

# Plant phylogeny determines host selection and acceptance of the oligophagous leaf beetle *Cassida rubiginosa*

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## Abstract

**BACKGROUND:** Predicting the host range of biocontrol agents is important for the safe and effective implementation of biocontrol of weeds. In this study, we examined the phylogenetic pattern of host selection and acceptance by the biocontrol beetle, *Cassida rubiginosa*. The beetle was released in New Zealand for control of *Cirsium arvense*, its primary host plant, but has potential to attack many Cardueae (thistles and knapweeds) species. We conducted a series of no-choice and choice experiments and modelled the responses of *Cassida rubiginosa* in relation to phylogenetic distance from *Cirsium arvense*.

**RESULTS:** The olfactory recognition (single odour) and preference (two odours) of the beetle showed a significant phylogenetic relationship. These relationships showed a high degree of correlation with 66.9% of the variation in olfactory recognition and 82.8% of the variation in olfactory preference explained by phylogeny. Where the beetle could contact plants, under no-choice conditions there was no phylogenetic pattern to host plant acceptance. However, under choice conditions, phylogenetic distance was a strong predictor of feeding and oviposition preference. These relationships showed a high degree of correlation, with 63.4% of the variation in feeding preference, and 89.0% of the variation in oviposition preference, explained by phylogeny.

**CONCLUSIONS:** As far as we are aware, this is the first demonstration of an herbivorous insect that exhibits a phylogenetic pattern to olfactory host plant selection. Host plant utilisation by *Cassida rubiginosa* in New Zealand will be mostly restricted to *Cirsium* and *Carduus* species, with minimal potential for impact on other Cardueae weeds.

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**Keywords:** host specificity; host range; spill-over; evolution of specialisation

## 1 INTRODUCTION

Most herbivorous beetles are specialised on a phylogenetically defined clade of plants, and the degree of specialisation can be described taxonomically, such as the host range being restricted to a family, tribe, or genus of plants. The associations between herbivorous beetles and their host plants are remarkably constant over evolutionary time, indicating that host range is phylogenetically constrained.<sup>1–3</sup> Major host shifts (e.g., to different plant families) in ecological time are rare, and contemporary evolution is limited within defined phylogenetic bounds. This broad constraint on host ranges has been exploited for classical biological control of weeds, since the host ranges of biocontrol agents can be adequately defined to ensure safety to unrelated plants.<sup>4–6</sup> In practice, the host range of biocontrol agents is determined through a series of no-choice and choice tests. No-choice tests are crucial for determining the fundamental host range of biocontrol agents (i.e., the set of all possible plant species on which the agent can complete development).<sup>7</sup> The fundamental host range typically defines the phylogenetic limits of host range, but seldom reflects the realised, or field host range, expressed by biocontrol agents.<sup>8,9</sup> Choice tests

can reveal preference hierarchies and better predict the realised host range, which is important for assessing risk to non-target species, and in some cases may be useful to assess the extent that biocontrol agents might control multiple related weed targets.

Host specificity testing is usually carried out using the 'centrifugal phylogenetic' method with a set of test plants that span from

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closely related to progressively more distantly related species to the target weed.<sup>10</sup> The assumption is that phylogenetic relatedness can provide a composite measure of trait similarity, and that host plant utilisation will decrease with increasing phylogenetic distance from the target weed. However, until recently, phylogenetic data was often limited, or unknown, and taxonomy was used as a close surrogate.<sup>11</sup> Nevertheless, the method has proved reliable, with very few weed biocontrol agents reported attacking non-target plants and even fewer (< 1%) that have caused long-term impacts to non-target plant populations.<sup>12</sup> Where non-target attack has occurred, in most cases it was predictable, since attack occurred on related plants in the same genus or family.<sup>13,14</sup> With increased availability of molecular phylogenies, the underlying assumption that phylogenetic relatedness can predict host range can be explored. To date, evidence is mixed on the usefulness of phylogenetic relatedness at lower taxonomic levels (within plant families) to predict the host range of specialised herbivores.<sup>15</sup> In some cases, phylogenetic distance is a strong predictor of host range<sup>16–18</sup>; however, in other cases, key traits that are independent of phylogeny, most notably chemical similarities, better predict host range.<sup>19–21</sup>

The phylogenetic pattern to host range might also depend on the type of host specificity test conducted and the responses measured. Most host specificity testing for biocontrol purposes only include experiments where biocontrol agents can contact the test plant. However, the behavioural responses of biocontrol agents can vary at the different stages of host selection.<sup>22,23</sup> The process by which herbivorous insects find, and ultimately accept, a host, is governed by a complex series of behaviours involving olfactory, visual, tactile, and gustatory senses that respond to both positive (attractant) and negative (repellent) plant stimuli.<sup>24,25</sup> The process can be broadly separated into pre-contact and post-contact stages. In the pre-contact stage, host selection involves long-range orientation towards possible hosts, and then short-range olfactory detection and discrimination. In the post-contact stage, tactile and gustatory cues are important for accepting a host plant for sustained feeding and oviposition.

The genetic control of the different host selection stages is not well understood,<sup>26</sup> but evidence indicates that the behavioural responses prior to contacting a plant compared to after contacting a plant are genetically independent.<sup>27</sup> Furthermore, host acceptance and performance can also differ between the larval and adult stages of holometabolous insects, although the general correlation between the larval and adult host ranges indicates strong selection towards a common host range that maximises fitness for both growth stages.<sup>28–30</sup> Therefore, including pre-contact tests, particularly with the adult stage, that assess host finding and recognition mechanisms, may help to better describe the host use patterns of biocontrol agents and explain some discrepancies between fundamental host ranges and realised field host ranges.

Previously, we examined the phylogenetic pattern to the host range of the larval stage of the oligophagous biocontrol beetle, *Cassida rubiginosa*.<sup>18</sup> The fundamental host range of the beetle is known to be restricted to the Cardueae tribe of Asteraceae, but the realised host range in Europe (native range) is much narrower, where it is mostly found on *Cirsium* and *Carduus* species.<sup>31</sup>

The beetle was released in New Zealand in 2007 primarily to control the thistle weed, *Cirsium arvense*, although it was recognised that it had the potential to control multiple thistle weeds in the Cardueae tribe.<sup>32,33</sup> The phylogenetic pattern to the host range was examined by testing the survival of *Cassida rubiginosa* larvae on a set of 16 Cardueae species. There was a strong phylogenetic signal to larval survival, whereby survival decreased on plant species

more distantly related to the beetle's primary field host, *Cirsium arvense*. However, the model for survival was improved when specific leaf area, a physical leaf trait independent of phylogeny, was included. These data indicated that specialisation of *Cassida rubiginosa* on *Cirsium arvense* has likely arisen through a combination of the beetle tracking phylogenetically conserved traits and responding to fitness trade-offs on hosts resulting from different physical defensive leaf traits (trichomes and specific leaf area).<sup>18,34</sup>

