A case of ecological specialization in ladybirds: *Iberorhyzobius rondensis* (Coleoptera: Coccinellidae), potential biocontrol agent of *Matsucoccus feytaudi* (Hemiptera: Matsucoccidae)

C. Tavares[1]*, H. Jactel[2,3], I. van Halder[2,3], Z. Mendel[4] and M. Branco[1]

[1]Centro de Estudos Florestais (CEF), Instituto Superior de Agronomia, University of Lisbon (ISA-UL), Tapada da Ajuda 1349-017 Lisbon, Portugal: [2]INRA, UMR1202, BIOGECO, F-33610 Cestas, France: [3]Univ Bordeaux, UMR1202, BIOGECO, F-33400 Talence, France: [4]Department of Entomology, Agricultural Research Organization, 50250 Bet Dagan, Israel

Abstract

Specialization is an important attribute of a biological control agent. The maritime pine bast scale, *Matsucoccus feytaudi* Ducasse (Hemiptera Matsucoccidae), is an invasive species in Southeast France and the North of Italy. *Iberorhyzobius rondensis* Eizaguirre (Coleoptera: Coccinellidae), is a recently described ladybird species. Both adults and larvae are predaceous, feeding on egg masses of *M. feytaudi*, and are strongly attracted to *M. feytaudi*'s sex pheromone. To evaluate the potential of *I. rondensis* as a biocontrol agent of the scale, we studied its niche breadth and prey range with emphasis on pine forests and hemipterans as tested prey. In this study, *I. rondensis* was found to achieve complete development only when fed on *M. feytaudi* egg masses (92.9% survival) and an artificial prey: eggs of *Ephestia kuehniella* Zeller (27.6% survival). From the 2nd instar onwards, complete development could be achieved using other prey species, although larvae had significantly higher mortality and slower development. In choice tests, *M. feytaudi* was the preferred prey. Surveys of the ladybird populations in the Iberian Peninsula revealed that it was found exclusively on *Pinus pinaster* Aiton, the sole host of *M. feytaudi*. The unusual specialization of *I. rondensis*, among other predaceous ladybirds, makes it an appropriate candidate for classical biological control of *M. feytaudi.*

Keywords: diet breadth, habitat specialization, predator–prey, coccidophagous

Introduction

Specialization is considered a critical criterion for classifying predator or parasitoid species as candidates for biological control, given that risk to non-target species, apparent competition, and competitive exclusion are expected to be minimal in specialized organisms (Dixon, 2000; Van Lenteren *et al.*, 2006). Furthermore, specialized predators are expected to display higher efficacy than generalist predators as the former

_____
*Author for correspondence
Phone: +351 21 365 3382
Fax: +351 21 365 31 95
E-mail: catarina.a.tavares@gmail.com

usually have higher prey searching ability and voracity (Dixon, 2000). Still, dietary specialization of predators is often conditioned to the availability of prey in time and space (Hodek & Honěk, 1996; Abrams & Ginzburg, 2000). Specialized predators are usually restricted to the prey's habitat, and their life cycles are often synchronized (Dixon, 2000; Sloggett & Majerus, 2000).

Processes of ecological specialization in insects have been well studied in herbivorous species, with special attention paid to herbivore–plant interactions and associated co-evolutionary processes (Thompson, 1995; Funk *et al.*, 2002; Scriber, 2010). Specialization in parasites, parasitoids, and predators has also been regularly studied from an evolutionary perspective and for practical biological control purposes (Bristow, 1988; Strand & Obrycki, 1996; Wiegmann *et al.*, 1996; Dixon, 2000; Diehl *et al.*, 2013). Studies on the specialization of arthropod predators cover a large range of orders such as Coleoptera, Diptera, Hemiptera, and Araneae (e.g., Pekár, 2004; Short & Bergh, 2004; Jackson *et al.*, 2010; Jałoszyński & Olszanowski, 2013; Vieira *et al.*, 2013).

Within Coleoptera, the species studied most often are Coccinellidae (Obrycki & Kring, 1998). Predaceous ladybirds show variable degrees of specialization, and have been widely used in biological control programs (Dixon, 2000). An example of the importance played by specialization in the suitability of a biological control is the contrast between the two species *Harmonia axyridis* Pallas and *Rodolia cardinalis* Mulsant. The first of the two is a generalist predator, which has been released to control aphids in Europe and North America, with negative impacts on native fauna, whereas the second species, which is coccidophagous, has provided highly targeted control of *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) in California and Europe (Caltagirone & Doutt, 1989; Koch & Galvan, 2008; Katsanis *et al.*, 2013).

In this study, we considered the predaceous ladybird *Iberorhyzobius rondensis* Eizaguirre (subfamily Coccidulinae) as a potential biological control agent of the pine bast scale *Matsucoccus feytaudi* Ducasse (Hemiptera: Matsucoccidae). *Matsucoccus feytaudi* is an extremely specialized scale insect which only feeds on maritime pine, *Pinus pinaster* Aiton. The native range of this insect species is restricted to the Southwestern part of the Mediterranean basin. This corresponds to the refuge areas of *P. pinaster* during the last glaciation (Burban & Petit, 2003), where the bast scale is thought to have evolved in close association with its host (Burban *et al.*, 1999). The only other *Matsucoccus* species naturally occurring in Europe is *Matsucoccus pini* Green feeding on *Pinus nigra* JF Arnold and *Pinus sylvestris* L. (Foldi, 2004). The distribution of *P. pinaster* has changed in the last two centuries, with afforestation of large areas especially in Southwest France, Corsica, and Italy. During the 20th century, *M. feytaudi* expanded its range to the Southeast of France and later also to the North of Italy, and to Corsica, becoming a major pest of maritime pine in those areas (Covassi *et al.*, 1991; Jactel *et al.*, 1996). In this context, the predaceous ladybird *I. rondensis*, if confirmed as a specialized predator, could be proposed as a good candidate for the classical biological control of *M. feytaudi* in invaded areas.

Iberorhyzobius rondensis is a recently discovered coccidophagous ladybird species (Raimundo *et al.*, 2006) and very little is known about its biology. To date this species has been collected only on maritime pines in Portugal and Spain (Eizaguirre, 2004; Raimundo *et al.*, 2006), despite focused search for natural enemies of *M. feytaudi* in Italy (Covassi *et al.*, 1991), the South of France and Corsica (Branco *et al.*, 2011). The new genus *Iberorhyzobius* is assumed to be closely related to *Rhyzobius*, based on morphological similarities (Raimundo *et al.*, 2006). The larvae of *I. rondensis* have been observed feeding on egg masses of *M. feytaudi* and found to respond to the sex pheromone of the scale insect (Branco *et al.*, 2006). Considering these characteristics, we hypothesized that *I. rondensis* is a specialized predator of *M. feytaudi*.

