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Feeding range studies of *Rodolia cardinalis* (Mulsant), a candidate biological control agent of *Icerya purchasi* Maskell in the Galápagos islands

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Abstract

The immediate threat of the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae), to the conservation of endangered flora in the Galápagos islands prompted conservation groups to assess the risks associated with the introduction of its natural enemy, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae). Although *R. cardinalis* has been widely used for controlling this exotic pest, little information was found to confirm its presumed narrow feeding range. Consequently, studies were deemed necessary to determine whether the introduction of *R. cardinalis* would harm the island's native invertebrate fauna, in particular rare or threatened species. Using no-choice trials, we tested neonate and third instar larvae of *R. cardinalis* against 16 and 11 potential prey species, respectively. Adults with prior feeding experience on *I. purchasi* were tested against eight non-target species and naïve adults (those that had not fed on *I. purchasi*) were tested against six. These trials included up to 35% of the Homoptera species of conservation value presumed to have the highest risk of being preyed upon by *R. cardinalis*. To maximize the range of species exposed to *R. cardinalis*, feeding trials were also carried out with some introduced species representative of groups containing potential non-target species that were not located for testing. *R. cardinalis* was unable to complete its life cycle on any of the test prey species and only fed on *Margarodes similis* Morrison (Homoptera: Margarodidae), a species closely related to the cottony cushion scale. *M. similis*, however, is subterranean and in its natural habitat is not at risk from foraging by *R. cardinalis*. Based on these trials, we believe that immature stages of *R. cardinalis* will have no impact on the non-target invertebrate fauna of the Galápagos islands because they specialize on Margarodidae. Although the limited nature of our testing prevents us from reaching a definitive conclusion about the prey range of *R. cardinalis* adults, our results indicate that it is also narrow. According to our field and laboratory studies, niche overlap with native predators of Homoptera will be minimal and intraguild predation should not occur.

Keywords: Galápagos; *Rodolia cardinalis*; *Icerya purchasi*; Biological control; Prey range; Non-target impacts; Risk assessment; Conservation

1. Introduction

The success of *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) in controlling the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae), in the citrus groves of California in the 1880s and the subsequent liberation of this beetle in many other countries at the beginning of the 20th century (Bennett et al., 1985; Caltagirone and Doutt, 1989) occurred before protocols had been developed to determine whether the introduction of this beetle would have any impact on native fauna. In the past, little importance was given to the impact that entomophagous biological control agents might have on non-target invertebrates. Thus, feeding range studies were not carried out before their release (Duan and Messing, 1997; Howarth, 1991; Kuhlmann et al., 1998; Secord and Kareiva, 1996; Simberloff and Stiling, 1996; Van Driesche and Hoddle, 1997). Recently some guidelines have been suggested for estimating host ranges of entomophagous insects (Barratt et al., 1999; Keller, 1999; Sands, 1998; Sands and Van Driesche, 2000; Van Driesche and Hoddle, 1997; Van Lenteren et al., 2003), but most emphasized parasitoids. Although the prey ranges of some predatory

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species have now been assessed (Kirk and Thistlewood, 1999; Lopez and Kairo, 2003; Zilahi-Balogh et al., 2002), standardized methods for studying the feeding range of entomphagous arthropods are still in the process of being developed.

While the effectiveness of *R. cardinalis* has been demonstrated by the many successful control programs conducted against the cottony cushion scale (Bartlett, 1978; Cressman, 1930; Quezada and Debach, 1973; Strand and Obrycki, 1996; Waterhouse, 1991), this is not necessarily an indication that this biological control agent has not had any impact on non-target organisms (Simberloff and Stiling, 1996). Many authors (e.g., Bartlett, 1978; Cressman, 1930; Waterhouse, 1991) have suggested that the range of prey attacked by this species is narrow, yet only Quezada and Debach (1973) provide evidence of prey choice from long-term field studies. Although observations of the life history of *R. cardinalis* suggest that this predator is specialized on Margarodidae, in reviewing literature and specimen labels we found that there was only limited evidence of stenophagicity. Life cycle completion or feeding by *R. cardinalis* has been reported on several genera of Margarodidae, including *Auloicerya* Morrison (V. Brancatini, pers. commun., 2003), *Crypticerya* Cockerell (Anon, 1939 cited in Kairo and Murphy, 1995), *Drosicha* Walker (Kuwana, 1922), *Gueriniella* Targioni Tozzetti (Balachowsky, 1932), *Icerya* Signoret (Bartlett, 1978; Gery, 1991; Moutia and Mamot, 1946; Ragab, 1995), *Monophlebus* Burmeister (Koebele, 1893 cited in Balachowsky, 1932), *Monophlebulus* Cockerell (V. Brancatini, pers. commun., 2002), and *Palaeococcus* Cockerell (Mendel et al., 1998). However, *R. cardinalis* has also been recorded feeding on a dactylopid in its native range of Australia (Frogatt, 1902). In other locations prey records include aphids, mealy bugs, and armored scales (specimens from BMNH, R. Booth, pers. commun., 1998; Muma, 1953-4, 1955 cited by Hodek, 1996; Thompson and Simmonds, 1965). Nevertheless, excluding the margarodids, most of these records are unconfirmed and some are questionable (Hodek, 1996, in his review of coccinellids found that adult behavior has often been misinterpreted). Yet, these reports could not be discarded without evidence to the contrary.

