Does phylogeny explain the host-choice behaviour of potential biological control agents for Brassicaceae weeds?

H.L. Hinz¹, M. Schwarzländer² and J. Gaskin³

Summary

Four invasive Brassicaceae are currently being studied at CABI Europe-Switzerland for biological control. A phylogenetic approach to host testing has so far been hampered by the fact that the evolutionary relationships of taxa within the Brassicaceae were unclear. Recently, a new phylogeny of the Brassicaceae, largely based on molecular studies, has been proposed. This presents a unique opportunity to relate host-range test results for some of our Brassicaceae agents to the new phylogeny. The host range of Ceutorhynchus scrobicollis Nerensheimer & Wagner, a root-crown mining weevil investigated as a potential agent for garlic mustard, Alliaria petiolata (Bieb.) Cavara & Grande, appeared to closely follow the new classification (significant linear relationship between phylogenetic distance and host-range test results). However, for Ceutorhynchus cardariae Korotyaev, a gall-inducing weevil considered as biocontrol agent for hoary cress, Lepidium draba L., phylogenetic distance of the test species to the target weed did not explain a significant amount of the variation in host preference or suitability. These results question the general applicability of the centrifugal phylogenetic method, where it is assumed that species more closely related to the target are at greater risk of attack than species more distantly related. The importance of other factors, specifically secondary metabolite profiles and morphological characteristics for the host-choice behaviour of C. cardariae are currently being investigated.

Keywords: Alliaria petiolata, Lepidium draba, centrifugal phylogenetic method.

Introduction

To date, no biological control agents have been released against weeds in the mustard family (Brassicaceae). The main reason for this is the family’s large number of economically important crop species and its many genera indigenous to North America. Four invasive Brassicaceae are currently being studied at CABI Europe-Switzerland for biological control. They are garlic mustard, Alliaria petiolata (Bieb.) Cavara & Grande, hoary cress, Lepidium draba L., perennial pepperweed, Lepidium latifolium L. (Hinz et al., 2008, this proceedings), and dyer’s woad, Isatis tinctoria L. (Cortat et al., 2008, this proceedings).

In biological control of weeds, it is generally assumed that species closely related to the target are at greater risk of attack than species more distantly related. However, a phylogenetic approach to host testing has so far been hampered by the fact that the evolutionary relationships of taxa within the Brassicaceae were unclear. The subdivision of the Brassicaceae at the tribal and subtribal levels has been a controversial aspect in the systematics of the family (Appel and Al-Shehbaz, 2003). Appel and Al-Shehbaz (2003) concluded that ‘in the absence of comprehensive, family-wide molecular data it is not regarded advisable to propose or recommend any classification system’. Recently, Al-Shehbaz et al. (2006) and Bailey et al. (2006) proposed the long-awaited new tribal alignment of the Brassicaceae based on molecular studies and careful evaluation of morphology and generic circumscriptions. This presented a unique opportunity to see whether host-range test results for some of our Brassicaceae agents correlated with the new phylogeny of the Brassicaceae. We used host-specificity test results of two currently studied potential biocontrol agents, viz., Ceutorhynchus scrobi-
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bicollis Nerensheimer & Wagner (Coleoptera, Curculionidae) on A. petiolata and Ceutorhynchus cardariae Korotyaev on L. draba, and correlated them with the genetic distance of test plant species to the respective target weed.

Methods and materials

The study species

A. petiolata is a strict biennial European herb introduced into North America that, by 2000, had spread to 34 states and four Canadian provinces (Blossey et al., 2001). A. petiolata is one of the few introduced herbaceous species that invades and dominates the understory of forested areas in North America. In 1998, a biological control program was started, and four weevils were prioritized as potential agents. One of these is the root-crown mining weevil, C. scrobicollis. Adults of this species aestivate in summer. Oviposition on A. petiolata rosettes begins in mid-September and lasts until the beginning of April of the following year (Gerber et al., 2007). Eggs are laid into petioles and leaves, and the growing point and larvae mine through petioles towards the roots and develop mainly in root crowns, occasionally also in shoot bases. Mature larvae leave host plants in spring to pupate in the soil, and the next generation of adults emerges in May and June.

L. draba [=Cardaria draba (L.) Desv.], with its two subspecies, L. draba spp. draba and L. draba spp. chalapense, and its close relative Lepidium appelium Al-Shehbaz [=Cardaria pubescens (C.A. Mey.) Jarm.], are perennial mustards (Brassicaceae) of European origin that were introduced in the USA in the late 19th century (Lyons, 1998). Since then, they spread throughout the western and the northeastern states and are now declared noxious weeds in 16 states and three Canadian provinces (Rice, 2005). Because they are difficult to control sustainably using mechanical or chemical methods, a consortium was established in Spring 2001 to investigate the scope for classical biological control. Five insect species are currently being studied for the biological control of L. draba: four weevils and one flea beetle. One of these is the gall-inducing weevil C. cardariae Korotyaev. Females of C. cardariae lay their eggs in the leaf midribs, petioles and developing shoots of L. draba from early spring until mid-June (Hinz et al., 2007). Oviposition induces the formation of galls, in which the larvae mine and develop. Mature larvae leave the plants to pupate in the soil, and the next generation of adults emerges from May to July.

Host-specificity tests

A. petiolata–C. scrobicollis: Between 1999 and 2006, sequential no-choice oviposition tests were conducted with C. scrobicollis. A mated pair of C. scrobicollis was placed into a transparent plastic cylinder (11 cm diameter, 15 cm high) and alternately offered cut leaves of A. petiolata, then those of a test-plant species. After 3 to 4 days, the plant material was removed, checked for feeding marks, dissected for eggs and the weevils provided with fresh plant material. Each exposure period was treated as one replicate. A replicate for test plants was only regarded as valid when the female laid at least one egg into the test plant or into an A. petiolata plant following a test plant.