Two main mechanisms have been proposed to explain ecological specialization in ladybirds: diet specialization and habitat specialization. The two are often linked and ladybirds, as with other predators, are usually restricted to the habitat of their main prey (Sloggett & Majerus, 2000). Therefore, given that specialized predators are expected to have a narrow range of hosts and habitats (stenotopic), the main objectives of this study were to determine: (i) the habitat specialization of *I. rondensis*, (ii) its diet breadth by analysing its development and survival on different prey species, and (iii) its host preference with choice tests. In addition, we tested the possibility of rearing the predator with an artificial diet (sterilized eggs of *Ephestia kuehniella* Zeller).

Material and methods

Habitat preference of I. rondensis

We tested whether *I. rondensis* occurs only on *P. pinaster* trees or whether it is also present on other native pine species that are hosts of *M. pini* (Foldi, 2004). Plots of three native pine species were surveyed (*P. pinaster*, *P. sylvestris*, and *P. nigra*) within six regions of the Iberian Peninsula, where they naturally co-occur (fig. 1). Two other Iberian regions, where only *P. pinaster* occurs, were also sampled. In each region at least three plots of each pine species were sampled. Distance between sampled plots was, whenever possible, less than 10 km and never more than 50 km. Lures were made of rubber dispensers impregnated with 200 μg of female *M. feytaudi* sex pheromone, pinned to the tree trunk (N = 50 trees per sample area), 1 m above ground level, after smoothing the bark surface to create an arena (Branco *et al.*, 2006). Baited arenas were monitored for 1 h, in March, April, and May 2006–2012, which corresponds to *I. rondensis* larval activity and male *M. feytaudi* flight, both of which are attracted to the lures. Plots of *P. sylvestris* and *P. nigra* were only sampled from the end of April through the beginning of May, when the flight periods of male *M. pini* and *M. feytaudi* overlap (Foldi, 2004).

Because the data did not satisfy the assumptions of normality and homoscedasticity, differences in the abundance of *I. rondensis* between regions were analyzed with the non-parametric Kruskal–Wallis test followed by the Mann–Whitney test to compare each pair of regions. Pearson's correlation coefficient was then calculated to assess the relationship between *M. feytaudi* and *I. rondensis* abundances.

Diet preference of I. rondensis

Potential prey species of *I. rondensis* were selected using the centrifugal phylogenetic method (Van Lenteren *et al.*, 2006), starting with close relatives of the target prey and continuing with species from more distant taxa: e.g., subfamilies and families within the same order. We tested the target prey (*M. feytaudi*) and its congener, *Matsucoccus josephi* Bodenheimer & Harpaz, which is native to the East

Fig. 1. Distribution map of maritime pine (*Pinus pinaster*) [EUFORGEN 2009, www.euforgen.org] with the sampled sites in the Iberian Peninsula. A – Catalonia, B – Valencia region C – Andalusia, D – Madrid region, E – South Portugal, F – Setúbal/Sintra, G – Central Portugal, and H – North Portugal. Triangles represent sampled sites with only *P. pinaster* and circles sampled sites with all three pine species (*P. pinaster*, *Pinus nigra*, and *Pinus sylvestris*).

Mediterranean region and only inhabits *Pinus halepensis* Mill and *Pinus brutia* Ten. Some species from the family Monophlebidae, which is phylogenetically very close to the Matsucoccidae, were tested as well: *Palaeococcus fuscipennis* Burmeister, a scale insect which also feeds on *P. pinaster* trees; and *I. purchasi* and *Gueriniella serratulae* Fabricius, which feed on several hosts (Ben-Dov *et al.*, 2001). Different families sharing the same pine habitat as the target prey were tested: an Aphididae, *Cinara maritimae* Dufour and an Adelgidae, *Pineus pini* Koch. From the family Pseudococcidae, we tested *Planococcus citri* Risso, which feeds on *Citrus* trees. Finally, sterilized eggs of *E. kuehniella* (Lepidoptera: Pyralidae) were used in order to check whether ladybirds could be reared on artificial food (table 1).

These diets were tested with second, third, and fourth instar larvae of *I. rondensis* collected from *P. pinaster* plots in 2006, 2009, and 2010, using sex pheromone lures of *M. feytaudi*, as described above. In the laboratory, larvae were weighed and their body length measured in order to determine their instar. Each larva was put into an individual plastic vial for feeding tests. In 2011 and 2012, neonate larvae were reared under room conditions, from eggs laid by adults in the laboratory.

Prey preference in choice tests

Paired choice tests were performed allowing *I. rondensis* larvae to choose between *M. feytaudi* eggs, *M. josephi* eggs (*n* = 20), *C. maritimae* nymphs (*n* = 48), *P. citri* eggs (*n* = 118), *P. pini* eggs (*n* = 63), and *E. kuehniella* sterilized eggs (*n* = 34). Tests were performed in Petri dishes of 10 cm in diameter, with

a layer of filter paper, under natural conditions of light and a temperature of 22 ± 2°C. Food items were placed at maximum distance opposite each other, and their position was switched for each replicate. Larvae were starved 48 h before trials. Assays were monitored for up to 2 h and the test was considered finished when the larva selected one of the food items and began to eat it. Choice tests were performed with the 3rd and 4th instar larvae collected in the field in Portugal, in March and April 2009 and 2010, with the exception of the test between *M. feytaudi* and *M. josephi* which was performed with 1st instar larvae in 2011. This was because these trials with *M. josephi* were completed in Israel (as *M. josephi* is not present in Europe), where the number of *I. rondensis* larvae was limited; none were available in the 3rd and 4th instar to be used for the choice test. A χ^2 analysis was performed to test for differences in prey choice.