Laboratory studies have been carried out to test the response of *R. cardinalis* to a small number of alternate prey other than Margarodidae (Balachowsky, 1932; Kuwana, 1922). In these studies, eight species of Homoptera were tested from the families Aphididae (3), Coccidae (3), Ortheziidae (1), and Pseudococcidae (1). None of these were fed upon by *R. cardinalis*. However, these trials do not reveal much about *R. cardinalis*' feeding range, because only some of the stages of the predator and prey were tested and the methodologies were missing some important information (e.g., number of individuals tested and whether they had prior feeding experience, test arena used, and whether no-choice or choice tests were carried out).

The possibility of intraguild predation between *R. cardinalis* and other scale insect predators should also be considered when assessing the potential impacts of this biological control agent. In the laboratory, larvae of *R. cardinalis* killed larvae of *Rodolia iceryae* Jenson, despite the presence of available food (Mendel and Blumberg, 1991). Moreover, predation may have been involved in the displacement by *R. cardinalis* of the introduced species *Rodolia koebelei* Oliff on *I. purchasi* in California (Bartlett, 1978) and the native *Rodolia amabilis* Gorham on *I. purchasi* in India (Subramanian, 1953).

In the Galápagos, the cottony cushion scale was first reported in 1982. Dispersed by wind currents and humans among the islands, *I. purchasi* has now colonized 15 islands in the archipelago. This cosmopolitan, polyphagous pest restricts plant growth and in some cases kills plants of at least 62 native or endemic species. Sixteen of these species are listed as threatened in the IUCN Red List of Threatened Species, of which six are classified as Endangered or Critically Endangered (Causton, 2001; Causton, 2003). Furthermore, indirect effects on endemic Lepidoptera associated with a threatened plant have also been observed (L. Roque-Albelo, pers. commun., 2001). The Charles Darwin Foundation (CDF) and the Galápagos National Park Service (GNPS) considered *I. purchasi* to be a serious threat to the conservation of the islands that could not be mitigated using chemical control. Legislation for the release of exotic natural enemies in the Galápagos is still being developed, and at this time responsibility for importation decisions lay with the GNPS. In 1996, the CDF at the request of the GNPS formed a technical advisory committee to evaluate the possibility of employing biological control for the first time on the Galápagos islands. This committee was composed of eight non-resident scientists and one resident scientist (all CDF members and each with at least 15 years experience in the Galápagos), along with two senior GNPS employees. Following the submission of a preliminary analysis, the committee concluded that given the urgency of the problem, a risk evaluation of potential biological control agents should be carried out at the same time as studies of the impacts of *I. purchasi* to confirm that this pest merited the introduction of a biological control agent.

Rodolia cardinalis was identified as the most suitable agent. It was concluded, however, that there were insufficient data to fully demonstrate that *R. cardinalis* would not threaten any Galápagos species, especially endemic scales with small populations already in danger of extinction. Accordingly, a risk assessment that included feeding range tests was conducted before releasing *R. cardinalis* into the Galápagos.

This paper describes the procedures used to evaluate the potential threats of this predator to the conservation of insect fauna in the Galápagos islands. High costs and logistic difficulties in shipping non-target Galápagos species to another testing location led to the decision to conduct feeding range tests on the Islands in a quarantine facility. In March 1999, beetles were donated by CSIRO Entomology (Brisbane) and a colony was established in a newly constructed insect containment facility at the Charles Darwin Research Station. Feeding range tests were carried out with all stages of the predator, including individuals that had no prior feeding experience on *I. purchasi* to determine if: (1) *R. cardinalis* could complete development on other insect species in the Galápagos, (2) late instar larvae or adults were able to switch between prey and feed temporarily on native insects, and (3) intraguild predation might occur between *R. cardinalis* and native predators of scale insects.

2. Materials and methods

2.1. Description of test procedures and arenas

To identify taxa potentially at risk from predation, literature and museum specimens of *R. cardinalis* and other *Rodolia* species were reviewed to record putative prey species for comparison with species checklists from the Galápagos (Morrison, 1924; Williams, 1977). Galápagos checklists were found to be incomplete and were supplemented with additional records we collected during field surveys in 1999 and 2000. Non-target species felt to most likely be at risk from predation by *R. cardinalis* were species that are related phylogenetically to *I. purchasi* (following the centrifugal methodology of Harley and Forno, 1992; Wapshere, 1974), species that are taxonomically unrelated, but live in close proximity to prey of *R. cardinalis* (including predators) and species that are morphologically similar to *I. purchasi*. Although, information on the distribution of some of these "at risk" non-target species was scarce, an attempt was made to rank them according to their conservation importance. For example, single island endemics that are specialized feeders on rare plant species that are also attacked by *I. purchasi* were considered to be the most susceptible to any potential impacts that *R. cardinalis* might cause and were a priority for testing.

