± 68 to 64.9 ± 43.2 individuals per branch ($n = 15$) and continued to decline, reaching zero 85 days after *R. cardinalis* was released. With the exception of some recruitment in the first and second instars (between 29 and 42 days post-release of *R. cardinalis*), all developmental stages declined at each sampling date (Table 3). In contrast, in the exclusion cages, first instars and adult *I. purchasi* increased during the first month (January 22–February 21, 2002), causing a 9% increase in total population numbers from an average of 176.7 ± 91 to 192. 8 ± 130 individuals per branch ($n = 10$). Following this, population numbers on caged branches declined although recruitment was observed in the immature stages (1–3 instars) during the second month of the experiment (Table 3). In both the treatment and control, 28 days after the release of *R. cardinalis*, more than a half of the *I. purchasi* population was composed of adult females. This ratio was maintained in the caged branches for at least another month. In contrast, on uncaged branches, the mean proportion of *I. purchasi* adults declined steadily, reaching zero 70 days after the release of *R. cardinalis* (Table 3).

Rodolia cardinalis was present on 73% of uncaged branches one month after it was released with an average of 1.9 ± 2 *R. cardinalis* individuals per branch ($n = 15$) (Fig. 3). All immature stages of *R. cardinalis* were observed, demonstrating predator

Table 3 Mean counts (±SD) for *I. purchasi* by time after release of *R. cardinalis* on uncaged and caged branches ($n = 15$ and $n = 10$, respectively) at Punta Estrada, Santa Cruz Island

I. purchasi	Days after release of *R. cardinalis*											
	−3		28		42		56		70		85	
	No cage	Caged	No cage	Caged	No cage	Caged	No cage	Caged	No cage	Caged	No cage	Caged
Instar 1	31 ± 24.2	50.5 ± 52.6	15.3 ± 17	55.3 ± 55.4	19.4 ± 17.5	36.8 ± 36.2	6 ± 13.9	46.4 ± 92.9	1.9 ± 6.2	22.3 ± 32.8	0	0
Instar 2	29.6 ± 20.9	41.6 ± 39.2	5.5 ± 9.6	36.4 ± 36.9	10.1 ± 17.1	19.5 ± 18	4.5 ± 15.4	30.5 ± 69.4	2.1 ± 7.7	5.5 ± 8.5	0	0
Instar 3	19.6 ± 21.6	15.8 ± 17.7	7.9 ± 13	15.5 ± 15.6	3.8 ± 5	17.8 ± 23.5	0.1 ± 0.3	9.1 ± 17.6	0.1 ± 0.5	2.9 ± 5.3	0	0
Adult	39.6 ± 30.4	68.8 ± 44.7	36.2 ± 27.5	85.6 ± 40.4	19.1 ± 16.6	59.2 ± 23.7	2.7 ± 5.9	28.5 ± 32.9	0	15 ± 19.1	0	1.5 ± 3.5

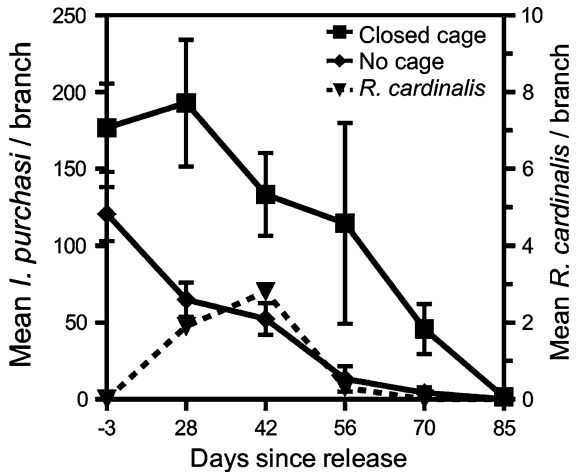

Fig. 3 Mean counts (±SE) for *I. purchasi* (all life stages combined) and *R. cardinalis* (larvae, pupae and adults combined) by days since release of *R. cardinalis* on uncaged and caged branches ($n = 15$ and $n = 10$, respectively) at Punta Estrada, Santa Cruz island

reproduction on the scale insect colonies. Predator numbers peaked 42 days after *R. cardinalis* was released with a mean of 2.8 ± 3.8 individuals per branch, after which numbers declined, reaching zero 70 days after release. Caged branches were not invaded by the predator at any time during the experiment.