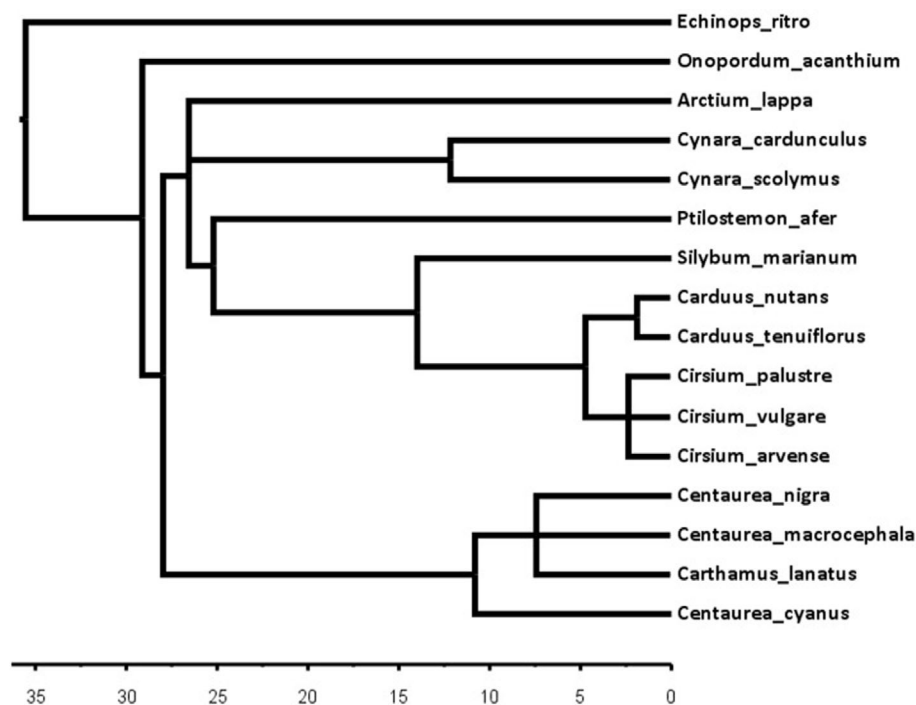
In the present study, we further describe the host range of *Cassida rubiginosa* through a series of experiments with the adult beetle. We used the same set of 16 Cardueae species to test the host plant finding and acceptance of the adult *Cassida rubiginosa* through a series of no-choice and choice experiments. Initially, we tested the olfactory recognition (single choice tests) and preference (dual choice tests) of the beetle, and then we tested the acceptance (no-choice tests) and preference (dual choice tests) of the beetle through feeding and oviposition experiments. We modelled the responses of *Cassida rubiginosa* in each of the no-choice and choice tests in relation to phylogenetic distance from its primary host, *Cirsium arvense*. We hypothesised that the correlation between beetle response (olfactory, feeding, and oviposition) and phylogenetic distance would strengthen where preference behaviours could be expressed. Specifically, we predicted (1) stronger correlations for feeding and oviposition responses compared to olfactory responses, and (2) stronger correlations for choice tests compared to no-choice tests.

## 2 MATERIALS AND METHODS

### 2.1 Study system

#### 2.1.1 Plant species

The Cardueae tribe is one of the largest in the family Asteraceae, comprised of five subtribes (Cardopatiinae, Carlininae, Echinopsinae, Carduinae, and Centaureinae), 72 genera, and approximately 2400 species.<sup>35</sup> The Cardueae are a monophyletic tribe that originated during the mid-Eocene, with subtribal diversification throughout the Oligocene – Miocene period.<sup>36</sup> For this study, 16 Cardueae species were selected: *Cirsium arvense* (L.) Scop., *Cirsium vulgare* (Savi) Ten., *Cirsium palustre* (L.) Scop., *Ptilostemon afer* (Jacq.) Greuter., *Carduus nutans* L., *Silybum marianum* (L.) Gaertn., *Carduus tenuiflorus* Curt., *Centaurea cyanus* L., *Centaurea macrocephala* Puschk., *Centaurea nigra* L., *Cynara cardunculus* L., *Cynara scolymus* L., *Onopordum acanthium* L., *Carthamus lanatus* L., *Arctium lappa* L., and *Echinops ritro* L. These species provide a good representation of the tribe, as they include species from three (Carduinae, Echinopsinae and Centaureinae) of the five subtribes and contain geographically widespread species-rich genera (e.g., *Cirsium*, *Centaurea*) as well as narrowly distributed species-poor genera (e.g., *Ptilostemon*, *Cynara*). The phylogeny of the 16 Cardueae test species was pruned from a comprehensive phylogeny of the Cardueae tribe based on nuclear ribosomal DNA and chloroplast DNA markers<sup>36</sup> (Fig. 1). The phylogenetic distances (in millions of years) were previously reported by Cripps *et al.* and calculated from the total branch lengths separating each species from *Cirsium arvense*. All the test species used in this study were either deliberately or inadvertently introduced species to New Zealand.<sup>33</sup> Three non-Cardueae species (*Taraxacum officinale*, *Inula hookeri*, and *Tragopogon porrifolius*) were also included based on the host range of Palearctic congeners of *Cassida rubiginosa*.<sup>37</sup> If genetic variation exists for host plant utilisation outside the Cardueae, then non-Cardueae plants used by related *Cassida* species are good candidates.<sup>38–40</sup>



**Figure 1.** Chronogram of the 16 Cardueae test plant species pruned from a comprehensive phylogeny of the tribe.<sup>36</sup> Branch length depicts phylogenetic distance in millions of years. This figure is reproduced from Cripps *et al.*<sup>18</sup> under a Creative Commons Attribution licence (CC BY 4.0).

### 2.1.2 *Cassida rubiginosa*

The tortoise beetle, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae), is an oligophagous feeder, specialised on host plants belonging to the tribe Cardueae.<sup>31</sup> Its primary host, *Cirsium arvense*, is a noxious weed found throughout temperate regions of the world.<sup>41</sup> The beetle is native to the Palearctic region and was deliberately introduced to New Zealand in 2007 as a biocontrol agent against *Cirsium arvense*, although it was recognised that it had potential to also control other thistle weeds.<sup>32,42</sup> The beetle is univoltine and both the adult and larvae are leaf-feeders. Adults overwinter under plant debris, in hedgerows or forest margins.<sup>43</sup> The adult undergoes an obligate diapause during winter before emerging in spring to seek host plants upon which they feed and deposit their egg masses (oothecae), mostly on the underside of the leaves.<sup>44</sup> The life cycle consists of five larval instars that are mostly confined to the plant where their eggs are laid, since they cannot move long distances to another host plant.<sup>45</sup>

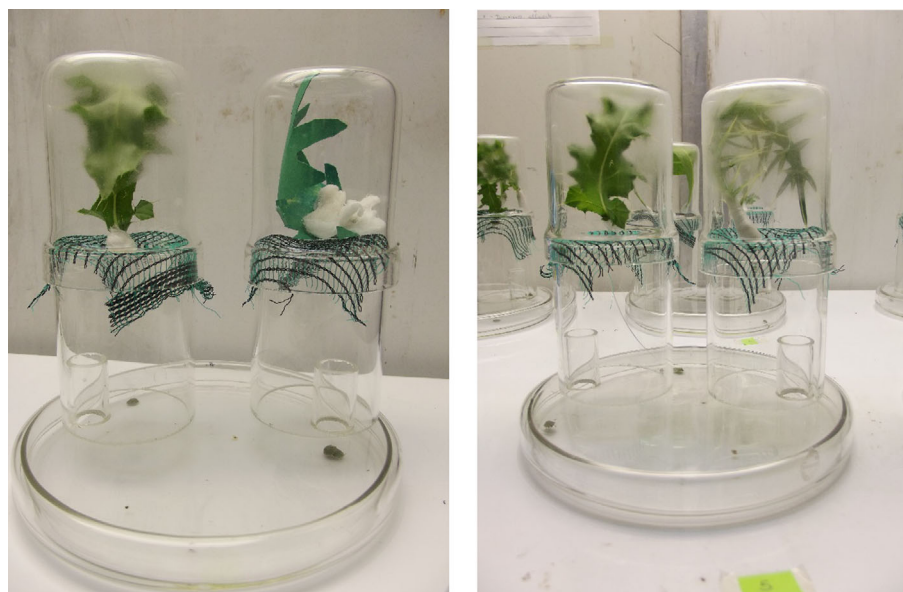
The beetles for this study were field collected in October (spring) from successful biocontrol release sites in the Wairarapa region, New Zealand. The beetles were maintained in 2 L ventilated plastic boxes (*c.* 40 beetles per box) kept inside a climate-controlled chamber (20 °C, 16 h:8 h light/dark photoperiod) and fed with *Cirsium arvense* shoot clippings. There are no key morphological features distinguishing the sexes of *Cassida rubiginosa* but there are significant weight differences between males and females. Ward and Pienkowski<sup>46</sup> reported the mean weight of males as 17.6 mg (range: 13.2–20.4 mg), and females as 24.4 mg (range: 20.5–29.0 mg). Based on this, beetles in this study were selected as males if they were ≤ 19 mg, and females if they were > 19 mg.