Survival of I. rondensis

Iberorhyzobius rondensis larval survival was analyzed for different feeding regimes. Larvae were reared in small tubes, with cotton as a lid. Feeding trials were performed for the entire life cycle, from the 1st instar larvae to the adult stage, starting from neonate larvae obtained in the laboratory (table 2) and also separately for each of the 2nd, 3rd, and 4th instar for larvae collected in the forest (table 3). *Matsucoccus feytaudi* eggs are difficult to obtain and not enough were found to feed all larvae from the 1st instar onwards; therefore larvae of later instars were obtained from the field. The number of larvae and food items available dictated the number of replicates for each regime. Also, 2nd and 3rd instar larvae were further pooled together because the number of 2nd instars captured in the field was very low. For 1st instars, experiments started when neonate larvae hatched. All larvae were reared in separate plastic test vials at 22°C, 14:10 L:D (ratio of light to dark), and 60% relative humidity. A small piece of cardboard was provided to allow them to hide, and the prey was exposed on a piece of paper. Individuals were checked and fresh food was added every 2 days, so that larvae were never limited by their food supply. Survival until adulthood was monitored for each individual and then analyzed with the Kaplan–Meier estimator, followed by a Log-Rank test based on *I. rondensis* larval stage. Pairwise comparisons of diet effects were also computed using the Log-Rank test. No statistical analysis was performed for the *P. fuscipennis* diet given the low number of replicates.

Growth rate

Performance under each regime was further evaluated by the relative growth rate (RGR), calculated for each individual as used by Matsuki & Jr.MacLean (1994):

$$RGR = \frac{(\ln(Wf) - \ln(Wi))}{(Df - Di)} \quad (1)$$

where *Wf* is the fresh weight at the final day of the test, *Wi* is the fresh weight at the first day, *Df* is the final day of the test, and *Di* is the first day of the test.

Larvae were checked every day and food was added every 2 days. Larvae were weighed every week, on the same day, and their instar stage was recorded by observing exuviae. Final weight (*Wf*) was measured after adult emergence, or otherwise we used the weight of the last day before the

Table 1. List of selected prey according to the centrifugal phylogenetic method (Van Lenteren *et al.*, 2006) to test host range of *Iberorhyzobius rondensis* Eizaguirre larvae.

Selected preys	Order	Family	Origin
Matsucoccus feytaudi egg masses	Hemiptera	Matsucoccidae	*Pinus pinaster* at Forest Unit INRA Bordeaux, France
Matsucoccus josephi egg masses	Hemiptera	Matsucoccidae	*Pinus halepensis* in Eshtao'l forest, Judean Hills, Israel
Palaeococcus fuscipennis eggs	Hemiptera	Monophlebidae	*P. pinaster* at Forest Unit INRA Bordeaux, France
Icerya purchasi eggs	Hemiptera	Monophlebidae	*Citrus sinensis* trees at the Agronomy Institute – Lisbon, Portugal
Planococcus citri eggs	Hemiptera	Pseudococcidae	Laboratory rearing on potato sprouts Agronomy Institute – Lisbon, Portugal
Gueriniella serratulae eggs	Hemiptera	Monophlebidae	*Molinia caerulea* Linnaeus at the Forest Unit of INRA – Bordeaux, France
Pineus pini egg masses	Hemiptera	Adelgidae	Young potted *P. pinaster* trees at Agronomy
Cinara maritimae nymphs	Hemiptera	Aphididae	Institute – Lisbon, Portugal
Sterilized eggs of *Ephestia kuehniella*	Lepidoptera	Pyralidae	Koppert France S.A.R.L., Cavaillon and Koppert España S.L., Almería.

Table 2. Number (*n*) and survival (%) of *Iberorhyzobius rondensis* larvae reared in the laboratory from neonate to adult on different prey treatments: egg masses of *Matsucoccus feytaudi*, *Matsucoccus josephi*, *Gueriniella serratulae*, *Pineus pini*, *Planococcus citri* and *Palaeococcus fuscipennis*, eggs of *Ephestia kuehniella*, and nymphs of *Cinara maritimae*. Within the 1st instar, results with the same letters were not significantly different ($P < 0.05$). With subsequent instars differences were not significant due to high survival rates.

	1st instar		2nd instar		3rd instar		4th instar	
	n	Surv. (%)	*n*	Surv.(%)	*n*	Surv. (%)	*n*	Surv. (%)
M. feytaudi	42	92.9[a]	41	95.1	40	97.5	40	97.5
M. josephi	–	–	15	86.7	13	100	13	100
E. kuehniella	29	27.6[b]	10	80	8	100	8	100
C. maritimae	14	0[c]	–	–	–	–	–	–
G. serratulae	13	0[c]	–	–	–	–	–	–
P. pini	16	0[c]	–	–	–	–	–	–
P. citri	17	0[c]	–	–	–	–	–	–
P. fuscipennis	5	0[1]	–	–	–	–	–	–

[1] Since *n* = 5, this diet was not statistically analyzed; all individuals were dead in 7 days.

Table 3. Number (*n*) and survival (%) of larvae collected in the forest and reared in the laboratory from 2nd, 3rd or 4th instar to adult with different prey treatments: egg masses of *Matsucoccus feytaudi*, *Gueriniella serratulae*, *Pineus pini* and *Planococcus citri*; *Icerya purchase*, and *Matsucoccus feytaudi*; nymphs of *Cinara maritimae* and control (No food). Within each instar, results with the same letters are not significantly different ($P < 0.05$).

	2nd/3rd instar		4th instar	
	n	Survival (%)	*n*	Survival (%)
M. feytaudi	49	91.5[a]	76	97.4[a]
C. maritimae	37	70.3[b]	26	100[a]
G. serratulae	–	–	16	100[a]
P. pini	–	–	12	91.7[ab]
P. citri	55	29.1[c]	43	95.3[a]
I. purchasi	53	5.7[d]	17	70.6[b]
No food	13	0[d]	7	85.7[ab]

larva died. RGR was calculated for each instar and diet, i.e., *C. maritimae* nymphs, eggs of *I. purchasi*, *M. feytaudi*, *P. citri*, *E. kuehniella*, plus a control with 'no food'. For the 1st instar, RGR was calculated only for the diets with *M. feytaudi* and *E. kuehniella* eggs due to the high mortality of neonate larvae on other prey regimes. In the 3rd instar, the 'no food' RGR was not calculated due to a lack of replicates. One-way analysis of variance (ANOVA) was used to test for differences in mean

RGR between diets for each larval stage. It was followed by a multiple comparisons Tukey's test to identify significant differences between diets. Normality and homogeneity of variances were assessed, prior to all analyses, using the Kolmogorov–Smirnov one sample and Levene's tests, respectively. For *t*-tests, the number of degrees of freedom was corrected whenever equal variance was not assumed.

Pre-imaginal development

Development time from neonate until adulthood was recorded with two diets: *E. kuehniella* eggs and *M. feytaudi* eggs. The duration of each instar was recorded at each molt by observing exuviae. Independent sample *t*-tests were performed to compare the effect of the two feeding regimes on development time for each larval stage separately. Levene's test was used to test for equality of variances. All statistics were performed with SPSS version 20.