Seventeen endemic and native species of Homoptera in the Galápagos were considered potential non-target prey of *R. cardinalis* of conservation value: Ortheziidae (1), Margarodidae (1), Pseudococcidae (7), Eriococcidae (2), Diaspididae (3), and Aphididae (3). Three of these species are questionable, but were included anyway. Two of these species are unlikely to be found in the same habitat as *R. cardinalis*; *Margarodes similis* Morrison (Margarodidae) the closest relative of *I. purchasi*, and a pseudococcid, which are suspected to be obligate root feeders. While *Paracoccus solani* Ezzat and McConnell (Homoptera: Pseudococcidae) is probably an introduced species. Ten species of coccinellids and a neuropteran were also identified as potential non-target prey. These species were selected as a result of field surveys that we carried out between 1996 and 2000 to determine whether any insect predators fed on *I. purchasi* and whether native and endemic predators of Homoptera are likely to be found in some of the habitats used by *R. cardinalis*. Species tested as potential prey for larvae or adults of *R. cardinalis* are shown in Tables 1 and 2. In some cases, when we were unable to locate a potential non-target species, an alternative related species was tested, even if it was an introduced species. Additionally, in order to maximize the range of species exposed to *R. cardinalis*, some species of Coccidae were tested even though they are not represented by native species. Where possible, prey species were tested on a variety of plant species to allow for the possible influence of plant kairmones used in host recognition and plant defense strategies such as pubescence or trichomes.

Due to physical and economic restraints, field-collected insects were tested rather than laboratory-reared individuals. This method was deemed acceptable because few species in Galápagos have parasitoids or pathogens. This allowed testing of a higher number of species in a shorter time. Test species used in the trial were reared after the trial to check for parasites. Unused specimens were also checked. Three prey species (1 Diaspididae and 2 Aphididae) were parasitized. The data from these trials were eliminated from the final results even though *R. cardinalis* has been known to eat parts of *I. purchasi* that are not parasitized by *Cryptochaetum iceryae* (Williston) in times of prey scarcity (Quezada, 1969). Two test prey species (1 Pseudococcidae and 1 Eriococcidae) were contaminated with fungi and were also excluded from the final analysis.

Trials were designed to test the response of a starved predator to a non-target species. This method is commonly known as no-choice testing where the survival of the agent on a test species (treatment) is compared with individuals fed on the target prey (control). For testing of adult *R. cardinalis*, our trials included two types of control (following the methodology of Lopez and Kairo, 2003), one being the target prey (*I. purchasi*) to provide baseline data to compare responses to non-target species and the other control being just water, to estimate mortality under starvation conditions. The number of replicates and species tested depended on the availability of the different developmental stages of *R. cardinalis* and the test species. All stages of the prey were used unless indicated in Tables 1 and 2. An indeterminate number of prey were used in the trials, because counting individual Homoptera proved to be time consuming and because

Table 1
Suitability of potential non-target prey for the development of immature stages of *R. cardinalis*

Test prey species[a,b]	Development of *R. cardinalis* larvae					
	Neonates			Third instars[c]		
	Feeding[c]	Development[c]	*n*	Feeding[c]	Development[c]	*n*
Homoptera						
Ortheziidae						
Orthezia insignis (I)	–	–	15	–	–	10
Orthezia sp. (?)	–	–	21	Nt	Nt	Nt
Margarodidae						
Margarodes similis (E) (cysts)•	–	–	88	+	–	26
M. similis (emerged females)•	+	–	94	–	–	3
Pseudococcidae						
Antonina graminis (N?)	–	–	57	–	–	45
Pseudococcus n. sp. # 2 New sp.•	–	–	20	–	–	14
Pseudococcus n. sp. # 3 New sp.•	–	–	44	–	–	22
Pseudococcus sp. (?)	–	–	26	–	–	17
Paracococcus solani (N?)•	–	–	15	Nt	Nt	Nt
Eriococcidae						
Eriococcus papillosus (E)•	–	–	69	–	–	15
Coccidae						
Saissetia coffeae? (I)	–	–	11	Nt	Nt	Nt
Parasaissetia nigra (I)	–	–	20	Nt	Nt	Nt
Diaspididae						
Selenaspidus articulatus (I)	–	–	20	–	–	31
Aspidiotus excisa (I?)	–	–	15	Nt	Nt	Nt
Aphididae						
Sitobion sp? (E?)• (All stages except eggs)	–	–	69	–	–	25
Coleoptera						
Coccinellidae						
Pentilia sp. (E?)• (mature larvae, pupae, and adults)	–	–	8	–	–	28
Neuroptera						
Chrysopidae						
Ceraeochrysa cincta (E?)• (eggs not tested on third instar larvae)	–	–	26	–	–	24