### 2.2 Host plant finding: olfactometer experiments

Single choice and dual choice olfactometer experiments were conducted to determine if there was a relationship between

olfactory response and phylogeny of the Cardueae plants. A glass still-air olfactometer was modified from that of Van Tol *et al.*<sup>47</sup> and the experimental protocol adapted to the behaviour of *Cassida rubiginosa* (Fig. 2). This type of olfactometer was chosen because *Cassida rubiginosa* adults generally showed slow response times (*i.e.*, hours). Furthermore, this simple olfactometer apparatus was easily replicated to allow for testing a large number of plant species at the same time. The olfactometer consisted of a large glass Petri dish (140 mm diameter) with two small circular openings (13 mm diameter) that were 82 mm apart. Attached to each opening was a short tube (26 mm long, 13 mm diameter) which led into a cylindrical holding chamber (69 mm long × 52 mm diameter). Above each holding chamber was a glass cup (75 mm long, 59 mm diameter) that held the plant material. The holding chamber and the glass cup were separated by a fine green mesh barrier (1 mm × 1 mm) that prevented the beetle from contacting the plant material and minimised visual cues. The base Petri dish became the ‘test arena’ where adult beetles were placed at the beginning of each experiment. The beetle’s choices were recorded based on the number of beetles found in the holding chambers. All olfactometer tests were conducted between 10.00 and 16.00 h. The tests were conducted in a climate chamber at 23 °C from November 2015 to February 2016 (southern hemisphere spring to summer). The humidity inside the olfactometer was maintained at 55–60% relative humidity (RH) to avoid responses to humidity.<sup>48</sup>

The first experiment consisted of a set of single choice tests, where female beetles were exposed to the odour of a leaf from a single plant species, in comparison to a control with no plant material. Only female beetles were used, since they must choose the oviposition sites, and are often more responsive than males to olfactory cues.<sup>49,50</sup> Leaves of similar age (second or third youngest fully expanded leaf) were cut from the petiole of each test species. The leaf petioles were immediately covered with damp



**Figure 2.** Glass still-air olfactometer. Single choice test (single odour source) where a plant leaf was compared with a mimic (paper leaf and moist cotton ball) (Left). Dual choice test (two odour sources) where *Cirsium arvense* was compared with another test plant (Right).

cotton wool to prevent wilting and limit the emission of green leaf volatiles at the wound site. A leaf of each test species (16 Cardueae and three non-Cardueae species) was placed inside one of the glass cups of the olfactometer. The control cup contained damp cotton wool to maintain the same level humidity as in the other cup and a green paper leaf (hereafter 'paper mimic') (Fig. 2). Three adult female beetles were placed into the test arena of an olfactometer and given 6 h to make a choice, after which the number of beetles in each of the holding chambers was recorded. Any beetles that remained in the test arena were counted as non-responding and omitted from analyses. Ten replicates were conducted for each plant species (total of 570 adult female beetles tested). The leaf cup of the olfactometer was randomly selected for each test, and all beetles were tested only once. A dual choice olfactometer experiment was carried out as described earlier, except that female beetles were given a choice between *Cirsium arvense* (control) and one of the other 18 test plant species (15 Cardueae and three non-Cardueae species, and a total of 540 female beetles tested). The olfactometers were washed with water, distilled water, acetone (100%) and hexane (99.5%) prior to each replicate in order to make sure that they were void of impurities and odour.

### 2.3 Host plant acceptance: feeding and oviposition experiments

#### 2.3.1 No-choice experiment

A no-choice experiment was conducted in spring 2015 at AgResearch, Lincoln, New Zealand, in an outdoor enclosed compound (S 43° 38' 20.54"; E 172° 28' 28.2"). This experiment consisted of the 16 Cardueae species plus the three non-Cardueae species. All the plants were grown from seeds, either collected from the field or purchased from a commercial supplier (Kings Seeds NZ Ltd, Katikati, New Zealand). Seeds were sown 15 June 2015 (for biennial and perennial species) and 2 September 2015 (for annual species). Seedlings were grown in a glasshouse at AgResearch and then transplanted into 12 L plastic pots and shifted to the outdoor compound in October 2015 (spring). All plants were grown in a

standard potting mix (54% aged bark, 45% sand, 1% nutrients, by weight) containing added nutrients of Osmocote® 17–11–10 (N-P-K), lime, superphosphate, sulphate of potash, and calcium nitrate.

The 19 plant species were arranged in a randomised complete block design with four replicates. Individual plants were covered with a polyester mesh bag (50 cm × 125 cm) with two wire struts, holding the bag off the plant. Four adult beetles (two females and two males) were released on to the soil of each potted plant on 6 November 2015. At the time of beetle release, plants in the experiment were either large rosettes (biennial species) or bolting (annual species). Plants were inspected for the number of feeding holes and egg masses at six and 14 days (egg masses only) after release of the beetles. All egg masses found were collected and maintained inside Petri dishes on moist filter paper at constant 20 °C for 15 days, after which time no more larvae emerged. The mean number of larvae that emerged from the egg masses was recorded for each plant species.

#### 2.3.2 Dual-choice experiment

A dual choice experiment was conducted in spring 2016 on the campus of AgResearch, inside a shadehouse. As in the no-choice experiment, all the plants were grown from seed, sown on 9 August 2016 (biennial and perennial species) and 6 September 2016 (annual species). The same set of 16 Cardueae species plus the three non-Cardueae species were grown. Seedlings were grown in a glasshouse until October 2016, when they were transplanted into 5 L plastic pots containing the standard potting mix and shifted to the shadehouse. For the experiment, a potted *Cirsium arvense* plant and one of the other 18 test plant species were placed 15 cm apart inside rearing cages (60 cm length × 60 cm width × 60 cm height; Bugdorm, Meview Science Ltd, Taiwan). Two female and two male beetles were released between the pots in the centre of the cage. The number of feeding holes and egg masses on each plant species were recorded after 3 days. Egg masses were collected and incubated inside Petri dishes on moist filter paper at 20 °C for 15 days, after which

no more larvae emerged. The choice test was repeated ten times, each replicate with new plants and beetles. The first replicate was initiated on 18 November 2016, and subsequent replicates were conducted every 3 days until the final tenth replicate on 21 December 2016.

## 2.4 Statistical analyses

Preference indices (PIs) were calculated based on the number of beetles recorded in the olfactometer holding chambers. The PIs were calculated as:  $PI = (\text{beetles in plant chamber} - \text{beetles in control chamber}) / \text{total number of beetles}$ . Similarly, for the dual choice tests, the PI was calculated as:  $PI = (\text{beetles in test plant chamber} - \text{beetles in } \textit{Cirsium arvense} \text{ chamber}) / \text{total number of beetles}$ . Then, the relationship of the PI with phylogenetic distance from *Cirsium arvense* was modelled with non-linear regression, using a logistic equation:  $PI = 1 - 2 / (1 + \exp(a * \text{Phylogenetic distance} - b))$ . Since the PIs have a maximum value of +1 and the minimum value of -1, a logistic formula was chosen to conform to this restricted range of values.

For the no-choice experiment with mating pairs of *Cassida rubiginosa* on potted plants, the relationship of each count variable (feeding holes, oviposition, and larval emergence) to phylogenetic distance was examined using a negative binomial log-link generalised linear model (GLM), which consisted of only phylogenetic distance as a covariate.