Results

Habitat preference of I. rondensis

Larvae of *I. rondensis* were only found on *P. pinaster* (table 4); none were observed on *P. nigra* or *P. sylvestris*, although males of *Matsucoccus* sp. were observed in several of these plots. These were presumably *M. pini*, which typically

Table 4. Total number of *Iberorhyzobius rondensis* and Matsucoccus sp. males sampled in selected regions of Spain and Portugal, on different pine species (*Pinus pinaster*, *Pinus nigra*, and *Pinus sylvestris*).

Species	Country	Region	Number of plots	Sampled trees per stand	Number of *I. rondensis* larvae	Percentage of plots with presence of *I. rondensis*	Number of *Matsucoccus* sp. adult males
P. nigra	Spain	Catalonia	3	45	0	0	0
		Valence	3	50	0	0	1
		Andalusia	3	45	0	0	528
		Madrid	3	46	0	0	6
	Portugal	Centre	3	40	0	0	23
		North	3	40	0	0	0
P. pinaster	Spain	Catalonia	7	50	0	0	1
		Valence	21	18	4	9.5	8
		Andalusia	7	35	64	57.1	491
		Madrid	6	50	187	83.3	24
	Portugal	South	8	22	31	62.5	14
		Setúbal/Sintra	44	43	1539	88.6	1001
		Centre	7	30	51	85.7	45
		North	5	30	47	80	1
P. sylvestris	Spain	Catalonia	3	45	0	0	0
		Valence	3	45	0	0	2
		Andalusia	3	45	0	0	4
		Madrid	3	46	0	0	0
	Portugal	Centre	3	40	0	0	23
		North	6	45	0	0	0

reproduces on these two pine species (Foldi, 2004). *Matsucoccus feytaudi* and *I. rondensis* were found in *P. pinaster* from all examined regions except Catalonia.

Pooling of data at the regional level showed that most regions presented a low density of *I. rondensis* larvae per maritime pine tree, but with significant differences (Kruskal–Wallis test $\chi^2_7 = 549.19$; $P < 0.001$) in density between regions. Pairwise comparison of regions where the ladybird was found showed that larval density (mean larvae per tree ± standard error) was lowest in Valencia (0.01 ± 0.006) followed by Southern Portugal (Algarve) (0.16 ± 0.036). In the other extreme, Madrid and Setúbal/Sintra had the highest densities (0.62 ± 0.071 and 1.59 ± 0.111, respectively). A density-dependent relationship was found between the total number of larvae of *I. rondensis* and the total number of *M. feytaudi* males observed in each region ($r = 0.889$; $n = 8$; $P = 0.003$).

Prey preference in choice-tests

In paired choice tests (df = 1), *M. feytaudi* eggs were preferred by *I. rondensis* larvae to *E. kuehniella* eggs, *M. josephi* eggs, *C. maritimae* nymphs, and *P. citri* eggs, but not to *P. pini* eggs (fig. 2). Larvae did not make a choice in 60% of the trials.

Survival of *I. rondensis*

Neonate larvae reached adulthood only when fed *M. feytaudi* or *E. kuehniella* eggs, but *M. josephi* eggs were not tested with 1st instars (table 2). The survival rate of first instars significantly differed between prey species ($\chi^2_6 = 102.18$; $P < 0.001$). It was highest (92.9%) with *M. feytaudi* eggs, significantly lower (27.6%) with *E. kuehniella* eggs ($P < 0.001$) and null with all other food regimes (table 2). Survival of 2nd instars was also higher on *M. feytaudi* (95.1%) than on *E. kuehniella* (80%) and *M. josephi* (86.7%) but the differences
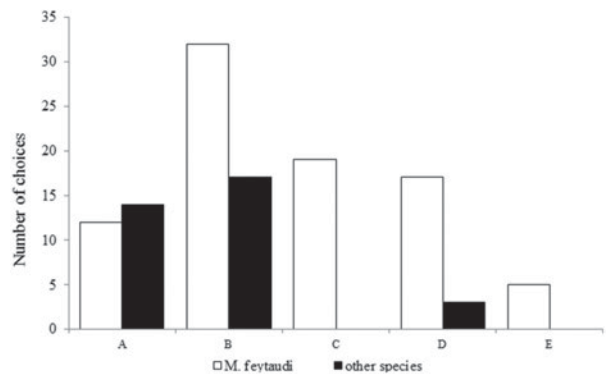

Fig. 2. Results of choice-tests between different prey regimes with larvae of *Iberorhyzobius rondensis*. All tested pairs presented significant differences $P < 0.05$ except pair A. Paired choices: (A) *Matsucoccus feytaudi* eggs vs. *Pineus pini* eggs ($n = 63$, $\chi^2 = 0.19$, $P = 0.660$), (B) *M. feytaudi* eggs vs. *Planococcus citri* eggs ($n = 118$, $\chi^2 = 4.61$, $P = 0.032$), (C) *M. feytaudi* eggs vs. *Cinara maritimae* nymphs ($n = 48$, $\chi^2 = 19.05$, $P < 0.001$), (D) *M. feytaudi* eggs vs. *Matsucoccus josephi* eggs ($n = 20$, $\chi^2 = 9.85$, $P = 0.002$) and (E) *M. feytaudi* eggs vs. *Ephestia kuehniella* eggs ($n = 34$, $\chi^2 = 4.33$, $P = 0.037$). All tested larvae were 3rd or 4th instar larvae, except for *M. feytaudi* vs. *M. josephi* for which we tested 1st instar larvae.

were not significant ($\chi^2_2 = 2.4551$; $P = 0.293$). For 3rd and 4th instars, survival was around 100% with the three prey items with no significant differences between them ($\chi^2_2 = 0.52$; $P = 0.769$).

For 2nd and 3rd instar larvae collected in the forest, the survival rate also differed between prey items ($\chi^2_4 = 102.417$, $P < 0.001$). It was highest when larvae were reared on the *M. feytaudi* eggs (91.5%), followed by the *C. maritimae* nymphs (70.3%). Only a small percentage of individuals survived until

Table 5. Relative Growth Rate (RGR) and development time (DT) in days (mean ± standard error) of *Iberorhyzobius rondensis* instars fed with different prey regimes. Within each instar, values followed by the same letters are not significantly different ($P < 0.05$).