A significant difference in mean counts per branch between experimental treatments was found for all life stages of *I. purchasi* (range in F values: 4.1–8.1, $df = 1, 115$, $P \leq 0.05$ for 1–3 instars, $F = 22.6$, $df = 1, 115$, $P < 0.0001$ for adults, and $F = 13.96$, $df = 1, 115$, $P < 0.0003$ for all life stages of *I. purchasi* combined) with consistently higher *I. purchasi* counts in the exclusion cages (Tables 3 and 4). It is important to note that there was significant variability (approximately one fifth of the residual variation) in *I. purchasi* counts between branches for all life stages. Scale insect populations at all life stages declined in both treatments over time (range in F values: 5.4–33.7; $df = 5, 115$; $P \leq 0.0002$); however, for all life stages there was evidence of an experimental treatment × time interaction, indicating that the time effect was different between experimental treatments. The P-values for this interaction were less than 0.25 for 1–3 instars and significantly different for adult females ($F = 3.89$; $df = 5, 115$; $P = 0.003$) and when all life stages were combined ($F = 2.52$; $df = 5, 115$;

Table 4 Summary of mixed model results for the predator exclusion studies where the effects of treatment (caged vs. uncaged) and day after release of *R. cardinalis* were tested

Effect	*df*	Instar 1		Instar 2		Instar 3		Adult		All life stages	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1, 115	8.12	0.005	5.67	0.02	4.08	0.05	22.63	<0.0001	13.96	0.0003
Days	5, 115	5.4	0.0002	7.31	<0.0001	7.42	<0.0001	33.7	<0.0001	16.67	<0.0001
Treatment × days	5, 115	1.37	0.24	1.7	0.14	1.69	0.14	3.89	0.003	2.52	0.03

Table 5 Summary of slope estimates (change in *I. purchasi* count per day) for uncaged branches (reference) and increment in slope for caged branches for response variables using data less than 56 days after the introduction of *R. cardinalis*

Treatment	Estimate (SE)				
	Instar 1	Instar 2	Instar 3	Adult	All life stages
No cage	−0.39 (0.22)	−0.41 (0.16)	−0.33 (0.09)	−0.60 (0.16)	−1.74 (0.47)
Caged (increment)	0.25 (0.34)	0.14 (0.24)	0.26 (0.14)	−0.01 (0.25)	0.64 (0.74)

$P = 0.03$) (Table 4). When slopes were estimated for *I. purchasi* counts for treatment and control groups, over the period up to 56 days after the release of *R. cardinalis,* a population decrease of 1.7 (SE = 0.47) scale insects per day was observed in uncaged branches compared to 1.1 (SE = 0.87) among caged branches, indicating that *I. purchasi* declined at a faster rate when it was exposed to the predator, *R. cardinalis* (Table 5). A steeper negative slope was evident for the uncaged branches for all developmental stages (except for adult females) demonstrating a faster rate of population decrease when scale insects were exposed to predators, but this was not statistically significant at the 0.05 level.

Impact of R. cardinalis under unmanipulated field conditions

On mangrove branches where adult female *I. purchasi* densities were followed after predator release, without the use of cages, *I. purchasi* numbers showed an initial increase but then after day 25 post-release, declined steadily, reaching almost zero at day 83 (a 99.8% reduction in two months) (Fig. 4). Twenty five days after the release of the predator, *I. purchasi* numbers had increased by 35% to a mean density of 61.9 ± 38.6 adult females per branch ($n = 24$), with up to 168 adult females found on one branch. At this time, a mean of 1.2 ± 3.7 *R. cardinalis* per branch were observed. Thirty-nine days after the biological control program was initiated, *I. purchasi* populations had declined to an average of 54.4 ± 37.6 adult females per branch and continued to decline, with only two adult females left 83 days after release. Predator numbers peaked 39 days after *R. cardinalis* was released with a mean of 2.2 ± 3.9 individuals per branch, following which numbers declined, with only one individual counted at 83 days post-release (Fig. 4).