For the dual choice experiment, where mating pairs of *Cassida rubiginosa* were given a choice between *Cirsium arvense* and a test species, PIs were calculated similar to the olfactometer experiments. The relationship of each PI with phylogenetic distance was examined using non-linear regression analysis with the same logistic formula. The three non-Cardueae species were not included in the modelled relationships because phylogenetic distances from *Cirsium arvense* are not available for these species. All statistical analyses were carried out with Minitab 17.0 or SAS version 9.3.

## 3 RESULTS

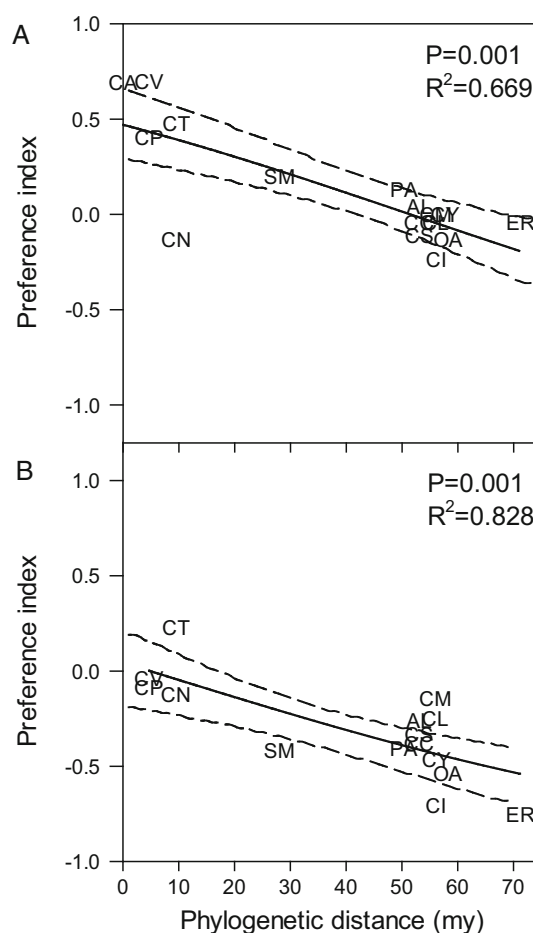
### 3.1 Host plant finding: olfactometer experiments

#### 3.1.1 Single choice experiment

Given a single choice (single odour source), the olfactory attraction of adult female *Cassida rubiginosa* showed a significant phylogenetic relationship, with attraction decreasing as the distance from the primary host plant increased ( $t_{14} = 4.35$ ,  $P = 0.001$ , Fig. 3(a)). The modelled relationship indicated that 66.9% of the variation in the beetle's olfactory attraction towards a potential host plant was explained by phylogeny.

#### 3.1.2 Dual-choice experiment

Given a choice between *Cirsium arvense* and a test plant (two odour sources), the olfactory preference of adult female *Cassida rubiginosa* showed a significant phylogenetic relationship, with preference decreasing as the distance from *Cirsium arvense* increased ( $t_{13} = 4.01$ ,  $P = 0.001$ , Fig. 3(b)). The relationship showed a strong correlation with 82.8% of the variation in the beetle's olfactory preference explained by phylogeny. The PI values showed a consistent pattern of preference for *Cirsium arvense* (i.e., negative PI values), except in the test with *Carduus tenuiflorus* ( $PI = 0.23$ ), indicating greater preference for this species (Fig. 3(b)).

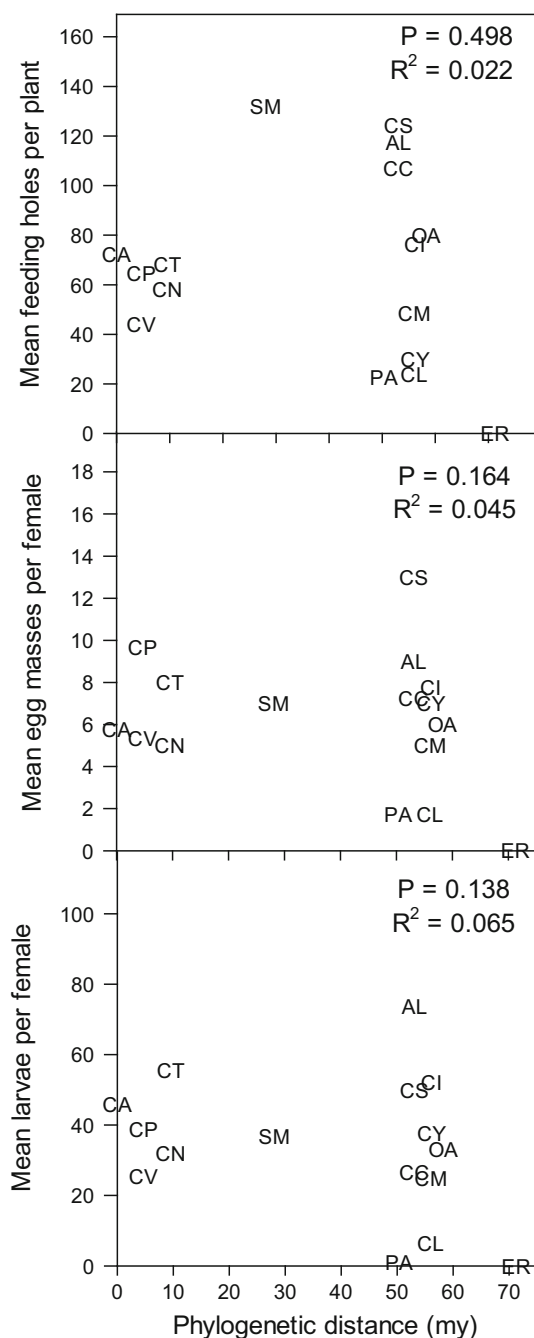


**Figure 3.** Olfactory recognition and preference tests with adult female *Cassida rubiginosa* in relation to phylogenetic distance (PD) in millions of years (my) from its primary host plant, *Cirsium arvense*. (a) Single choice olfactory recognition (single odour source) tests where plant species were compared to no plant (a control with moist cotton and paper leaf mimic). The relationship is given by preference index ( $PI = 1 - 2 / (1 + \exp(-0.0198 * PD + 1.02146))$ ). (b) Dual choice preference (two odour sources) tests where plant species were compared to the primary host plant, *Cirsium arvense*. The relationship is given by  $PI = 1 - 2 / (1 + \exp(-0.0181 * PD + 0.08864))$ . Dashed lines represent the 95% confidence intervals. CA—*Cirsium arvense*, CV—*Cirsium vulgare*, CP—*Cirsium palustre*, CT—*Carduus tenuiflorus*, CN—*Carduus nutans*, SM—*Silybum marianum*, AL—*Arctium lappa*, CL—*Carthamus lanatus*, CS—*Cynara scolymus*, CC—*Cynara cardunculus*, PA—*Ptilostemon afer*, CY—*Centaurea cyanus*, OA—*Onopordum acanthium*, CI—*Centaurea nigra*, CM—*Centaurea macrocephala*, ER—*Echinops ritro*.