Instar	Prey	N	RGR	Development time
1st instar	*Matsucoccus feytaudi*	15	0.528 ± 0.0116^a	8.4 ± 0.35^a
	Ephestia kuehniella	5	0.187 ± 0.0349^b	10.8 ± 1.30^b
2nd instar	*M. feytaudi*	23	0.179 ± 0.0017^a	4.0 ± 0.13^a
	E. kuehniella	7	0.095 ± 0.0058^b	7.8 ± 0.98^b
	Icerya purchasi	11	0.04 ± 0.0036^{bc}	–
	Cinara maritimae	2	0.018 ± 0.0203^{bd}	–
	Planococcus citri	24	-0.01 ± 0.0017^d	–
	No food	7	-0.04 ± 0.0058^d	–
3rd instar	*M. feytaudi*	24	0.083 ± 0.0016^a	5.7 ± 0.24^a
	E. kuehniella	6	0.092 ± 0.0075^a	8.9 ± 0.74^b
	I. purchasi	37	0.005 ± 0.0012^b	–
	C. maritimae	26	-0.002 ± 0.0017^b	–
	P. citri	28	-0.009 ± 0.0017^b	–
4th instar	*M. feytaudi*	18	0.003 ± 0.0007^a	14.3 ± 0.23^a
	E. kuehniella	4	-0.009 ± 0.0032^{ab}	13.9 ± 0.55^a
	I. purchasi	11	-0.013 ± 0.0012^b	–
	C. maritimae	14	-0.014 ± 0.0009^b	–
	P. citri	18	-0.014 ± 0.0007^b	–
	No food	4	-0.024 ± 0.0031^b	–
Pupae	*M. feytaudi*	18	–	9.4 ± 0.23^a
	E. kuehniella	4	–	9.3 ± 0.49^a

adulthood on other treatments and none of the 2nd and 3rd instar larvae survived with the control 'no food' treatment (table 3). For the 4th instar larvae collected in the forest, survival was relatively high for all treatments (>70%, table 3), although significant differences between treatments were still found ($\chi^2_6 = 23.151$, $P < 0.001$). Larvae fed with eggs of *M. feytaudi* again exhibited a high survival rate (97.4%). It is worth noting that these larvae were probably close to pupation, which may explain the high survival rate (85.7%) of the 'no food' regime.

Growth rate

The mean RGR varied significantly between prey for the 1st, 2nd, 3rd, and 4th instars respectively: ($F_{(1.19)} = 14.33$; $P = 0.001$), ($F_{(5.73)} = 60.98$; $P < 0.001$), ($F_{(4.128)} = 22.72$; $P < 0.001$), and ($F_{(5.68)} = 4.55$; $P < 0.001$). For all instars, larvae fed with *M. feytaudi* showed higher RGR than larvae fed on other diets, with the only exception being the 3rd instar fed with *E. kuehniella* (table 5). This diet provided the second best food, although displaying high variability. The RGR coefficient of variance (CV%) was 8.5 and 41.8 for first instar larvae fed with *M. feytaudi* and *E. kuehniella*, respectively. In all other diets, with the exception of *C. maritimae* in the 2nd instar and *I. purchasi* in the 2nd and 3rd instar, larvae showed decrease in their weight which was highest with the 'no food' diet (table 5).

Pre-imaginal development

Development time averaged 10 days longer for *I. rondensis* larvae fed *E. kuehniella* eggs than on *M. feytaudi* eggs (table 5). The difference was significant for the 1st ($t_{9.19} = 2.32$, $P = 0.045$), 2nd ($t_{7.32} = 4.23$, $P = 0.004$), and 3rd instars ($t_{45} = 5.43$, $P < 0.001$). No significant differences were found between the two diets for the 4th instar ($t_{45} = -0.90$, $P = 0.371$) and the pupal stage ($t_{45} = -0.21$, $P = 0.837$).

Discussion

So far, no detailed information on the biology and host range for the recently described ladybird species *I. rondensis* was available (Raimundo *et al.*, 2006). The present findings support the hypothesis that *I. rondensis* is specialized on *M. feytaudi* as indicated by its habitat, prey choice, survival, and development rate on different prey species. On the Iberian Peninsula, *I. rondensis* was found only on *P. pinaster* trees, even when other pine species co-occurred. We thus reject the hypothesis that it is likely to naturally prey on *M. pini* which can only reproduce on *P. sylvestris* and *P. nigra*. As for the majority of coccinellids, if neonate larvae can only feed on a specific prey, adult females are most likely to lay eggs in the vicinity of that prey (Ferran & Dixon, 1993; Dixon, 2000).

Full development of *I. rondensis* was only achieved with two prey items, *M. feytaudi*'s eggs and sterilized eggs of *E. kuehniella*. However, survival was much lower for 1st instar larvae fed with eggs of the moth *E. kuehniella* compared to those fed with *M. feytaudi* egg masses. Survival on this artificial food is not unusual since other ladybirds such as *Cryptolaemus montrouzieri* Mulsant (Hodek & Honěk, 2009) have been successfully reared on *E. kuehniella* eggs, which they would never normally find in their natural habitat (Hodek & Honěk, 1996). After the 2nd instar, larval survival increased considerably on alternative prey items. For the 4th instar, survival was almost 100% with all tested prey. This trend suggests a broadening of diet breadth as larvae grow, which may imply the use of alternative food sources towards the end of the developmental season, when *M. feytaudi* egg masses become scarce. Even when left without food, 85.7% of the 4th instar larvae were able to pupate. As observed by Hodek & Honěk (1996) larvae of ladybirds can complete their development when a certain weight is achieved, although this may be delayed by nutritional deficiency and adults may emerge smaller. Thus it can be hypothesized that, in the absence of *M. feytaudi*, late instars of *I. rondensis* can survive and complete

their development on alternative prey occurring in the same habitat, such as *C. maritimae* or *P. pini*.

In two-choice tests, a consistent preference of *M. feytaudi* over the other tested prey was observed, except for *P. pini* egg masses. Like *M. feytaudi*, females of *P. pini* lay egg masses in cottony ovisacs in tree trunks and twigs. It is possible that *P. pini* is an alternative food prey for 3rd and 4th instar larvae when there is a lack of *M. feytaudi*. By contrast, *C. maritimae*, also occurring on *P. pinaster* trees, is highly mobile, able to defend itself or escape and is a myrmecophilous species, which makes it an improbable prey for *I. rondensis*. *Matsucoccus feytaudi* was preferred to *M. josephi*, the only other tested species of the *Matsucoccus* genus. However, this prey was only tested with 1st instar larvae in the two-choice test, and thus we cannot conclude about its use by older instars.