[a] All stages tested unless indicated.
[b] E, endemic; I, introduced; N, native; •, high risk potential prey of conservation value.
[c] –, negative response; +, positive response; Nt, not tested.

the emergence of crawlers from eggs and recently molted nymphs during the trials prevented us from accurately counting the number of prey in the test arena. All developmental stages of *R. cardinalis* were observed at least two times daily for indications of feeding activity. Test prey were examined for signs of predation and fresh material added every 3 days. Trials were terminated at least 7 days after all the individuals that had been exposed to the test prey species or the control with only water (CC) had died. Trials were only considered valid when more than 75% of the controls fed on *I. purchasi* (C) survived. All trials were conducted at temperatures between 24 and 26 °C, with a 12:12 dark/light photoperiod in the Insect Containment Facility of the Charles Darwin Research Station, Santa Cruz, Galápagos.

2.2. Feeding range tests for larvae

2.2.1. No-choice tests with neonate larvae

This methodology was based on recommendations of V. Brancatini (pers. commun., 1999) following similar experiments with *Rodolia limbata* (Blackburn). Mature *R. cardinalis* adults that had been previously exposed to *I. purchasi* were placed in plastic containers (11 cm diam.) with cotton wool balls to encourage oviposition. Adults were fed on honey and water. After 3 days, the cotton wool with eggs was removed and placed in a sterile container. One recently emerged neonate was selected with a fine paint brush dipped in alcohol and allowed to dry. The larva was transferred to an Eppendorf tube (mouth = 1 cm diam, 4.2 cm high) with one adult female

Table 2
Survival (number of days) of "conditioned" and "naïve" adult *R. cardinalis* fed on a test prey species compared with individuals given only water (CC)

Test prey species[a]	Survival (days ± SD)[b]							
	Naïve				Conditioned			
	Test	*n*	CC	*n*	Test	*n*	CC	*n*
Homoptera								
Margarodidae								
Margarodes similis (E) (emerged female)•	**10.5 ± 3.8****	10	**3.8 ± 1.0**	10	**5.8 ± 4.3**	10	**3.1 ± 0.5**	10
M. similis (cysts)•	**5.5 ± 1.3**	10	**4.7 ± 1.3**	10	**2.8 ± 0.3**	10	**3.4 ± 0.4***	10
	7.8 ± 1.1	11	**7.6 ± 2.0**	11	Nt		Nt	
Pseudococcidae								
Paracoccus solani (N?)•	**6.7 ± 0.9***	12	**5.4 ± 1.0**	11	**2.0 ± 1.6**	17	**1.9 ± 0.7**	17
	Nt		Nt		**3.0 ± 0.7**	17	**2.9 ± 0.8**	17
Pseudococcus sp. #3 New Sp.•	Nt		Nt		**3.6 ± 1.2**	14	**2.8 ± 0.8**	13
Pseudococcus sp. #6 New Sp.•	**3.9 ± 0.8**	8	**4.8 ± 1.3**	7	**2.0 ± 0***	5	**1.2 ± 0.4**	5
Eriococcidae								
Eriococcus papillosus (E)•	**5.9 ± 1.8**	9	**4.6 ± 1.4**	10	**4.2 ± 1.0***	4	**2.3 ± 0.6**	3
Coccidae								
Ceroplastes ?rusci (I)	**6.3 ± 1.1**	9	**6.4 ± 1.7**	9	**4.1 ± 0.6**	7	**3.8 ± 0.9**	7
	Nt		Nt		**4.4 ± 0.5**	4	**3.9 ± 0.2**	4
Diaspididae								
Aspidiotus excisa (I?)	Nt		Nt		**3.1 ± 0.7**	13	**3.4 ± 0.6**	13
Neuroptera								
Chrysopidae								
Ceraeochrysa cincta(E?)•	**2.5 ± 1.5**	16	NA		**3.6 ± 1.3**	5	NA	
	Nt		Nt		**1.2 ± 0.4**	6	NA	

[a] E, endemic; N, native; I, introduced; •, potential prey of conservation value.
[b] Sample means compared using independent samples *t* test for data with equal variance and Mann–Whitney *U* test in the event of unequal variation. NA, not applicable; *, significant ($P < 0.05$); **, highly significant ($P < 0.001$).

I. purchasi (control) or the test species. A plug made from Kimwipes was pushed into the tube, leaving a 1 cm space for the larva to move in and tubes were inverted to prevent larvae from burrowing through the plug. Representatives from three insect orders and nine families (16 species) were tested in 47 trials. The variables recorded were: whether there was feeding, the number of days of larval survival, and number of molts achieved.