### 3.2 Host acceptance: feeding and oviposition

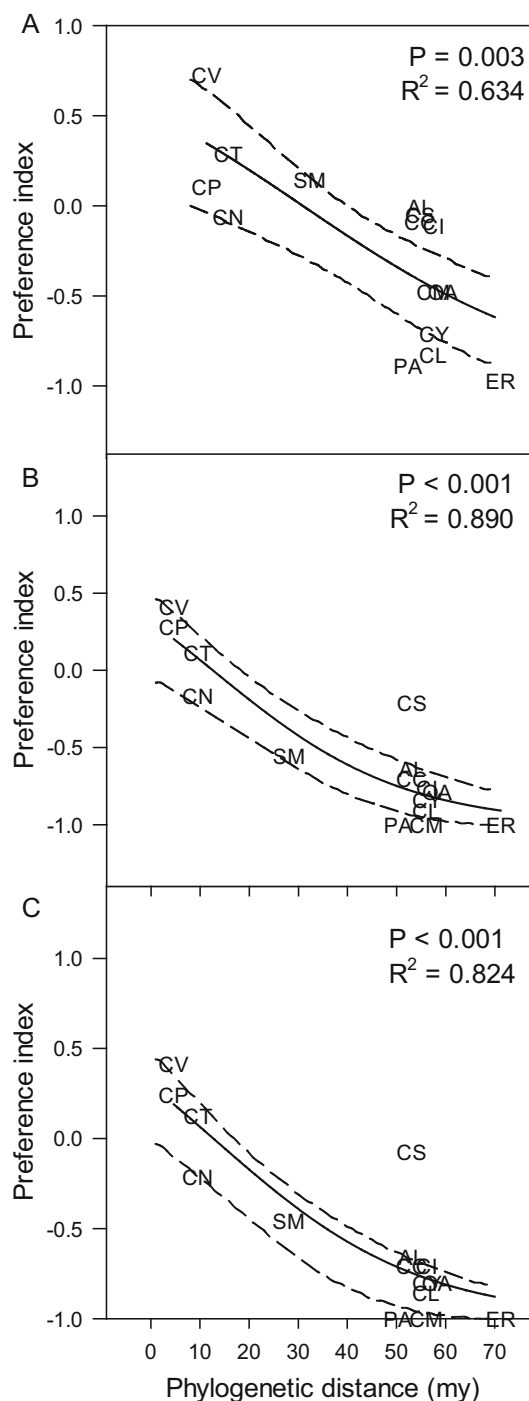
#### 3.2.1 No-choice experiment

When mating pairs of *Cassida rubiginosa* were placed on potted plants in a no-choice experiment, there was no significant relationship between any of the measures of host plant acceptance (feeding holes  $\chi^2 = 0.46$ ,  $df = 1$ ,  $P = 0.498$ ; oviposition  $\chi^2 = 1.94$ ,  $df = 1$ ,  $P = 0.164$ ; larval emergence  $\chi^2 = 2.20$ ,  $df = 1$ ,  $P = 0.138$ ) and phylogenetic distance from *Cirsium arvense* (Fig. 4). Furthermore, there were no correlations between the measures of host acceptance and phylogenetic distance with < 7% of the variation explained by phylogenetic distance in each case. Most test plants were accepted by the beetle, as indicated by feeding holes, oviposition and larval emergence numbers. The exceptions to this were *Carthamus lanatus* and *P. afer*, which showed a low degree of



**Figure 4.** No-choice acceptance (feeding and oviposition) test of *Cassida rubiginosa* in relation to phylogenetic distance (PD) in millions of years (my) from the primary host plant, *Cirsium arvense*. (a) The mean number of feeding holes per plant, (b) the mean number of egg masses per female and (c) the mean number of larvae per female that emerged from the egg masses. CA—*Cirsium arvense*, CV—*Cirsium vulgare*, CP—*Cirsium palustre*, CT—*Carduus tenuiflorus*, CN—*Carduus nutans*, SM—*Silybum marianum*, AL—*Arctium lappa*, CL—*Carthamus lanatus*, CS—*Cynara scolymus*, CC—*Cynara cardunculus*, PA—*Ptilostemon afer*, CY—*Centaurea cyanus*, OA—*Onopordum acanthium*, CI—*Centaurea nigra*, CM—*Centaurea macrocephala*, ER—*Echinops ritro*.

acceptance. On these two species there was a low number of feeding holes (mean  $\pm$  standard error of  $24.0 \pm 5.2$  and  $22.8 \pm 5.0$  holes per plant for *Carthamus lanatus* and *P. afer*, respectively), egg masses ( $1.8 \pm 0.7$  egg masses per plant for both *Carthamus lanatus* and *P. afer*), and larval emergence



**Figure 5.** Caged dual choice preference tests with two mating pairs of *Cassida rubiginosa* in relation to phylogenetic distance (PD) in millions of years (my) from its primary host plant, *Cirsium arvense*. (a) Feeding preference calculated from the mean number of leaf holes recorded on each test plant species, preference index (PI) =  $1 - 2/(1 + \exp(-0.0332 \cdot PD + 0.8780))$ . (b) Oviposition preference calculated from the mean number of egg masses per female recorded on each test plant species, PI =  $1 - 2/(1 + \exp(-0.0477 \cdot PD + 0.6073))$ . (c) The mean number of larvae per female that emerged from the egg masses, PI =  $1 - 2/(1 + \exp(0.0518 \cdot PD + 0.6514))$ . Dashed lines represent the 95% confidence intervals. CA—*Cirsium arvense*, CV—*Cirsium vulgare*, CP—*Cirsium palustre*, CT—*Carduus tenuiflorus*, CN—*Carduus nutans*, SM—*Silybum marianum*, AL—*Arctium lappa*, CL—*Carthamus lanatus*, CS—*Cynara scolymus*, CC—*Cynara cardunculus*, PA—*Ptilostemon afer*, CY—*Centaurea cyanus*, OA—*Onopordum acanthium*, CI—*Centaurea nigra*, CM—*Centaurea macrocephala*, ER—*Echinops ritro*.

( $4.5 \pm 1.5$  and  $0.5 \pm 0.4$  larvae per female for *Carthamus lanatus* and *P. afer*, respectively) compared to all other Cardueae plants that were accepted (Fig. 4). Of the Cardueae species, only *Echinops ritro* was not accepted as host with no feeding or oviposition recorded. There was no feeding or oviposition observed on any of the non-Cardueae species except for one egg mass recorded on *Tragopogon porrifolius*, from which no larvae emerged.

### 3.2.2 Dual-choice experiment

When mating pairs of *Cassida rubiginosa* were given a choice between *Cirsium arvense* and a test species, there were significant relationships between the beetle's feeding and oviposition preferences and phylogenetic distance from *Cirsium arvense* (Feeding:  $t_{13} = 3.61$ ,  $P = 0.003$ ; Oviposition:  $t_{13} = 5.07$ ,  $P < 0.001$ ; Larval emergence:  $t_{13} = 5.63$ ,  $P < 0.001$ ). These relationships showed a high degree of correlation, with 63.4% of the variation in feeding preference, and 89.0% of the variation in oviposition preference, and 82.4% of the variation in larval emergence, explained by phylogenetic distance (Fig. 5).

Positive PI values for feeding hole numbers were recorded for *Cirsium vulgare* (PI = 0.72), *Carduus tenuiflorus* (PI = 0.29), *S. marianum* (PI = 0.15), and *Cirsium palustre* (PI = 0.10), indicating greater feeding preference for these test plants compared to *Cirsium arvense*. Negative PI values were recorded for all other test plants indicating low feeding preference for these test plants compared to *Cirsium arvense*. The beetle exhibited very low feeding preference for *Carthamus lanatus* (PI = -0.84), and *P. afer* (PI = -0.88). Trace levels of feeding were observed on a single replicate of *Echinops ritro* ( $0.8 \pm 0.4$  feeding holes per plant, PI = -0.96) and *I. hookeri* ( $0.2 \pm 0.4$  feeding holes per plant, PI = -0.99). No feeding holes were detected on *Tragopogon porrifolius* and *Taraxacum officinale*.

Positive PI values for oviposition were recorded for *Cirsium vulgare* (PI = 0.42), *Cirsium palustre* (PI = 0.24), and *Carduus tenuiflorus* (PI = 0.12), indicating greater oviposition preference for these test plants. Negative PI values were recorded for all other test plants indicating lower oviposition preference for these test plants compared to *Cirsium arvense*. The beetle exhibited very low oviposition preference for *Carthamus lanatus* (PI = -0.86) with only one egg mass laid on a single replicate. No oviposition was observed on *P. afer*, *Centaurea macrocephala*, *Echinops ritro*, or any of the non-Cardueae species (PI = -1.0 for all species with no oviposition).

