# A PHYLOGENETIC APPROACH TO ASSESSING OLFACTORY HOST SELECTION IN *CASSIDA RUBIGINOSA* (CHRYSOMELIDAE)

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## Introduction

Herbivorous insects often exhibit a phylogenetically conserved pattern in host plant selection due to long evolutionary associations that have promoted adaptation to feeding on particular plant lineages (Winkler and Mitter, 2008). This implies that more closely related species share a greater number of phenotypic and ecological traits and should therefore be more suitable hosts. By assessing functional plant traits with putative roles for host plant specialization in phylogenetically controlled experiments, their role as selection factors may be determined (Rasmann and Agrawal, 2011).

As part of a larger study (see also Cripps et al., 2016) that investigates several plant traits relevant for host selection in the thistle tortoise beetle, *Cassida rubiginosa* (Chrysomelidae), we assessed the role of volatile organic compounds (VOC) as kairomones in a series of olfactometer experiments. The thistle tortoise beetle is considered oligophagous as most plants in the Cardueae tribe are potential hosts, although its preferred food plant is *Cirsium arvense* (Californian thistle) (Fujiyama et al., 2011; Cripps et al., 2013). We tested the hypotheses that *C. rubiginosa* shows strong preference for closely related plants (i.e. within *Cirsium* clade), reduced preference for more distantly related host plants, and no preference for non Cardueae plants.

## Methods

The following six plant species were selected according to their phylogenetic relationships (Figure 1) and their suitability in supporting larval development (which has been assessed in previous experiments, see Table 1): *C. arvense, Carduus nutans, Echinops ritro, Centaurea nigra, Ptilostemon afer.* The non-Cardueae species *Taraxacum officinale* (Cichorieae) was included as outgroup.

### *No-choice tests*

To determine the long-distance responses of female *C. rubiginosa* to plant VOCs, still-air olfactometers made of glass (Van Tol et al., 2002) were used. One chamber of the olfactometer contained an undamaged leaf (2nd or 3rd leaf from the top) from one of the seven plant species. The control chamber was supplied with damp cotton wool and a piece of green paper in the shape of a leaf (Figure 2). Preliminary experiments had indicated that the beetles were attracted to higher humidity. The cotton wool was therefore treated with an appropriate amount of water (1–1.5 ml) to adjust the relative humidity in the control chamber to the level measured in the leaf-containing test chamber, using a hygrometer with a remote sensor (McGregor's Digital Weather Station Thermometer. M440, AHM Ltd., New Zealand). Three adult females of *C. rubiginosa* were released in the bottom of the still-air olfactometer and allowed to choose a chamber within a 6-hour time period. The time had been determined by preliminary experiments. The growth room temperature and the humidity were kept constant at 25°C, 30 to 33% RH. Position of the olfactometers and the chambers containing the leaves were randomised. Eight replicates were conducted with each plant species using a total of 168 beetles. Olfactometers were washed with water, distilled water, acetone and hexane to clean them from impurities and odour each time they were used. Data were analysed using log-linear model analysis.

**Figure 1.**
Phylogeny of the Cardueae test plants pruned from a comprehensive phylogeny of the tribe (Barres et al., 2013). Plants that were used in the experiments are highlighted. Scale: millions of years.

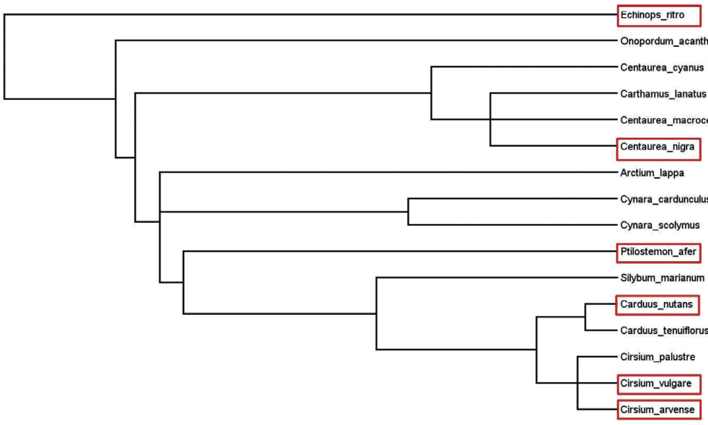
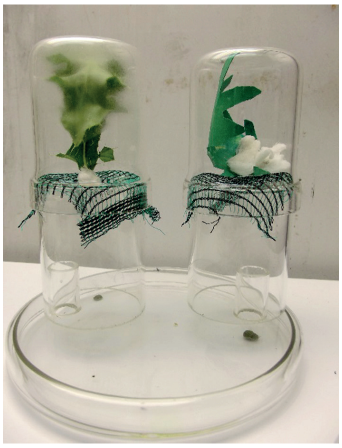


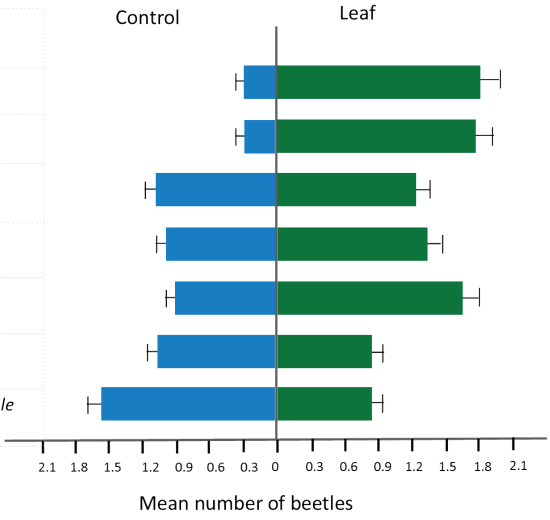
**Figure 2.**
Set-up of still-air olfactometer experiment.