2.2.2. No-choice tests with third instar larvae

Eggs were placed in 9 cm petri dishes and newly emerged larvae were reared on *I. purchasi* until they were late second or early third instars. Larvae were then moved with a fine paint brush (dipped in alcohol and then dried) to a 9 cm petri dish with an adult female *I. purchasi* (control) or the test species. Eleven species, from eight families and three orders of insects, were assessed as prey in a total of 21 trials. The variables recorded were: whether there was feeding, the number of days of larval survival, and number of molts achieved.

2.3. Feeding range tests for adults

Both "naïve" (unfed, mated beetles recently emerged from pupae) and "conditioned" (mated and previously fed on *I. purchasi*) beetles were tested on potential non-target species. Where possible, one female–male adult pair was used in each replicate. When only one sex was available it was ensured that the same sex was used for testing both the non-target prey species and controls. In each trial, beetles were tested with the test species (T), a control with two adult female *I. purchasi* (C), and a control with no food and just water (CC). Only two treatments (T and C) were used for the trials with the predator *Ceraeochrysa cincta* Williston (Neuroptera: Chrysopidae). The test arena was a 9 cm petri dish, which is an acceptable space for *R. cardinalis* to mate and lay eggs according to previous studies (Matsuka and Watanabe, 1980; Ragab, 1995). The number of replicates (petri dishes) varied according to the species tested. A 1 cm^2 pie of humid absorbent cotton cloth was placed in each petri dish and sprayed with water once a day. Petri dishes were checked twice daily and any dead individuals of *R. cardinalis* removed and sexed. The variables recorded were: whether there was feeding, the number of days of adult survival, and the number of eggs deposited. Fecal pellets were also counted, but were not used in the analysis because their presence was not a reliable indicator of feeding; both starved naïve and starved

conditioned beetles produced a small number of faeces in some of the trials.

2.3.1. No-choice tests with naïve adults

Two-day old *R. cardinalis* pupae were dipped in 1% Clorox solution and placed in a sterile 15 cm diam. petri dish or in a 11 cm diam. plastic container. On emergence, one mated pair of 1- to 2-day old adults was transferred to the test arena. A total of eight trials were carried out on six prey species from five families in two insect orders.

2.3.2. No-choice tests with conditioned adults

Adults were reared on *I. purchasi* for 1–2 weeks in a 11 cm diam. container and then separated from *I. purchasi* and given water, but no food for 1–2 days. Following which, adults were sexed and one female–male pair placed in the test arena. Eight prey species were tested, from six insect families in two orders with a total of 12 trials.

2.3.3. Data analysis for no-choice tests with adults

The average survival time was calculated for each treatment. Because the control groups fed on *I. purchasi* were terminated approximately 1 week after the beetles from the other treatments died and, as such, a normal frequency distribution was not obtained, Kruskal–Wallis was used to test for significant differences between survivorship. An independent sample t test analysis was used to determine significant differences between treatments with the test species (T) and controls with no food (CC) if equal variance was confirmed by the Kruskall–Wallis test. The Mann–Whitney U test was used in the event of unequal variance. The statistics were calculated with the SPSS system (Norusis, 1993).

3. Results

3.1. Feeding range of larvae

3.1.1. Neonate larvae

Neonate larvae died 1–2 days after they were exposed to 12 species of scale insects from five Coccoidea families (Ortheziidae, Pseudococcidae, Eriococcidae, Coccidae, and Diaspididae), a probably endemic aphid, and the probably endemic *Pentilia* sp. (Coccinellidae) and lacewing *C. cincta* (Table 1).

Neonate larvae exposed to non-target prey other than Margarodidae were initially very mobile, but became very weak and immobile in the first 24 h and died within 2 days. Even individuals that had fed on conspecifics before being transferred to the test arena (indicated by direct observation or by swollen abdomens) died within 2–3 days. On the other hand, some neonates exposed to *M. similis* females that had emerged from their protective cysts lived for up to 7 days ($\overline{X} = 1.7$ days, SD $= \pm 1.5$ days, $n = 94$) during which they fed on the scale. However, neonate *R. cardinalis* larvae that fed on *M. similis* did not molt to second instar and could not complete development. Larvae that fed on *I. purchasi* reached adulthood within 3–4 weeks.

3.1.2. Third instar larvae

Larvae were able to feed and complete development on females of *M. similis* that had emerged from their protective cysts. Body fluids were extracted from *M. similis*, and in some cases, females were torn apart. Due to the low numbers of individuals that were tested ($n = 3$), we were unable to observe whether they were able to reproduce on this alternate prey.