Larval emergence was a good indicator of the total number of eggs laid, and a further measure of adult female fitness on the test plants. Similar to oviposition, positive PI values for larval emergence were recorded for *Cirsium vulgare* (PI = 0.41), *Cirsium palustre* (PI = 0.28), and *Carduus tenuiflorus* (PI = 0.11). Negative PI values were recorded for all other test plants indicating lower oviposition preference for these test plants compared to *Cirsium arvense*. Five larvae emerged from the one egg mass on *Carthamus lanatus*, indicating very low oviposition preference for this plant (PI = -0.91).

## 4 DISCUSSION

This study demonstrates that *Cassida rubiginosa* exhibits a strong phylogenetic pattern to host plant selection. Our first prediction was that feeding and oviposition responses would be stronger than olfactory responses since greater sensory information is acquired when contact is made with the host plant.<sup>51,52</sup> However, contrary to this prediction, the olfactory responses showed a

similar pattern to the feeding and oviposition responses. This suggests that olfactory cues are important in the host finding and recognition process. The phylogenetic pattern was significant with only a single odour source in the single choice olfactometer experiment. We anticipated this relationship to be weak since the beetle might respond to any recognised plant within its fundamental range, or even respond to any volatile plant cues. For instance, the biocontrol beetle, *Lochmaea suturalis* (Chrysomelidae, heather beetle) was attracted to a non-host in a single choice olfactory test (single plant odour) but was able to discriminate between host and non-host in an olfactory choice test (two plant odours).<sup>53</sup> In contrast, *Cassida rubiginosa* appears to be finely tuned towards olfactory cues from *Cirsium* and *Carduus* with the olfactory signal weakening with phylogenetic distance from *Cirsium arvense*. In line with our prediction of stronger correlations for choice tests compared to no-choice tests, the strength of the relationship increased when offered a choice of two odour sources (*Cirsium arvense* versus a test species). This suggests that the dual-choice olfactometer tests may have presented additional sensory information, possibly both attraction and repellence cues. This might have caused more refined choices, and possibly explains the stronger correlation with phylogeny when olfactory preferences could be expressed.

For specialist herbivores, some degree of pre-contact recognition from olfactory and/or visual cues are generally required to find host plants.<sup>54</sup> This study demonstrates that female *Cassida rubiginosa* uses volatile chemical cues to find its host plants. However, the underlying chemical mechanism to the phylogenetic pattern remains uncertain. Interestingly, Susanna *et al.*<sup>55</sup> noted there were no clear chemical synapomorphies linking the Cardueae subtribes to a common ancestor. However, the olfactometer studies here suggest that there must be a phylogenetic chemical signal, and the strength of the signal weakens and/or the composition of the signal becomes blurred with phylogenetic distance. Several studies have shown that low volatility compounds from leaf surface waxes (e.g., volatile alkanes and free fatty acids) act as cues for host finding of chrysomelid beetles.<sup>56–59</sup> Possibly, this is also the type of volatile chemical cues being used by *Cassida rubiginosa* for finding its host plants.

In this study we did not assess visual cues, although many herbivorous insects use colour, shape, and size features of plants as part of their host finding mechanism.<sup>54</sup> In some cases, visual cues may be used in combination with olfactory cues to enhance host finding abilities, as was shown for the biocontrol weevil, *Mogulones borraginis*.<sup>23</sup> Therefore, it is possible that including visual cues might enhance the ability of *Cassida rubiginosa* to discern between hosts and strengthen the relationship with phylogeny.

In accordance with our second prediction, correlations with phylogeny were stronger for choice tests compared to no-choice tests. In fact, under no-choice conditions, there were no significant relationships, and no correlations (< 7% of variation explained by phylogeny), between the measures of plant acceptance (feeding, oviposition, and larval emergence) and phylogeny of host plants. Given no choice, most Cardueae plants were accepted equally as hosts, with only a few exceptions. *Ptilostemon afer* and *Carthamus lanatus* consistently showed a low degree of acceptance, and *Echinops ritro* was not accepted as a host. The fact that *Echinops ritro* was not accepted fits with the phylogenetic pattern since it was the most distantly related Cardueae species included in this study belonging to a distinct subtribe, separate from the Carduinae-Centaureinae clade.<sup>36</sup> These results also correspond well with the feeding tests conducted by Zwölfer and

Eichhorn<sup>31</sup> who found inconsistent 'nibbling' on *Echinops sphaerocephalus* and *Carthamus tinctorius*. As expected, none of the non-Cardueae plants were accepted as hosts, under no-choice or choice conditions.

As predicted, the strongest correlations between host acceptance and phylogeny were found under choice conditions, where the beetle could contact the test plants. Once in contact with the plant many cues are available that can cause the initiation of feeding, allow for continued feeding, and possibly result in oviposition. Under choice conditions, feeding showed the weakest relationship, where phylogeny explained 63.4% of the variation in the number of feeding holes. Feeding is a crude measure of plant acceptance since many factors can influence the amount consumed, such as nutritional quality, and various chemical and physical defensive features of the plant.<sup>60</sup> Oviposition is the ultimate measure of acceptance since this determines where the offspring must survive and develop. This is particularly important for many holometabolous insects that are not highly mobile and are confined to the host plant where they hatch, as is the case for *Cassida rubiginosa*. In the choice experiment, 89.0% of the variation in oviposition was explained by phylogeny, the strongest correlation found in this study. Similarly, the number of larvae that emerged from egg masses showed a significant and strong correlation with 82.4% of the variation explained by phylogeny. The number of larvae emerged is a good indication of the number of eggs per egg mass and therefore an additional measure of beetle fitness on the test plants. Given full access to all pre-contact and contact cues, host plant selection by *Cassida rubiginosa* follows a highly predictable phylogenetic pattern.

In this study there appeared to be few aberrant or indiscriminate responses that can sometimes result from the state of the insect or the experimental conditions.<sup>61</sup> The state of an insect being tested, including degree of hunger, egg load, and prior experience, can influence its responses under choice or no-choice conditions. In this study we used adult beetles that were field collected on the primary host, *Cirsium arvense*, and maintained on this plant prior to placement on the experimental plants. The absence of feeding on *Echinops ritro* and the non-Cardueae species in the no-choice experiment is not likely explained by a lack of hunger since the beetles were on the plants for 14 days, and beetles readily commenced feeding on most other host plants. Under no-choice conditions there was little evidence of the phenomenon of egg 'dumping' on non-hosts,<sup>62</sup> despite the beetle being well-fed on its primary host in a colony with males and females (i.e., females were likely gravid) prior to the experiment. The exception being one egg mass recorded on *I. hookeri*; but in this case no larvae emerged, indicating that the egg mass contained no eggs, or was possibly misidentified. The results suggest that plant contact cues are likely required to stimulate oviposition.

Some studies have noted that proximity of host plants and test plants in choice experiments can result in feeding on plants that are typically not hosts.<sup>22,61</sup> The trace levels of feeding found on *Echinops ritro* and *I. hookeri* under the choice conditions may have been due to the presence of *Cirsium arvense* providing sufficient stimulation to cause feeding. This is a likely explanation considering there was no feeding on these plants under no-choice conditions.

Prior experience on host plants can condition some insects to prefer these plants over plants they have not experienced.<sup>63</sup> It is possible that the results of our study might differ if the beetle was only exposed (from adult eclosion to reproductive maturity) to host plants other than *Cirsium arvense* prior to placement on test plants. However, it seems unlikely that this would cause

anything other than a temporary difference since most evidence indicates host affinities of herbivorous insects are stable.<sup>4</sup> A plausible result of rearing on another host plant prior to experimentation is that it would be suboptimal, causing reduced performance across its range of hosts.