## Results

The primary host plant and closely related species (i.e. other *Cirsium* spp.) were strongly preferred (*C. arvense*: P = 0.011 and *C. vulgare*: P = 0.016) in comparison to the control chamber. The beetles showed no significant preference (P > 0.05) for any other plant species (Figure 3).

**Figure 3.**
Olfactory preferences of *C. rubiginosa* in no-choice tests. Bars represent mean numbers and SEM of beetles choosing one of the chambers of the olfactometer. P-values indicate the significance of the log-linear model analysis. Response indicates proportion of beetles that made a choice. N = 8 per plant species.



| Plant Species | P-value | Response |
|---|---|---|
| *Cirsium arvense* | 0.011 | 75% |
| *Cirsium vulgare* | 0.016 | 71% |
| *Carduus nutans* | 0.819 | 79% |
| *Echinops ritro* | 0.493 | 79% |
| *Ptilostemon afer* | 0.187 | 83% |
| *Centaurea nigra* | 0.618 | 67% |
| *Taraxacum officinale* | 0.257 | 79% |

## Discussion

In the present study we correlated host plant phylogeny and larval performance to make predictions about the host selection behaviour of female beetles. Host plant selection can be imagined as a multistep process which starts by homing in on long-range cues such as plant volatiles (Bruce et al., 2005; Visser, 1988). Further steps on the plant surface involve processing gustatory cues and non-chemical factors. In theory, volatile blends should be characterised by a certain degree of specificity that allows the herbivore to identify its host. With increasing phylogenetic distance we expect a lower degree of similarity in the composition of volatile compounds and subsequently a gradual decrease in attractiveness. The latter assumption was tested in a series of no-choice olfactometer tests.

In accordance with our hypotheses, we demonstrated that *C. rubiginosa* had a strong olfactory preference for the primary host, *C. arvense*, and its congener, *C. vulgare*. We also confirmed that phylogenetically distant plant species, which support very low or no level of larval development (*E. ritro, P. afer, T. officinale*) were not attractive to the beetles. In contrast to our prediction, however, this was equally found for more closely related host plants outside the *Cirsium* clade (*C. nigra, C. nutans*). Both plants, although suboptimal, supported larval development in the range of 40–60% survival and therefore one could expect that female beetles in search of feeding and oviposition substrates, while lacking alternatives, would cue in on the volatiles of both species. Since this was not observed, it raises the question whether other factors such as visual cues or random search plays a crucial role in detecting *C. nigra* and *C. nutans*. Field surveys show that *C. rubiginosa* is infrequently found on other Cardueae species (Zwölfer and Eichhorn, 1966) thus corroborating our results. Alternatively, we cannot exclude that host preference could have been influenced by the beetles' previous experience as they have been reared on their primary host before being tested in the olfactometer experiments (Scheirs et al., 2000; Agrawal, 2000). Further work is in progress to test the influence of previous experience on beetle behaviour. Work is also underway to characterize the emitted thistle volatiles and include the full range of species listed in Table 1. The results from these analyses should allow us to establish whether plant volatile composition is phylogenetically conserved, in which case we can expect the detection and use of alternative hosts to be more likely, because closely related plants emit similar odours.

Apart from a better understanding of the factors that shape host plant specialisation, assessing the likelihood of switching to secondary hosts can be of practical importance in weed biological control. In the case of *C. rubiginosa*, utilisation of alternative thistle hosts could be beneficial since all Cardueae plants are introduced to New Zealand, and most are considered invasive weeds.

**Table 1**.
Mean and standard error (SEM) of survival rate of *C. rubiginosa* larvae on different plant species (Michael Cripps, unpublished data). Species marked with an asterisk were selected for the olfactometer experiments.

| Plant species | Tribe/Sub tribe | Survival [%] | ± SEM |
|---|---|---|---|
| *Carduus tenuiflorus* | Carduinae | 85.0 | 4.6 |
| *Cirsium arvense** | Carduinae | 78.3 | 5.4 |
| *Cirsium palustre* | Carduinae | 71.7 | 5.9 |
| *Cirsium vulgare** | Carduinae | 66.7 | 6.1 |
| *Arctium lappa* | Carduinae | 64.2 | 4.4 |
| *Silybum marianum* | Carduinae | 61.7 | 6.3 |
| *Carduus nutans** | Carduinae | 60.0 | 6.4 |
| *Cynara cardunculus* | Carduinae | 58.3 | 6.4 |
| *Cynara scolymus* | Carduinae | 46.7 | 6.5 |
| *Centaurea cyanus* | Centaureinae | 43.3 | 6.5 |
| *Centaurea nigra** | Centaureinae | 40.0 | 6.4 |
| *Onopordum acanthium* | Carduinae | 40.0 | 6.4 |
| *Centaurea macrocephala* | Centaureinae | 33.3 | 6.1 |
| *Echinops ritro** | Echinopsinae | 8.3 | 3.6 |
| *Carthamus lanatus* | Centaureinae | 0.0 | 0.0 |
| *Ptilostemon afer** | Carduinae | 0.0 | 0.0 |

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