Mature larvae did not feed on any of the eight test species of Homoptera from the five other families representative of groups with endemic species in the Galápagos or on cysts of *M. similis* (Table 1). Late instar larvae, reared on *I. purchasi* and then transferred to other prey species as second to third instars lived for as long as 15 days. Larvae searched continuously for the first week or so, gradually slowing down before becoming immobile and dying. Some late second instars were able to molt to third instar, but these were exceptions and were most likely individuals on the verge of molting when transferred to the experiment. Molts to fourth instar did not occur when larvae had only non-target prey as food. On the other hand, larvae that were fed on *I. purchasi* completed development to adults within 15 days. Similarly, mature larvae and pupae of the coccidophagous predator *Pentilia* sp. (Coleoptera: Coccinellidae) were not attacked. However, on one occasion the two species were found with their mandibles locked together. In contrast, larvae of the lacewing *C. cincta* attacked *R. cardinalis,* and lacewing larvae were often observed extracting the fluids from dead or dying beetle larvae.

3.2. Feeding range of adults

Extended dry periods limited the number of non-target species available for testing. Unidentified cecidomyiids were found associated with two mealybug species (*P. solani* and *Pseudococcus* n. sp. # 6), but the data from these trials have been included because it is not sure whether these flies are predators of scale insects or scavengers.

Recently emerged (naïve) *R. cardinalis* adults and adults that had been previously fed on *I. purchasi* (conditioned) were observed actively feeding on females of *M. similis* that had emerged from cysts (Table 2). Naïve, mated *R. cardinalis* adult pairs given emerged *M. similis* females (treatment T) lived for an average of 10.5 days (SD $= \pm 3.8$ days, $n = 10$), significantly longer ($P < 0.001$) than starved individuals (treatment CC)

($\overline{X} = 3.8$ days, SD $= \pm 1.0$ days, $n = 10$). Moreover, 65% of the beetles survived for more than 13 days on *M. similis,* at which stage experiments had to be terminated due to a shortage of the test species. The longevity of beetles fed previously on *I. purchasi* and then exposed to *M. similis* ($\overline{X} = 5.8$ days, SD $= \pm 4.3$ days, $n = 10$) was not significantly different from that of the control beetles fed only water ($\overline{X} = 3.1$ days, SD $= \pm 0.5$ days, $n = 10$) (Table 2). Only three (15%) of the conditioned beetles survived for more than 8 days (11, 15, and 24 days, respectively) on *M. similis*. On the other hand, neither conditioned or naïve beetles could break open the hard waxy cysts that typically protect *M. similis* females and the presence of the cysts in the test arena did not result in beetles living longer than individuals that were starved (Table 2).

Rodolia cardinalis were not observed feeding on any scale insect other than the margarodid. Beetles continuously moved around the petri dishes, but rarely settled on the prey. Obvious signs of predation such as punctured ovisacs or desiccated prey were not observed. Conditioned *R. cardinalis* adults tested against four endemic and native species (3 Pseudococcidae and 1 Eriococcidae), and two introduced species (1 Coccidae and 1 Diaspididae) lived for an average of 3.1 days (SD $= \pm 1.3$ days, $n = 81$) and lived as long as the controls with water ($\overline{X} = 2.7 \pm 1.0$, $n = 79$) in 75% of the trials (Table 2). In the remaining two trials, adult beetles tested against *Eriococcus papillosus* Morrison (Homoptera: Eriococcidae) and a new species of *Pseudococcus* (#6) lived significantly longer ($P < 0.05$) than beetles starved on water within the same trial. However, only beetles tested against *E. papillosus* lived longer ($\overline{X} = 4.2 \pm 1.0$, $n = 4$) than the average for controls given only water from all the trials. Naïve *R. cardinalis* adults were tested on fewer scale insect species ($n = 4$) and lived for an average of 5.9 days (SD $= \pm 1.6$ days, $n = 38$) when tested against an endemic and native pseudoccoccid, an endemic eriococcid and an introduced coccid. Adults tested against three of these species lived as long as the controls given only water, while adults tested against *P. solani* lived significantly longer ($\overline{X} = 6.7 \pm 0.9$, $n = 12$, $P < 0.05$) than their water-fed counterparts (Table 2). Beetles offered *P. solani* also lived longer than the average for water-fed controls pooled from all the trials ($\overline{X} = 5.4$ days, SD $= \pm 1.5$, $n = 37$).

Similarly, adult *R. cardinalis* did not feed on mature *C. cincta* larvae. In contrast, adults that were weakened by a lack of food were often attacked by this scale insect predator.

Conditioned beetles laid a maximum of four eggs ($\overline{X} = 1.8$ eggs, SD $= \pm 1.2$, $n = 13$) only in test arenas with the following species: *M. similis* cysts, *Pseudococcus* new sp.#3, (Homoptera: Pseudococcidae), *Aspidiotus excisa* Green (Homoptera: Diaspididae), and *Ceroplastes ?rusci* (L.) (Homoptera: Coccidae). In trials with the same species, where conditioned beetles were given only water a maximum of nine eggs was deposited ($\overline{X} = 2.1$ eggs, SD $= \pm 2.3$, $n = 16$). Naïve beetles did not lay eggs in any of the trials unless they were fed *I. purchasi*.