The value of this study is that we examined the beetle responses in a phylogenetic context, which offers an evolutionary explanation for the pattern of host use, and some predictive ability for the host plants likely to be used in nature. The phylogenetic pattern of host plant selection suggests that the evolution of specialisation has arisen via the beetle tracking overall trait similarity (i.e., phylogeny) rather than key traits independent of phylogeny such as secondary chemicals, as found in some similar studies.<sup>19,20</sup>

The phylogenetic pattern of the adult choices also indicates a strong evolutionary selection pressure on the adult stage of the beetle to make optimal choices for larval survival. While the adult beetle can utilise most Cardueae species equally, its oviposition choices closely follow that which is best for larval survival,<sup>18</sup> as found with many other specialised herbivorous insects.<sup>28</sup>

The results of the choice experiments also correspond well with the relative performance thresholds (on target versus non-target species) calculated by Paynter *et al.*<sup>9</sup> for predicting field utilisation of plants by weed biocontrol agents. The relative performance thresholds for *Cassida rubiginosa* predicted full field utilisation of *Cirsium* and *Carduus* species, and spill-over attack on the non-target, *Cynara scolymus* (artichoke). This generally matches well with the PI values for plants with a phylogenetic distance of more than 20 million years separation from *Cirsium arvense*. However, in our study, *Cynara scolymus* had an oddly high oviposition PI value compared to other test species of similar phylogenetic distance. Being an agricultural plant, it is possible that artificial selection for desirable crop traits has also resulted in traits enhancing its palatability and acceptance to insect herbivores.<sup>64,65</sup>

Given the strong phylogenetic relationship found in this study, it would be possible to estimate the preference value for any Cardueae species based on its phylogenetic distance from *Cirsium arvense* and use this as a predictor of field utilisation. In the case of *Cassida rubiginosa* in New Zealand, attack on other thistle weeds was viewed as a potential benefit.<sup>32,33</sup> However, it seems unlikely that there will be any substantial attack on plants outside the *Cirsium-Carduus* group. This matches with field host records in the native range indicating that *Cassida rubiginosa* is primarily associated with *Cirsium* and *Carduus*, and generally only found on Carduinae, rarely on Centaurinae, and never found on Carlineae or Echinopsinae.<sup>31</sup> Predicting the pattern of field host utilisation is important for the safe release of biocontrol agents, particularly in the case of oligophagous species that have potential to attack a large number of species. Including olfactometer experiments in host specificity testing can improve the predicted field host range of biocontrol agents by elucidating the mechanisms behind the host plant selection process.

A question that deserves further exploration is the evolutionary stability of the pattern of host plant selection by the adult stage of biocontrol species. That is, what ecological selection pressures might operate in the novel introduced ranges of biocontrol agents that could alter the pattern of host plant utilisation.<sup>4</sup> In the introduced range of New Zealand, the beetle has been observed to obtain outbreak populations, possibly due to release from specialised predators and parasitoids. While the beetle is restricted to the Cardueae tribe, novel conditions such as enemy-free space and increased competition for resources could result in selection pressures for altered patterns of host use.



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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## REFERENCES

- Farrell BD, "Inordinate fondness" explained: why are there so many beetles? *Science* **281**:555–559 (1998).
- Winkler IS and Mitter C, The phylogenetic dimension of insect-plant interactions: a review of recent evidence, in *The Evolutionary Biology of Herbivorous Insects: Specialization, Speciation, and Radiation*, ed. by Tilmon KJ. Berkeley, University of California Press, pp. 240–263 (2008).
- Futuyma DJ, Evolutionary constraint and ecological consequences. *Evolution* **64**:1865–1884 (2010).
- van Klinken RD and Edwards OR, Is host-specificity of weed biological control agents likely to evolve rapidly following establishment? *Ecol Lett* **5**:590–596 (2002).
- Müller-Schärer H, Bouchemousse S, Litto M, McEvoy PB, Roderick GK and Sun Y, How to better predict long-term benefits and risks in weed biocontrol: an evolutionary perspective. *Curr Opin Insect Sci* **38**:84–91 (2020).
- Hinz HL, Winston RL and Schwarzländer M, A global review of target impact and direct nontarget effects of classical weed biological control. *Curr Opin Insect Sci* **38**:48–54 (2020).
- Schaffner U, Host range testing of insects for biological weed control: how can it be better interpreted? *Bioscience* **51**:951–959 (2001).
- Hinz HL, Schwarzländer M, Gassmann A and Bouchier RS, Successes we may not have had: a retrospective analysis of selected weed biological control agents in the United States. *Invasive Plant Sci Manage* **7**:565–579 (2014).
- Paynter Q, Fowler SV, Gourlay AH, Peterson PG, Smith LA and Winks CJ, Relative performance on test and target plants in laboratory tests predicts the risk of non-target attack in the field for arthropod weed biocontrol agents. *Biol Control* **80**:133–142 (2015).
- Wapshere AJ, A strategy for evaluating the safety of organisms for biological weed control. *Ann Appl Biol* **77**:201–211 (1974).
- Briese DT, Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedures. *Biol Control* **35**:208–214 (2005).
- Suckling DM and Sforza RFH, What magnitude are observed nontarget impacts from weed biocontrol? *PLoS One* **9**:e84847 (2014).
- Pemberton RW, Predictable risk to native plants in weed biological control. *Oecologia* **125**:489–494 (2000).
- Hinz HL, Winston RL and Schwarzländer M, How safe is weed biological control? A global review of direct nontarget attack. *Q Rev Biol* **94**:1–27 (2019).
- Wheeler GS and Schaffner U, Improved understanding of weed biological control safety and impact with chemical ecology: a review. *Invasive Plant Sci Manage* **6**:16–29 (2013).
- Wheeler GS and Madeira PT, Phylogeny within the Anacardiaceae predicts host range of potential biological control agents of Brazilian peppertree. *Biol Control* **108**:22–29 (2017).
- Rasmann S and Agrawal AA, Evolution of specialization: a phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophthalmus*). *Am Nat* **177**:728–737 (2011).
- Cripps MG, Jackman SD, Roquet C, van Koten C, Rostás M, Bourdôt GW *et al.*, Evolution of specialization of *Cassida rubiginosa* on *Cirsium arvense* (Compositae, Cardueae). *Front Plant Sci* **7**:1261 (2016).
- Wheeler GS, David AS and Lake EC, Volatile chemistry, not phylogeny, predicts host range of a biological control agent of old-world climbing fern. *Biol Control* **159**:104636 (2021).
- Rapo CB, Schaffner U, Eigenbrode SD, Hinz HL, Price WJ, Morra M *et al.*, Feeding intensity of insect herbivores is associated more closely with key metabolite profiles than phylogenetic relatedness of their potential hosts. *PeerJ* **7**:e8203 (2019).
- Wheeler GS, Chawner M and Williams DA, Predicting the host range of *Nyctalea ebalea*: secondary plant chemistry and host selection by a surrogate biological control agent of *Schinus terebinthifolia*. *Biol Control* **73**:39–49 (2014).
- Marohasy J, The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. *Biocontrol News Inf* **19**:13N–20N (1998).
- Park I, Eigenbrode SD, Cook SP, Harmon BL, Hinz HL, Schaffner U *et al.*, Examining olfactory and visual cues governing host-specificity of a weed biological control candidate species to refine pre-release risk assessment. *Biocontrol* **63**:377–389 (2018).
- Miller JR and Strickler KL, Finding and accepting host plants, in *Chemical Ecology of Insects*, ed. by Bell WJ and Carde RT. Sinauer Associates, Inc., Publishers, Sunderland, MA (1984).
- Eigenbrode SD and Bernays EA, Evaluation of factors affecting host plant selection, with an emphasis on studying behaviour, in *Methods in Agricultural and Ecological Entomology*, ed. by Dent DR and Walton MP. CAB International, Wallingford, UK, pp. 147–170 (1997).
- Hardy NB, Kaczkvinsky C, Bird G and Normark BB, What we Don't know about diet-breadth evolution in herbivorous insects. *Annu Rev Ecol Syst* **51**:103–122 (2020).
- Forister ML, Ehmer AG and Futuyma DJ, The genetic architecture of a niche: variation and covariation in host use traits in the Colorado potato beetle. *J Evol Biol* **20**:985–996 (2007).
- Gripenberg S, Mayhew PJ, Parnell M and Roslin T, A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol Lett* **13**:383–393 (2010).
- Forister ML, Independent inheritance of preference and performance in hybrids between host races of *Mitoura* butterflies (Lepidoptera: Lycaenidae). *Evolution* **59**:1149–1155 (2005).
- Craig TP and Itami JK, Evolution of preference and performance relationships, in *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects*, ed. by Kelley T. University of California Press, Berkeley, pp. 20–28 (2008).
- Zwölfer H and Eichhorn O, The host ranges of *Cassida* spp. (Col. Chrysomelidae) attacking Cynareae (Compositae) in Europe. *Z Entomol* **58**:384–397 (1966).
- Bourdôt GW, Fowler SV, Edwards GR, Kriticos DJ, Kean JM, Rahman A *et al.*, Pastoral weeds in New Zealand: status and potential solutions. *N Z J Agric Res* **50**:139–161 (2007).
- Cripps MG, Bourdôt GW and Fowler SV, Sleeper thistles in New Zealand: status and biocontrol potential. *New Zealand Plant Prot* **66**:99–104 (2013).
- Cripps MG, Jackman SD, Rostás M, Van Koten C and Bourdôt GW, Leaf traits of congeneric host plants explain differences in performance of a specialist herbivore. *Ecol Entomol* **40**:237–246 (2015).
- Susanna A and Garcia-Jacas N, Cardueae (Carduoideae), in *Systematics, Evolution, and Biogeography of Compositae*, ed. by Funk VA, Susanna A, Stuessy TF and Bayer RJ. International Association for Plant Taxonomy, Vienna, pp. 293–313 (2009).
- Barres L, Sanmartín I, Anderson CL, Susanna A, Buerki S, Galbany-Casals M *et al.*, Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). *Am J Bot* **100**:867–882 (2013).
- Borowiec L and Świętojańska J, *Cassidinae of the World: An Interactive Manual (Coleoptera: Chrysomelidae)*. University of Wrocław, Poland (2020).
- Futuyma DJ, Keese MC and Funk DJ, Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evolution* **49**:797–809 (1995).
- Salem H, Kirsch R, Pauchet Y, Berasategui A, Fukumori K, Moriyama M *et al.*, Symbiotic digestive range reflects host plant breadth in herbivorous beetles. *Curr Biol* **30**:2875–2886.e2874 (2020).
- Simmons W and Blossy B, Host plant phylogeny does not fully explain host choice and feeding preferences of *Galerucella*