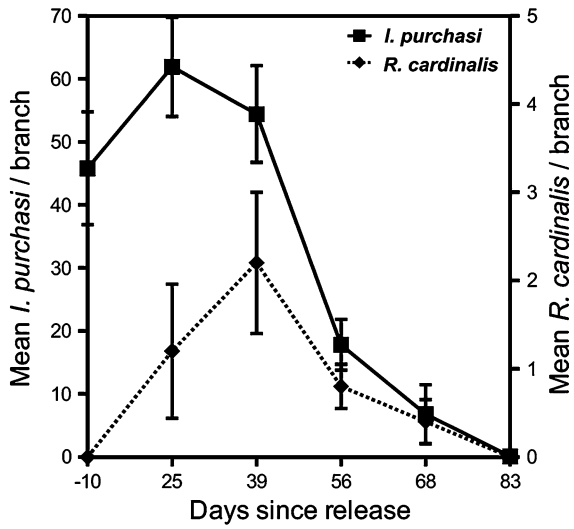

Fig. 4 Mean counts (±SE) for *I. purchasi* adult female and *R. cardinalis* (larvae, pupae and adults combined) by days since release of *R. cardinalis* on marked, but unmanipulated branches ($n = 24$) in Puerto Ayora, Santa Cruz Island

Discussion

The coccinellid *R. cardinalis*, released for the classical biological control of *I. purchasi* in the Galapagos Islands in 2002, has established and dispersed throughout the archipelago. Furthermore, it has adapted to a wide range of habitats and has been shown experimentally to be at least partially responsible for reducing *I. purchasi* populations on one important native plant (white mangrove).

Rodolia cardinalis has established successfully in most parts of the archipelago despite the release of relatively low numbers (2,206 individuals). This is particularly notable for Fernandina Island, a semi-pristine island of 248 mi^2 where populations of *R. cardinalis* originated from just 26 individuals. *Rodolia cardinalis* has also proven to be a good intra- and inter-island disperser. Island-wide surveys indicate that *R. cardinalis* is now widely present in many areas and habitats (including urban, agricultural, and natural areas). It has also been found preying on *I. purchasi* on islands where it was not originally released (i.e., Baltra, North Seymour, and Champion Island). Furthermore, our studies on Santa Cruz Island demonstrated that it dispersed quickly after its release and exhibited a high searching capacity, similar to that observed in its native range (Australia), where it was able to detect and destroy cottony cushion scales located 500 m from other *I. purchasi* colonies within a week (Prasad 1990).

Reproducing populations of *R. cardinalis* were observed on *I. purchasi* at two study sites on Santa Cruz Island within a month of the beetle being released. At Punta Estrada (site of the exclusion study) and on the coast of Puerto Ayora, *I. purchasi* populations that were exposed to the predator declined by 99–100% within three months of *R. cardinalis* release. Within a month after the release of its natural enemy, *I. purchasi* populations had been almost halved at Punta Estrada, the site with the highest number of beetles (200) released. At both study sites, the trends of *I. purchasi* and *R. cardinalis* followed the classical predator–prey response curve with the predator increasing in response to prey densities and then declining once pest populations diminished locally (van Driesche et al. 2008). The rapid drop in scale insect numbers, suggests that the predator had an impact on *I. purchasi* populations. Nevertheless, it was hard to isolate the effects of the predator from other factors because survivorship also decreased in populations of *I. purchasi* that were protected from the predator by cages. When *I. purchasi* populations are protected from natural enemy attacks because of exclusion cages, populations should become significantly higher in comparison to populations that are not protected from foraging natural enemies (Prasad 1989).

