Overwintering habitat and the survival of the biocontrol beetle, *Cassida rubiginosa*

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Summary   The leaf-feeding beetle, *Cassida rubiginosa* Müller, was introduced to New Zealand in 2007 as a biological control agent against the pasture weed, *Cirsium arvense* (L.) Scop. (Californian thistle). The beetle is now established in most regions, but outbreak populations have only been observed in one region (Wairarapa). The overwintering habitat type might affect the survival of the beetle and its ability to achieve outbreak populations. We tested the overwintering survival of *C. rubiginosa* in three different habitats: radiata pine forest, native forest, and pasture. In autumn 2016, adult diapausing *C. rubiginosa* were placed in overwintering cages established in the three habitats, and in spring their survival was assessed. Temperature and relative humidity was recorded in each habitat, and predatory spiders were also sampled. The total recovery rate of overwintered beetles was greatest in the native forest (42 ± 7.9%), followed by ungrazed pasture (32.5 ± 7.5%), and pine forest (7.5 ± 4.2%). The total percentage recovered was not significantly different between the native forest and pasture habitats, but both of these habitats had greater recovery rates than the pine habitat. The native forest provided the best insulation against temperature and humidity extremes on a daily basis, and across the winter season, and is the likely explanation for the greater recovery rates in that habitat. This study has provided initial evidence that the particular overwintering habitat may contribute to the success of this biocontrol agent, and suggests that native forest is likely to provide a better habitat than pine forest.

Keywords   Green thistle beetle, Californian thistle, biological control.

INTRODUCTION

Cassida rubiginosa Müller (Coleoptera: Chrysomelidae) is a leaf-feeding beetle of European origin that was introduced to New Zealand in 2007 as a biological control agent against *Cirsium arvense* (L.) Scop. (Californian thistle) (Cripps *et al.* 2011). Under controlled experimental conditions the beetle has been shown to reduce the density, size, and biomass of the weed, indicating that it has potential as a biocontrol agent (Cripps *et al.* 2010). However, in the field it seldom reaches population densities capable of exerting a regulating influence on its host plant. In Europe, and in North America (where the beetle was inadvertently introduced prior to 1901), this is primarily attributed to high rates of predation and parasitism (Ang and Kok 1995). However, in the novel ecosystem of New Zealand that has no native thistle species the beetle apparently experiences a high degree of enemy-free space, which allows for population outbreaks (Cripps 2013).

The beetle is now established in most regions of New Zealand, although its abundance is variable ranging from low, but persistent populations, to outbreak populations of hundreds of individuals per thistle shoot (M. Cripps, pers. obs.). One possible explanation for the variable abundance is the suitability of overwintering habitat. In the native range, the beetle is known to migrate from meadows, where it feeds on thistle species, to nearby broadleaf forests where it overwinters under leaf litter, which is thought to better insulate against environmental extremes (Kosior and Klein 1970). Life table studies in the native range have indicated that the overwintering survival rate of *C. rubignosa* is between 16 and 34%, and is a severe bottleneck in its population increase, although the causes for the low survival rate are unknown (e.g. predation, or environmental factors) (Koji *et al.* 2012). Therefore, understanding the overwintering conditions that enhance the survival of this beetle might help promote its effectiveness as a biocontrol agent. To this end, we tested the overwintering success of *C. rubignosa* in three different habitats typical of New Zealand agricultural landscapes: native forest, high-producing perennial pasture, and planted radiata pine forest.

MATERIALS AND METHODS

In autumn 2016 (18 April), 120 overwintering adult beetles were collected from two field cages located at the Lincoln campus of AgResearch (43°38′21″ S; 172°28′28″ E) and transported to the trial location at Tangihanga Station, near Gisborne (38°33′11″ S;

177°52′59″ E). The native forest was estimated to have 80% canopy cover and was composed of *Pittosporum eugenioides* A.Cunn. (lemonwood), *Alectryon excelsus* Gaertn. (tītoki), *Corynocarpus laevigatus* J.R.Forst. et G.Forst. (karaka), *Piper excelsum* G.Forst. (kawakawa), and *Melicytus ramiflorus* J.R.Forst. et G.Forst. (māhoe). The ground cover was a layer of leaf litter (4–6 cm deep) with no live vegetation. The pasture was completely open (i.e. no shading), and had 100% ground cover that was predominately composed of *Lolium perenne* L. (perennial ryegrass), with a small amount of *Agrostis capillaris* L. (browntop) and a *Crepis* sp. (hawksbeard). The pine forest was a mature plantation of *Pinus radiata* D.Don, visually assessed to have approximately 70% canopy cover. The ground cover was a layer of pine needle litter (2–3 cm deep), and the New Zealand native grass, *Microlaena stipoides* (Labill.) R.Br. (ca. 10% ground cover).