- birmanica*, a promising biological control herbivore of *Trapa natans*. *Biol Control* **180**:105201 (2023).
- 41 Tiley GED, Biological flora of the British isles: *Cirsium arvense* (L.) Scop. *J Ecol* **98**:938–983 (2010).
  - 42 Cripps M, Gassmann A, Fowler S, Bourdôt G, McClay A and Edwards G, Classical biological control of *Cirsium arvense*: lessons from the past. *Biol Control* **57**:165–174 (2011).
  - 43 Kosior A and Klein J, Migrations and wintering of cassids (Coleoptera, Chrysomelidae, Cassidinae) under the climatic conditions of the Ojców National Park. *Acta Zool Cracov* **15**:315–341 (1970).
  - 44 Koji S and Nakamura K, Seasonal fluctuation, age structure, and annual changes in a population of *Cassida rubiginosa* (Coleoptera: Chrysomelidae) in a natural habitat. *Ann Entomol Soc Am* **99**:292–299 (2006).
  - 45 Tipping PW, Field studies with *Cassida rubiginosa* (coleoptera: Chrysomelidae) in Canada thistle. *Environ Entomol* **22**:1402–1407 (1993).
  - 46 Ward RH and Pienkowski RL, Biology of *Cassida rubiginosa*, a thistle-feeding shield beetle. *Ann Entomol Soc Am* **71**:585–591 (1978).
  - 47 Van Tol R, Visser J and Sabelis M, Olfactory responses of the vine weevil, *Otiorhynchus sulcatus*, to tree odours. *Physiol Entomol* **27**:213–222 (2002).
  - 48 Martinez AS and Hardie J, Hygroreception in olfactometer studies. *Physiol Entomol* **34**:211–216 (2009).
  - 49 Kühnle A and Müller C, Differing acceptance of familiar and unfamiliar plant species by an oligophagous beetle. *Entomol Exp Appl* **131**:189–199 (2009).
  - 50 Sutton G, Paterson I, Compton S and Paynter Q, Predicting the risk of non-target damage to a close relative of a target weed using sequential no-choice tests, paired-choice tests and olfactory discrimination experiments. *Biocontrol Sci Technol* **1-14**:364–377 (2017).
  - 51 Schoonhoven LM, Van Loon JJ and Dicke M, *Insect-Plant Biology*. Oxford University Press on Demand, Oxford (2005).
  - 52 Mitchell B, The chemosensory basis of host-plant recognition in Chrysomelidae, in *Novel Aspects of the Biology of Chrysomelidae*, Springer, Dordrecht, pp. 141–151 (1994).
  - 53 Effah E, Svendsen L, Barrett DP and Clavijo McCormick A, Exploring plant volatile-mediated interactions between native and introduced plants and insects. *Sci Rep* **12**:15450 (2022).
  - 54 Fernandez P and Hilker M, Host plant location by Chrysomelidae. *Basic Appl Ecol* **8**:97–116 (2007).
  - 55 Susanna A, Garcia-Jacas N, Soltis DE and Soltis PS, Phylogenetic relationships in tribe Cardueae (Asteraceae) based on ITS sequences. *Am J Bot* **82**:1056–1068 (1995).
  - 56 Malik U and Barik A, Free fatty acids from the weed, *Polygonum orientale* leaves for attraction of the potential biocontrol agent, *Galerucella placida* (Coleoptera: Chrysomelidae). *Biocontrol Sci Technol* **25**:593–607 (2015).
  - 57 Mitra S, Sarkar N and Barik A, Long-chain alkanes and fatty acids from *Ludwigia octovalvis* weed leaf surface waxes as short-range attractant and ovipositional stimulant to *Altica cyanea* (Weber) (Coleoptera: Chrysomelidae). *Bull Entomol Res* **107**:391–400 (2017).
  - 58 Mukherjee A, Sarkar N and Barik A, Leaf surface n-alkanes of *Momordica cochinchinensis* Spreng as short-range attractants for its insect pest, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Allelopathy J* **36**:109–122 (2015).
  - 59 Müller C and Hilker M, Host finding and oviposition behavior in a chrysomelid specialist—the importance of host plant surface waxes. *J Chem Ecol* **27**:985–994 (2001).
  - 60 Matsuda K, Feeding stimulants of leaf beetles, in *Biology of Chrysomelidae*, Springer, Dordrecht, pp. 41–56 (1988).
  - 61 Withers T and Barton Browne L, Possible causes of apparently indiscriminate oviposition in host specificity tests using phytophagous insects, in *Pest Management—Future Challenges, Sixth Australasian Applied Entomology Research Conference*, University of Queensland, Brisbane, Vol. 1, pp. 565–571 (1998).
  - 62 Sheppard AW, van Klinken RD and Heard TA, Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. *Biol Control* **35**:215–226 (2005).
  - 63 Bernays EA, Effects of experience on host-plant selection, in *Chemical Ecology of Insects 2*, ed. by Cardé RT and Bell WJ. Springer US, Boston, MA, pp. 47–64 (1995).
  - 64 Turcotte MM, Turley NE and Johnson MTJ, The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytol* **204**:671–681 (2014).
  - 65 Franks SJ, Genovese N, Stockdale M, Weber JJ, Ansaldi BH and Wilgenburg E, The effects of artificial selection for rapid cycling in *Brassica rapa* on herbivore preference and performance. *Int J Plant Sci* **179**:175–181 (2018).