RGR was highest when *I. rondensis* larvae were reared on *M. feytaudi* eggs for all instars except for the 3rd, for which RGR was approximately the same as with *E. kuehniella* eggs. When larvae were reared on other prey, RGR was in most cases null or even negative, demonstrating a detrimental effect. The lower suitability of other prey is also indicated by the higher variability of RGR with other prey regimes, particularly *E. kuehniella*, compared to *M. feytaudi*. Interestingly, when fed with *I. purchasi* egg masses, 2nd and 3rd instar larvae had a positive RGR but survival was extremely low (5.7%). Apparently, the larvae were able to convert assimilated food into biomass but died, possibly due to some toxic effect. Also, neonate larvae were observed to feed voraciously when *P. citri* eggs were offered, but would die a few days later, without gaining weight and unable to complete molting. These results suggest physiological adaptations of *I. rondensis* to *M. feytaudi*. According to the trade-off specialization theory it is expected that a specialist will perform poorly (development and survival) on other kinds of prey, even if the different prey are taxonomically similar (Ferran & Dixon, 1993). This has been observed in a large number of studies. *Coccinella septempunctata* L. is known to feed on *Aphis sambuci* L. although this is a highly unsuitable prey (Hodek & Honěk, 1996). Nine aphid species were tested as prey for the aphidophagous *Calvia quatuordecimguttata* L. but only six were 'essential' food, and three were unsuitable, causing 100% mortality in fed larvae (Kalushkov & Hodek, 2001). The specialist *R. cardinalis* was tested on 16 prey species before its introduction in Galapagos and it was only able to complete its life cycle with one, *Margarodes similis* Morrison which, like the target prey *I. purchasi*, belongs to the family Monophlebidae (Causton *et al.*, 2004). According to Hodek & Honěk (1996) most cases of unsuitable prey concern herbivores that derive chemical protection from their toxic plants (e.g., Mendel *et al.*, 1992).

Iberorhyzobius rondensis distribution overlaps with that of its prey in the Iberian Peninsula where the latter found refuge during the last ice age (Burban *et al.*, 1999). Apparently, the ladybird did not follow its prey when the latter expanded its range to new pine forest areas in Southern France and Northern Italy. Densities of *I. rondensis* were generally low, as are densities of *M. feytaudi*, in its native geographical distribution (Riom & Gerbinot, 1977). However, differences could be observed from one region to another. *Iberorhyzobius rondensis* showed the highest abundance in one particular region (Setúbal/Sintra) where *M. feytaudi* was also reported to be particularly abundant (M. Branco, personnel observation). In agreement with a density-dependent relationship,

a significant and positive correlation was found between the number of ladybird larvae and the number of *M. feytaudi* males caught per region. This result suggests a numerical response of the predator to the density of its prey populations (Abrams & Ginzburg, 2000).

Additional evidence of *I. rondensis* specialization is seen in its foraging behavior. Other coccinellids are known to be attracted to the odor of their prey, e.g., *H. axyridis* to *Aphis citricola* van der Goot (Obata, 1986), *Cryptolaemus montrouzieri* to mealybugs (Heidari & Copland, 1992), *Chilocorus nigritus* F. to *Abgrallaspis cyanophylli* Signoret in conjunction with the host plant (Ponsonby & Copland, 1995) and *Hippodamia convergens* Guérin-Méneville which responds positively to (E)-b-farnesene, the alarm pheromone released by aphids (Acar *et al.*, 2001). In the case of *I. rondensis*, it is the species-specific sex pheromone of *M. feytaudi* which acts as kairomone for *I. rondensis* larvae. Using these olfactory cues, ladybirds are able to find the prey on a pine tree within a short period of time which greatly reduces their foraging time (Branco *et al.*, 2011).

The results of this study suggest that *M. feytaudi* is the optimal prey for *I. rondensis*: the highest survival of larvae was achieved when fed with egg masses of *M. feytaudi* and in choice tests *M. feytaudi* was consistently preferred over other prey items. These results, together with its restriction to *P. pinaster* forest habitat, lead us to suggest that *I. rondensis* is specialized on *M. feytaudi*. Due to logistic constraints, we were unable to test the full development of *I. rondensis* on *M. josephi* egg masses. However, this scale species does not occur in the natural range of *I. rondensis*. In contrast, it would be interesting to test the possibility of development on *M. pini* since this prey species can be found in the natural habitat of the ladybird.

Rhyzobius, which is considered the ladybird genus closest to *Iberorhyzobius*, appears to be more generalist in terms of prey and habitat. Two *Rhyzobius* species are native to Europe, *Rhyzobius chrysomeloides* Herbst and *Rhyzobius litura* Fabricius; both are polyphagous species feeding on Aphididae and Coccidae, present in several Mediterranean forest ecosystems and arable lands (Ricci, 1986; Toccafondi *et al.*, 1991). Three species are of Australasian origin: *Rhyzobius lophanthae* Blaisdell, *Rhyzobius forestieri* Mulsant, and *Rhyzobius ventralis* Erichson. The third species is monophagous and stenotopic, feeding only on the genus *Eriococcus* (Hemiptera: Coccoidea) and present mostly on *Eucalyptus* species (Pope, 1981). *Rhyzobius forestieri* is oligophagous, feeding on several species in the Coccidae and present on different tree species such as *Casuarina* spp., *Citrus* spp., and *Acacia* spp. (Richards, 1981). *Rhyzobius lophanthae* is polyphagous, feeding on Diaspididae and Pseudococcidae and is present in many different ecosystems, having been introduced in many areas around the world for biological control (Stathas, 2000). Furthermore, in general specificity does not appear to be characteristic of the taxonomic group Coccidulinae, but rather more of the coccidophagous guild: e.g., *Coelophora quadrivittata* Fauvel, *Hyperaspis egregia* Fürsch, *Hyperaspis pantherina* Fürsch, *Rodatus major* Blackburn, *Scymnus mediterraneus* Iablokoff-Khnzorian, and *R. cardinalis* (Chazeau, 1981; Richards, 1985; Ragab, 1995; Ackonor & Mordjifa, 1999; Fowler, 2004). Some exceptions are *Diomus thoracicus* Fabricius which is myrmeco-phagous with a parasitic mode of living (Vantaux *et al.*, 2010), and *Anisolemnia dilatata* Fabricius which feeds on the woolly aphid *Ceratovacuna silvestrii* Takahashi on bamboo plants (Majumder & Agarwala, 2013).

The fact that late instars of *I. rondensis* can feed on other prey is also common to other specialized predators. Even the

successful biological control agent *R. cardinalis* can at least partially develop on the eggs of *P. fuscipennis* in its natural environment (Mendel *et al.*, 1998). The ability of *I. rondensis* to feed and develop on *E. kuehniella* eggs is of interest since it might allow mass rearing under laboratory conditions. Nevertheless, we need to consider that at least for the first instar of *I. rondensis*, *M. feytaudi* egg masses will be needed, as the survival rate was very low in our experiments with *E. kuehniella* eggs.