On 20 April, four replicate overwintering cages were established at each of the three habitats. The cages were made of Lumite® mesh material (BioQuip, USA) dug into the ground around the edges and supported by wire struts. The established cages were 50 × 90 cm, and approximately 30 cm high. A small rosette of *Cirsium vulgare* (Savi) Ten. (ca. 10 to 15 cm diameter) was collected from a nearby paddock and transplanted into each of the cages. This was planted as a food source for the beetle since in late winter to early spring the beetle enters a post-diapause quiescent state, and occasionally feeds when weather conditions are favourable (M. Cripps, pers. obs.). Ten adult beetles were placed inside each cage (40 individuals total per habitat). A data logger (Digitech QP-6013) contained inside a protective weather shield was placed inside each cage. Data loggers were placed on the ground surface to record temperature and relative humidity hourly. On 4 October 2016 the cages were examined for the presence of *C. rubignosa* first by physically searching through the vegetation and litter material in the caged area. Second, the caged area was sampled with a battery-powered vacuum (14.4V wet–dry Hoover Handivac) for any additional *C. rubiginosa* individuals. Potential predators were sampled (ca. 50 cm perimeter from the cage edge with a leaf-blower vacuum) in autumn at the time of cage set up, and again in spring at the time of assessment. Since nearly all predators collected were spiders we focused on identifying this group.

Unfortunately, the rosettes of *C. vulgare* in the forest habitats did not survive until spring, likely as a result of insufficient sunlight. The lack of a food source appeared to have resulted in the death of some beetles. Therefore, the recovery of beetles were grouped into two categories, 'alive' (active beetles), and 'recently dead', which were beetles that were dead or moribund (inactive), but still with normal colouration, and no indication of decay, such as fungal growth.

The relationship of the adult *C. rubignosa* recovery rate with each environmental variable (relative humidity (RH) and temperature) was analysed using a generalised linear model (GLM) with a binomial distribution through a logit-link function. Statistically significant relationships were assessed with the Akaike Information Criterion (AIC) in order to determine the relationship that had the closest fit to the data (smaller AIC values indicate a closer fit to the data.). Once the relationship with the closest fit to the data was determined, another variable was added, and assessed if it significantly improved the relationship. The assessment of these improvements was made using analysis of deviance. The environmental extreme variables included in the analyses were the time above 10°C and time below 70% RH since 10°C is the predicted developmental threshold for *C. rubignosa* (Ward and Pienkowski 1978), and since <10% of RH observations were below 70%.

RESULTS

The mean (±SE) percent recovery rate of alive beetles was greatest in the pasture (22.5 ± 6.7%), followed by native forest (10.0 ± 4.8%), and the pine forest (0%). However, the total recovery rate (alive plus recently dead) was greatest in the native forest (42 ± 7.9%), followed by pasture (32.5 ± 7.5%), and pine forest (7.5 ± 4.2%). The percentage of alive beetles recovered was not significantly different between the native and pasture habitats, but alive recovery in both of these habitats were significantly greater than the pine habitat (P=0.002). Similarly, the total percentage recovered was not different between the native and pasture habitats, but both of these habitats had greater recovery rates than the pine habitat (P=0.001 and 0.010, respectively). The recovery rate of recently dead beetles was significantly greater in the native habitat than either the pasture or pine habitats (P=0.019 and 0.010, respectively).

The total recovery rate of *C. rubignosa* decreased with increasing temperature and decreasing RH. The best models (AIC values <130) explaining *C. rubignosa* recovery rates were those based on:

1. maximum daily RH range; and
2. number of hours when temperature >1°C and RH <70%.

All of these models showed a negative relationship with the total recovery rate (Figure 1, P<0.001).

A total of ten different spider species were found, half of them introduced species (Table 1). In the native forest, three of the five spider species found were
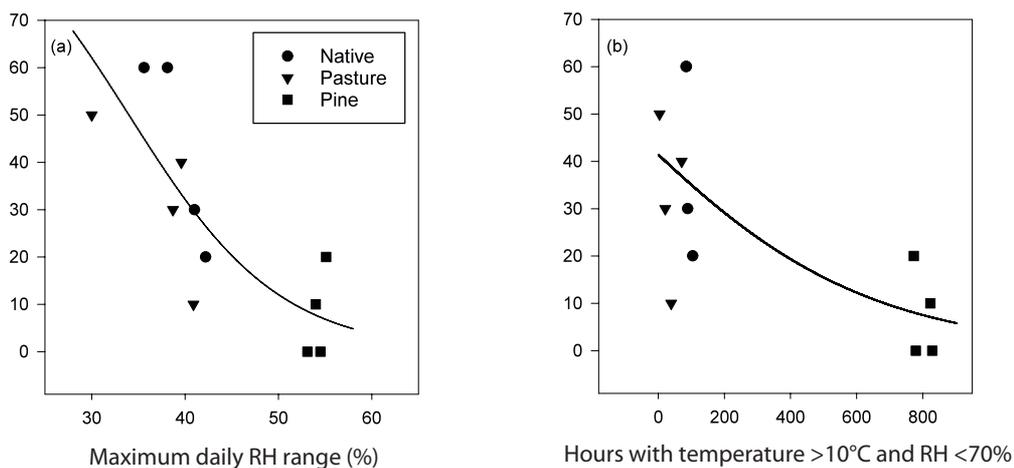
Figure 1. The two best models (AIC values <130) predicting the total recovery rate of overwintered *Cassida rubiginosa* from three different test habitats (native forest, pasture, or pine forest). The best predictors of recovery rate (%) from temperature and relative humidity (RH) recorded inside the cage over the study period (20 April to 4 October 2016) in order of the closest fit to the data are:

(a)   maximum daily RH range: $Y = 100/(1 + \exp(-4.2181 + 0.1241*X))$; and

(b)   hours with temperature >10°C and RH <70%: $Y = 100/(1 + \exp(0.3505 + 0.0027*X))$.

endemic, and comprised 68% of the individuals. In contrast, seven spider species were found in the pasture of which five were introduced species that comprised 80% of the individuals. The pine forest was depauperate in both species and abundance with only an individual each of two species collected. The introduced linyphiid, *Tenuiphantes tenuis* Blackw., was the only species common to all three habitats (Table 1).

DISCUSSION

Many insects complete diapause in mid-winter, but remain in a quiescent state until temperatures warm before becoming active (Danks 1991, MacMillan and Sinclair 2011). The quiescent state can be easily departed and returned to and it is possible for the overwintering beetle to temporarily become active and feed in small amounts before quickly returning to an inactive state when the temperature cools. The total recovery rate of beetles was influenced most strongly by temperature and RH extremes (time above 10°C and below 70% RH), and the native forest habitat provided the best insulation against these extremes on a daily basis, and across the winter season. As such, it is likely that the native forest habitat was more conducive for maintaining a quiescent state and therefore the beetle could survive longer there even without a food source. Leaf litter was also shown to be the preferred overwintering substrate in a multi-choice experiment (Spring and Kok 1999), conferring with the

Table 1. Spider species collected from vacuum samples taken in the three overwintering test habitats (native forest, pine forest and pasture) for *Cassida rubiginosa*. The total number of each spider species (#) and its endemic (E) or introduced (I) status to New Zealand are indicated.

Habitat	Species	#
Native forest	*Anoteropsis hilaris* (L. Koch) (E)	1
	Dunedinia denticulate Mill. (E)	2
	Rinawa pula Forster (E)	1
	Steatoda capensis Hann (I)	5
	Tenuiphantes tenuis (Blackw.) (I)	1
Pasture	*Anoteropsis hilaris* (L. Koch) (E)	2
	Cryptachaea blattea (Urquh.) (I)	1
	Erigone wiltoni Locket (I)	2
	Mermessus fradeorum (Berl.) (I)	1
	Steatoda capensis Hann (I)	2
	Tenuiphantes tenuis (Blackw.) (I)	1
	Theridiidae sp. (E)	2
Pine forest	*Tenuiphantes tenuis* (Blackw.) (I)	1
	Desidae sp. (E)	1

observed natural overwintering habitat, and the greater survival rates under leaf litter in native New Zealand forest found in our study. While the pasture habitat did not have a significantly different recovery rate of *C. rubignosa* compared to the native forest it is important to note that this was ungrazed pasture. At the time of cage establishment in the pasture habitat, the sward had been recently grazed to 3–4 cm, and by the time of assessment the sward had grown to approximately 30 cm in height. If the pasture had continued to be grazed it is likely that the recovery rate of *C. rubignosa* would have been reduced by this disturbance, but nevertheless this suggests that ungrazed habitats could also be suitable for overwintering. In contrast, the pine forest was the worst habitat for the overwintering of *C. rubiginosa*, and had the most extreme temperature and RH conditions (Figure 1).

To date, no predators of *C. rubiginosa* have been documented in New Zealand, although generalist predators such as spiders are certain to consume some, and have been observed feeding on adult *C. rubiginosa* in rearing cages and field trial sites (M. Cripps, pers. obs.). Pasture boundary habitats can influence the species composition and abundance of spiders in New Zealand and therefore could affect the overwintering survival of *C. rubiginosa* in different habitats (Bowie *et al.* 2014). However, the overwintering success of *C. rubiginosa* in our study did not appear to be influenced by spider predators, regardless of habitat.

This study provides initial evidence that the particular overwintering habitat available locally to *C. rubignosa* may contribute to the success of this biocontrol agent, and suggests that native forest with a leaf litter layer is likely to provide a better habitat than pine forest. Overall, the results suggest that the overwintering conditions for *C. rubignosa* are improved when insulated against extreme flucuating temperature and humidity. However, in order to generalise across New Zealand it would be necessary to repeat this experiment with increased replication in space and time with a greater number of beetles. Future studies should also include winter surveys for overwintering beetles in different habitats.

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