Cages in our experiment did initially show increased *I. purchasi* population growth, but after one month numbers started to decline. A deterioration in the cage environment may have been responsible for scale insect mortality. The exclusion study was conducted in the hot, rainy season, and 2002, the year of the experiment, was a particularly wet year because of an el Niño event. High rainfall and high temperatures are likely to have increased humidity within the cages, which in turn may have promoted the growth of pathogens: an unidentified white fungus was found on many *I. purchasi* towards the end of the experiment. Between January and April, 459 mm of rainfall were recorded with more than 75% falling in the last two months of the trial. The months of highest rainfall were also associated with the highest temperatures of the year (CDF 2011). Previous collection records found that lower population numbers of *I. purchasi* were recorded between January and May (Causton 2001; Roque-Albelo and Causton 1999), suggesting that *I. purchasi* does not thrive in periods of hot, wet weather.

A marked difference in the rate of decline between *I. purchasi* populations that were exposed to the predator (uncaged) and populations that were protected from the predator (caged) does, however, suggest a predator effect. Although, initial total population counts for *I. purchasi* were similar for both experimental treatments, the rate of decline of *I. purchasi* over the 12 week experiment was greater on branches that were exposed to *R. cardinalis.* Furthermore, additional recruitment was prevented because the proportion of reproducing adults in the uncaged population declined quickly.

The results from the exclusion experiment on white mangrove, though not conclusive, suggest that *R. cardinalis* played a key role in reducing *I. purchasi* populations on this ecologically important species. It is possible that high rainfall and temperature also contributed to this decline. Mangrove forests are important refuges for littoral and terrestrial fauna, as

well as important nesting areas for marine and terrestrial birds. Within a year, substantial recovery of white mangroves was observed along the coast of Puerto Ayora and at Punta Estrada. Highly stressed mangroves that had been covered with *I. purchasi* and a black sooty mold that grows on the honeydew excreted by the cottony cushion scale, had returned to a lush green colour and new growth was notable (see van Driesche et al. 2010). Such fast recuperation was not only encouraging to conservation managers, but permitted the local community to see these changes and experience first-hand the results of conservation science in action. This was especially important because until then most of the large conservation projects in the Galapagos had been conducted on uninhabited islands and some residents expressed the feeling that nothing was ever done to help local communities.

From a conservation management perspective, *R. cardinalis* has demonstrated characteristics of an effective natural enemy and should be highly beneficial for the archipelago's natural ecosystems. Self-dispersal mechanisms have enabled *R. cardinalis* to track *I. purchasi* into ecologically sensitive areas that are remote and difficult to reach, making for a highly cost effective management program. The presence of *R. cardinalis* on a wide range of plant species damaged by *I. purchasi* suggests that the predator is mitigating the impact of this invasive species on other valued plant species. Continuing and future surveys of these affected species will over time enlarge the record of *R. cardinalis'* benefits.

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Author Biographies

Carolina Calderon Alvarez conducted the evaluation of *Rodolia cardinalis* in 2002. These studies formed part of her undergraduate thesis at Pontifica Universidad Javeriana, Bogota, Colombia.

Charlotte E. Causton has worked with the Charles Darwin Foundation for the Galapagos Islands since 1997. She has extensive experience with the management of invasive invertebrates in areas of conservation importance. She coordinated the biological control program against *Icerya purchasi* with the ladybird, *Rodolia cardinalis*, and along with Mark Hoddle, Christina Hoddle, Roy van Driesche, and other cooperators is currently evaluating this classical biological control program.

Mark S. Hoddle is a biological control specialist with the Department of Entomology, and Director of the Center for Invasive Species Research at the University of California Riverside, USA. He has worked on various biological control projects of agricultural pests and weeds, and on the biological control of invasive arthropods of conservation importance.

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