From an applied perspective, our findings suggest that *I. rondensis* may be an effective and safe biological control agent of *M. feytaudi* due to its high level of specialization at both the dietary and habitat level. In particular, acclimation of *I. rondensis* could be envisaged in recently colonized areas of Corsica and Northern Italy where the invasive scale is still spreading and causing important forest damage. However, further studies are needed to estimate the predation effectiveness of *I. rondensis* before deciding on its introduction.

Acknowledgements

References

Abrams, P.A. & Ginzburg, L.R. (2000) The nature of predation: prey dependent, ratio dependent or neither? *Trends in Ecology and Evolution* **15**, 337–341.

Acar, E.B., Medina, J.C., Lee, M.L. & Booth, G.M. (2001) Olfactory behavior of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). *Canadian Entomologist* **133**, 389–397.

Ackonor, J. & Mordjifa, D. (1999) Parasitism and predation in *Planococcoides njalensis* (Laing) (Homoptera: Pseudococcidae) on cacao in Ghana. *Tropical Agriculture* **76**, 269–274.

Ben-Dov, Y., Miller, D.R., & Gibson, G.A.P. (2001) ScaleNet, Catalogue Query Results. 13 June 2013. Available online at http://www.sel.barc.usda.gov/scalenet/scalenet.htm

Branco, M., Franco, J.C., Dunkelblum, E., Assael, F., Protasov, A., Ofer, D. & Mendel, Z. (2006) A common mode of attraction of larvae and adults of insect predators to the sex pheromone of their prey (Hemiptera: Matsucoccidae). *Bulletin of Entomological Research* **96**, 179–185.

Branco, M., van Halder, I., Franco, J.C., Constantin, R. & Jactel, H. (2011) Prey sex pheromone as kairomone for a new group of predators (Coleoptera: Dasytidae, Aplocnemus spp.) of pine bast scales. *Bulletin of Entomological Research* **101**, 667–674.

Bristow, C.M. (1988) What makes a predator specialize? *Tree* **3**, 1–2.

Burban, C. & Petit, R.J. (2003) Phylogeography of maritime pine inferred with organelle markers having contrasted inheritance. *Molecular Ecology* **12**, 1487–1495.

Burban, C., Petit, R.J., Carreff, E. & Jactel, H. (1999) Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. *Molecular Ecology* **8**, 1593–1602.

Caltagirone, L. & Doutt, R. (1989) The history of the vedalia beetle importation to California and its impact on the development of biological control. *Annual Review of Entomology* **34**, 1–16.

Causton, C.E., Lincango, M.P. & Poulsom, T.G.A. (2004) Feeding range studies of *Rodolia cardinalis* (Mulsant), a candidate biological control agent of *Icerya purchasi* Maskell in the Galapagos islands. *Biological Control* **29**, 315–325.

Chazeau, J. (1981) Biology of *Coelophora quadrivittata* (Col.: Coccinellidae), predator of *Coccus viridis* (Hom.: Coccidae) in New-Caledonia. *Entomophaga* **26**, 301–312.

Covassi, M., Binazzi, A. & Toccafondi, P. (1991) Studies on the entomophagous predators of a scale of the genus Matsucoccus Cock. in Italy. I. Faunistical-ecological notes on species observed in pine forests in Liguria and Tuscany. *Redia* **74**, 575–597.

Diehl, E., Sereda, E., Wolters, V. & Birkhofer, K. (2013) Effects of predator specialization, host plant and climate on biological control of aphids by natural enemies: a meta-analysis. *Journal of Applied Ecology* **50**, 262–270.

Dixon, A.F.G. (2000) *Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control*. p. 288. New York, USA, Cambridge University Press.

Eizaguirre, S. (2004) Revisión de la tribu Coccidulini en la Península Ibérica (Coleoptera: Coccinellidae) *Estudios del Museo de Ciencias Naturales de Álava* **18–19**, 153–170.

EUFORGEN (2009) Distribution map of maritime pine (*Pinus pinaster*). Available online at www.euforgen.org.

Ferran, A. & Dixon, A.F.G. (1993) Foraging behaviour of ladybird larvae (Coleoptera: Coccinellidae). *European Journal of Entomology* **90**, 383–402.

Foldi, A.I. (2004) The Matsucoccidae in the Mediterranean basin with a world list of species (Hemiptera: Sternorrhyncha: Coccoidea). *Annales de la Société entomologique de France* **40**, 145–168.

Fowler, S.V. (2004) Biological control of an exotic scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), saves the endemic gumwood tree, *Commidendrum robustum* (Roxb.) DC. (Asteraceae) on the island of St. Helena. *Biological Control* **29**, 367–374.

Funk, D.J., Filchak, K.E. & Feder, J.L. (2002) Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica* **116**, 251–267.

Heidari, M. & Copland, M. (1992) Host finding by *Cryptolaemus montrouzieri* (Col., Coccinellidae) a predator of mealybugs (Hom., Pseudococcidae). *Entomophaga* **37**, 621–625.

Hodek, I. & Honěk, A. (1996) *Ecology of Coccinellidae*. p. 464. Dordrecht, The Netherlands, Kluwer Academic Publishers.

Hodek, I. & Honěk, A. (2009) Scale insects, mealybugs, whiteflies and psyllids (Hemiptera, Sternorrhyncha) as prey of ladybirds. *Biological Control* **51**, 232–243.

Jackson, R.R., Salm, K. & Nelson, X.J. (2010) Specialized prey selection behavior of two East African assassin bugs, *Scipinnia repax* and *Nagusta* sp. that prey on social jumping spiders. *Journal of Insect Science* **10**, 1–19.

Jactel, H., Ménassieu, P. & Burban, C. (1996) Découverte en Corse de *Matsucoccus feytaudi* Duc (Homoptera: Margarodidae), cochenille du pin maritime. *Annals of Forest Science* **53**, 145–152.

Jałoszyński, P. & Olszanowski, Z. (2013) Specialized feeding of *Euconnus pubicollis* (Coleoptera: Staphylinidae: Scydmaeninae) on oribatid mites: prey preferences and hunting behaviour. *European Journal of Entomology* **110**, 339–353.

Kalushkov, P. & Hodek, I. (2001) New essential aphid prey for *Anatis ocellata* and *Calvia quatuordecimguttata*

(Coleoptera: Coccinellidae). *Biocontrol Science and Technology* **11**, 35–39.