4. Discussion

4.1. Can R. cardinalis complete its life cycle on Galápagos insects?

Even though species inventories are not complete and little is known about the ecology of the high risk potential prey of *R. cardinalis* in the Galápagos, a sufficiently wide range of phylogenetically and ecologically related species were tested against neonate *R. cardinalis* larvae to indicate that this life stage has a narrow prey range. Lack of survival of recently emerged larvae on these non-target species suggests that this biological control agent would be unable to survive for any long period or reproduce on any species other than *I. purchasi* in the Galápagos. Using some native and endemic, and some introduced test species we were able to test larvae against at least one species from each Homoptera family that is represented by an endemic species potentially at risk from predation in Galápagos. Moreover, larvae of *R. cardinalis* were tested on 35% of the homopteran species classified as potential non-target prey of conservation value. These test species included the endemic *M. similis*, the closest relative to *R. cardinalis*' target prey, *I. purchasi*. They also included four species of mealybugs, the group most likely to be encountered by *R. cardinalis* and the group of non-target prey with the largest number of Galápagos endemics, some of which are associated with threatened plant species.

The feeding range of *R. cardinalis* larvae is most likely defined when the adults select prey for oviposition. In our feeding tests and in the field (Quezada, 1969; Ragab, 1995), *R. cardinalis* laid eggs in or on its target prey, *I. purchasi*. This behavior suggests host specialization and has been observed in other species of coccinellids (Booth et al., 1995; Kairo and Murphy, 1995; Lopez and Kairo, 2003). Although, oviposition was observed in some of the test arenas occupied by alternate prey, eggs were deposited haphazardly and beetles also laid eggs in the arenas with only water. This suggests that factors other than the presence of non-target species were responsible for stimulating oviposition such as egg storage capacity in the oviduct (Dixon, 2000).

On the other hand, mated *R. cardinalis* beetles that had never been exposed to *I. purchasi* did not lay eggs on any occasion until after they had fed on the target prey. According to Frazer (1988), female coccinellids can only deposit eggs after they have eaten a sufficient amount of prey. The findings of Matsuka et al. (1982) with newly

emerged *R. cardinalis* fed only water or artificial diets, imply that normal fecundity cannot be achieved in the absence of suitable prey. Our results are in keeping with both of these studies and indicate that *R. cardinalis* needs to feed on sufficient quantities of *I. purchasi* or another suitable prey before egg production can occur. All of which suggests that the species tested against naïve *R. cardinalis* including *M. similis* were not suitable alternative prey for reproduction.

4.2. Will R. cardinalis temporarily switch to Galápagos insects in times of prey scarcity?

Temporary foraging on alternative food sources may prevent a biological control agent from dying out between population explosions of its target prey and may not affect the population numbers of the non-target prey (Sands, 1997). In island ecosystems such as the Galápagos, however, short-term feeding could have a lasting impact on species with small populations, particularly those that are already threatened (e.g., monophagous scale insects that feed on endangered plant species). Consequently, it is important to assess whether a candidate biological control agent can use high risk potential prey of conservation value as a temporary food source and not only look at whether the candidate biological control agent can complete its life cycle on a given species.

Feeding trials in this study demonstrated that early instar larvae *R. cardinalis* pose no threat to non-target species tested because larvae are initially weak and immobile and are typically found feeding inside the egg sac of adult *I. purchasi* Mature larvae and adults on the other hand, are more likely to come into contact with non-target species especially if they are found on the same host plants as *I. purchasi*. In our studies, late instar larvae continuously moved around the test arenas without target prey for at least 1 week before showing signs of weakness indicating that individuals are likely to search for extended periods of time in the absence of their target prey. Nonetheless, mature larvae did not feed on test species from five of six families that contain high-risk species of conservation value. Feeding occurred only on the congeneric soil-dwelling species, *M. similis*. Our results suggest that neither young nor old larvae will switch to other prey unless that prey is a species of margarodid. Therefore, *R. cardinalis* larvae will be unable to use Galàpagos insect species as temporary food sources.

Adult *R. cardinalis* have a high searching capacity (Prasad, 1990), and according to our studies are able to survive for a considerable length of time (up to 15 days) in the absence of food. Our results suggest that the prey range of both conditioned and naïve beetles is narrow. We were able to test conditioned adults against 25% of the high-risk species of conservation value excluding Margarodidae, and naïve adults against 19% of these species. A small number of introduced species was also tested. Survivorship of both naïve and conditioned *R. cardinalis* adults was no better than that of starved individuals in 83% of these trials. In the remaining trials, significant differences in lifespan were noted between the tests and the controls, but these differences were not consistent between naïve and conditioned adults. No obvious signs of feeding were observed. Adults may have fed on honeydew or attempted to feed on the test prey, but were not able to use these species as alternative prey. Nonetheless, with the limited number of prey species available for testing, conclusions cannot be drawn for these species or other Galápagos species that could not be tested. For example, we were unable to test any species from the Ortheziidae, one of the closest families to that of the target prey. Although we were unable to test aphids because of parasitization, this distantly related group is unlikely to be used as a temporary food source if *R. cardinalis* did not feed on species closely related to its target prey.

Our only test species that supported some feeding was the endemic species, *M. similis*, the only other Margarodidae in the Galápagos. However, the biology of this species makes it an unlikely alternate prey for *R. cardinalis*. Field studies carried out since our feeding range studies with *R. cardinalis* have shown that *M. similis* are typically found encased within waxy secretions that form hard cysts found at least 15 cm below the ground surface (Lincango and Causton, unpublished data). *M. similis* appears to only reproduce parthenogenetically and would therefore not need to migrate to the soil surface to mate with males. Moreover, during a year and a half of surveys, females that had emerged from the cysts were never observed above ground and live cysts were rarely observed (Lincango and Causton, unpublished data). Exposed live cysts would not be at risk from predation by *R. cardinalis* because our tests demonstrated that *R. cardinalis* larvae and adults are incapable of breaking open the hard casing and are only able to feed on emerged females.

4.3. Are damaging interactions likely with native scale insect predators?

Specialist predators of margarodids are unlikely to be found in the Galápagos, unless they are adapted to feeding on the subterranean *M. similis.* Throughout 3 years of monitoring in the Galápagos, only two insect species were found preying on *I. purchasi*, both generalists: the endemic neuropteran *C. cincta* and *Pyroderces rileyi* Walsingham (Lepidoptera: Cosmopterigidae).The latter is a new record for Galápagos, and we do not know if it is an introduced or native species.

Ceraeochrysa cincta is common during the rainy season in Galápagos, but negative interactions with *R. cardinalis* are improbable because larvae of *C. cincta*

will attack *R. cardinalis* in captivity. This behavior has been found elsewhere. Coccinellids are susceptible to interspecific predation by Chrysopidae (Ceryngier and Hodek, 1996), which has been reported feeding on early instars of Coccinellidae by several authors (Balduf, 1935; Bartlett, 1978; Sengonca and Frings, 1985; Waterhouse, 1991). On the other hand, *P. rileyi* larvae are most commonly known as scavengers of detritis rather than predators. Larvae of this lepidopteran were commonly observed in the rearing containers of *R. cardinalis*, but were not fed on by *R. cardinalis*.

The immature stages of coccidophagous coccinellids are likely to be encountered by adult *R. cardinalis*, but native coccinellids are not predators of *I. purchasi*. Therefore, habitat overlap and intraguild predation should be rare because *R. cardinalis* is not expected, based on our tests, to feed on the prey used by native or endemic coccinellids. Native coccinellids are more likely to come into contact with *R. cardinalis* if their prey were found on a host plant shared with *I. purchasi* or if the native coccinellids were abundant. Even when there is habitat overlap, the size of the individual may determine whether predation occurs, and if it does, which species is the predator and which is the prey (Dixon, 2000). In our trials, all stages of *R. cardinalis* ignored larvae and pupae of one of the most commonly encountered predators, the pseudococcid specialist *Pentilia* sp. Only on one occasion were larvae of the two species found with their jaws locked. Likewise, larvae of a *Diomus* sp. were not attacked by *R. cardinalis* during preliminary observations of this species.

5. Conclusions

Our use of field-collected specimens in this study allowed us to quickly and cost-effectively test a wide variety of species. The only limitation this seemed to impose was that parasitization caused us to eliminate a small number of test species all together. It is hoped that the documentation of these procedures will contribute towards a standardization of methods for conducting feeding range studies on predators.

The negative responses we observed by all life stages of *R. cardinalis* with or without prior feeding experience to a wide range of prey indicate that this biological control agent should not be a threat to the conservation of insect biodiversity in the Galápagos islands. Immature stages of *R. cardinalis* pose no threat to other insect species in the Galápagos aside from *I. purchasi* because life cycle development and feeding is almost certainly restricted to margarodids. Difficulties in locating species for testing prevented us from assessing fully the impact for all the families of potential prey of adult *R. cardinalis*. However, our results showed that adult *R. cardinalis* were unable to use a small range of Homoptera as temporary sources of food, suggesting a narrow prey range. Intraguild predation by *R. cardinalis* should be uncommon and unlikely to adversely affect native predators for the following reasons: (1) *R. cardinalis* feeds specifically on Margarodidae; (2) native coccinellids and most other scale insect predators in the Galápagos do not feed on scales from the family Margarodidae; (3) there is little habitat overlap between the prey of native coccinellids and *R. cardinalis*; and (4) *R. cardinalis* did not attack four commonly encountered Galápagos species in the laboratory. Post-introduction monitoring is recommended to corroborate the results of these trials.

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