Katsanis, A., Babendreier, D., Nentwig, W. & Kenis, M. (2013) Intraguild predation between the invasive ladybird *Harmonia axyridis* and non-target European coccinellid species. *BioControl* **58**, 73–83.

Koch, R. & Galvan, T. (2008) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. pp. 23–35 in Roy, H. & Wajnberg, E. (*Eds*) *From Biological Control to Invasion: the Ladybird Harmonia axyridis as a Model Species*. Netherlands, Springer.

Majumder, J. & Agarwala, B. (2013) Biology and population dynamics of giant ladybird predator *Anisolemnia dilatata* (F.) (Coleoptera: Coccinellidae): a specialized predator of woolly aphids of bamboo plants in Northeast India. *World Journal of Zoology* **8**, 55–61.

Matsuki, M. & MacLean, S.F. Jr (1994) Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia* **100**, 141–152.

Mendel, Z., Blumberg, D., Zehavi, A. & Weissenberg, M. (1992) Some polyphagous Homoptera gain protection from their natural enemies by feeding on the toxic plants *Spartium junceum* and *Erythrina corallodendrum* (Leguminosae). *Chemoecology* **3**, 118–124.

Mendel, Z., Assael, F., Zeidan, S. & Zehavi, A. (1998) Classical biological control of *Palaeococcus fuscipennis* (Burmeister) (Homoptera: Margarodidae) in Israel. *Biological Control* **12**, 151–157.

Obata, S. (1986) Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* [Coleoptera: Coccinellidae]. *Entomophaga* **31**, 303–311.

Obrycki, J.J. & Kring, T.J. (1998) Predaceous Coccinellidae in biological control. *Annual Review of Entomology* **43**, 295–321.

Pekár, S. (2004) Predatory behavior of two european ant-eating spiders (Araneae, Zodariidae). *Journal of Arachnology* **32**, 31–41.

Ponsonby, D. & Copland, M. (1995) Olfactory responses by the scale insect predator *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae). *Biocontrol Science and Technology* **5**, 83–94.

Pope, R.D. (1981) '*Rhyzobius ventralis*' (Coleoptera: Coccinellidae), its constituent species, and their taxonomy and historical roles in biological control. *Bulletin of Entomological Research* **71**, 19–32.

Ragab, M.E. (1995) Adaptation of *Rodolia cardinalis* (Mulsant) (Col., Coccinellidae) to *Icerya aegyptiaca* (Douglas) (Hom., Margarodidae) as compared with *Icerya purchasi* Mask. *Journal of Applied Entomology* **119**, 621–623.

Raimundo, A., Canepari, C., Mendel, Z., Branco, M. & Franco, C. (2006) *Iberorhyzobius* Raimundo & Canepari gen. nov., for *Coccidula rondensis* Eizaguirre (Coleoptera: Coccinellidae). *Zootaxa* **1312**, 49–58.

Ricci, C. (1986) Seasonal food preferences and behavior of *Rhyzobius litura*. pp. 119–123 in Hodek, I. (*Ed.*) *Ecology of Aphidophaga*. Dordrecht, The Netherlands, Academia, Prague & Dr. W.Junk.

Richards, A.M. (1981) *Rhyzobius ventralis* (Erichson) and *R. forestieri* (Mulsant) (Coleoptera: Coccinellidae), their biology and value for scale insect control. *Bulletin of Entomological Research* **71**, 33–46.

Richards, A.M. (1985) Biology and defensive adaptations in *Rodatus major* (Coleoptera: Coccinellidae) and its prey, *Monophlebulus pilosior* (Hemiptera: Margarodidae). *Journal of Zoology* **205**, 287–295.

Riom, J. & Gerbinot, B. (1977) Étude biologique et écologique de la cochenille du pin maritime *Matsucoccus feytaudi* Ducasse, 1942 (Coccoidea, Margarodidae, Xylococcinae) dans le Sud-Est de la France. 1. Biologie générale et phénologie. *Annales de Zoologie Ecologie Animale* **9**, 11–50.

Scriber, J.M. (2010) Integrating ancient patterns and current dynamics of insect–plant interactions: taxonomic and geographic variation in herbivore specialization. *Insect Science* **17**, 471–507.

Short, B.D. & Bergh, J.C. (2004) Feeding and egg distribution studies of *Heringia calcarata* (Diptera: Syrphidae), a specialized predator of woolly apple aphid (Homoptera: Eriosomatidae) in Virginia Apple Orchards. *Journal of Economic Entomology* **97**, 813–819.

Sloggett, J.J. & Majerus, M.E.N. (2000) Habitat preferences and diet in the predatory Coccinellidae (Coleoptera): an evolutionary perspective. *Biological Journal of the Linnean Society* **70**, 63–88.

Stathas, G.J. (2000) *Rhyzobius lophanthae* prey consumption and fecundity. *Phytoparasitica* **28**, 203–211.

Strand, M.R. & Obrycki, J.J. (1996) Host specificity of insect parasitoids and predators. *BioScience* **46**, 422–429.

Thompson, A.J.J.N. (1995) Trade-offs and the evolution of host specialization. *Evolutionary Ecology* **9**, 82–92.

Toccafondi, P., Covassi, M. & Pennacchio, F. (1991) Studies on the entomophagous predators of scale insects of the genus Matsucoccus Cock. in Italy. II Bio-ethological notes on *Rhyzobius chrysomeloides* (Herbst) in pine forests of Liguria (Coleoptera: Coccinellidae). *Redia* **74**, 599–620.

Van Lenteren, J., Bale, J., Bigler, F., Hokkanen, H. & Loomans, A. (2006) Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of. Entomology* **51**, 609–634.

Vantaux, A., Roux, O., Magro, A., Ghomsi, N.T., Gordon, R.D., Dejean, A. & Orivel, J. (2010) Host-Specific Myrmecophily and Myrmecophagy in the Tropical Coccinellid *Diomus thoracicus* in French Guiana. *Biotropica* **42**, 622–629.

Vieira, L., Salom, S., Montgomery, M. & Kok, L. (2013) Field-cage evaluation of the survival, feeding and reproduction of *Laricobius osakensis* (Coleoptera: Derodontidae), a predator of *Adelges tsugae* (Hemiptera: Adelgidae). *Biological Control* **66**, 195–203.

Wiegmann, B., Mitter, C. & Farrell, B. (1996) Diversification of carnivorous parasitic insects: extraordinary radiation or specialized dead end? *American Naturalist* **142**, 737–754.