Plant species accepted for oviposition were subsequently exposed in no-choice oviposition and development tests. Two to three females and one to two males were released onto individually potted, gauze-covered rosettes of A. petiolata or onto test species. To verify that females were fertile, one pair was offered a cut leaf of garlic mustard in a cylinder for 2 to 3 days after which plant material was dissected for eggs. Only females that laid eggs on these leaves were used for the tests. For each no-choice experiment, two to 13 garlic mustard plants were infested concurrently as controls. After 2 to 4 weeks, weevils were retrieved and plants re-covered with gauze bags. In late spring of the following year, all plants were searched regularly for emerging adults until emergence ceased.

L. draba–C. cardariae: Between 2003 and 2006, no-choice oviposition and development tests were conducted with C. cardariae. One to five females and one to four males, depending on plant size, were placed onto individually potted, gauze-covered test plants or L. draba (control plants). All females were tested for egg-laying before use in tests (see above). Each time a series of test plants was infested, two to four L. draba plants were infested concurrently as controls. After 8 to 12 days, weevils were retrieved from the plants, and feeding, oviposition and gall formation were recorded. To ensure that females had a chance to feed and oviposit on L. draba in between tests, all weevils were placed into cylinders for a couple of days and provided with cut plant material of L. draba before beginning a new series of tests. About 1 month after infestation, plants were re-checked for gall development. After about 12 weeks, all plants were checked for adult emergence.

Molecular and statistical analyses

Leaf material of plant species within the family Brassicaceae used in tests with C. scrobicollis and C. cardariae that were not included in the phylogenetic tree of Beilstein et al. (2006) was collected and subjected to molecular analysis. Genomic DNA was isolated using a cetyl trimethylammonium bromide method. Polymerase chain reaction (PCR) amplification of the chloroplast ndhF region was done with same primers and conditions as in Beilstein et al. (2006). PCR products were purified using QIAquick PCR Purification kit (Qiagen) before sequencing in a Beckman CEQ 2000XL automated sequencer using standard protocols including the LFR-1 method of injection time and volt-
age. Sequences were aligned manually using Se-Al (Rambaut, 1996). Maximum parsimony (MP) analysis was performed and uncorrected (p) distances of the data set were determined using PAUP* v. 4.0b8 (Swofford, 2000). For estimation of the most parsimonious phylogenetic trees, the heuristic MP search employed 500 random taxon addition sequences and the tree-bisection-reconnection branch-swapping algorithm. All characters were weighted equally. A 10,000-replicate, fast stepwise-addition, bootstrap analysis was conducted to assess clade support. The phylogenetic analysis was included to illustrate evolutionary relationships of the plant taxa, while the distance measurements were correlated with host-specificity measurements.

To relate results of host-specificity tests (for C. scrobicollis, the number of eggs laid and the number of offspring produced per female; for C. cardariae, the number of galls induced and offspring produced per female) with the genetic distances generated for test plants of each target weed species, we used simple linear regression analysis. The two target weeds were not included in the analyses, as the aim was to test whether plants more closely related to the respective target weed would be preferred by female weevils for oviposition and/or would be more suitable for weevil development. When data on the genetic distance for a test-plant species was not available, we used congeners of known genetic distance from the target to extrapolate the missing value to the precision of two decimal places.

### Results

**A. petiolata–C. scrobicollis**

Of the 28 plant species and varieties for which data on both oviposition-test results and genetic distance were available, 18 were accepted for oviposition by C. scrobicollis females (Fig. 1A). As expected, females of C. scrobicollis laid more eggs on plants more closely related to A. petiolata ($r^2 = 0.298, F_{1,27} = 11.02$,
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$P = 0.003$) than on less closely related plants. The two plant species that received the most eggs, the European species, *Peltaria alliacea* Jacq. and *Thlaspi arvense* L., are in the same tribe as *A. petiolata* (Thlaspeae), according to the new molecular phylogeny (Al-Shehbaz et al., 2006; Fig. 2). No plant species outside the family Brassicaceae supported normal oviposition behaviour of *C. scrobicollis* (Gerber et al., 2005).

Of the 21 plant species and varieties for which data on both results of no-choice development tests and genetic distance were available, adults emerged from five species other than *A. petiolata*, viz. the European species, *Nasturtium officinale* R.Br., *P. alliacea*, and *T. arvense*, and the North American species, *Rorippa sinuata* (Nutt.) A.S. Hitchc. In addition, a single adult in a single replicate emerged from the commercially grown cabbage variety *Brassica oleracea sabauda* ‘Paradisler’. However, none of the other six *B. oleracea* varieties we offered were attacked (Gerber et al., 2005), and moreover, no attack occurred under multiple-choice cage conditions. This suggests that *B. oleracea* and its various cultivars are outside the fundamental

![Phylogeny of Alliaria petiolata and test-plant species used in oviposition and development tests with Ceutorhynchus scrobicollis. The figure is a strict consensus of the ten most parsimonious trees, each 615 steps in length, derived from 1965 aligned bases of the chloroplast gene ndhF. Bootstrap values (>50%) are shown above branches. Taxa that supported adult development are shown in bold font. An asterisk indicates that the DNA sequence was provided by M. Beilstein.](image-url)
The host range of *C. scrobicollis*. We therefore consider the emergence of a single adult an artefact of experimental conditions. Females of *C. scrobicollis* produced more offspring on plants more closely related to *A. petiolata* ($r^2 = 0.237, F_{1,20} = 5.91, P = 0.025$; Figs. 1B and 2) than on less closely related species.

It is worth noting that the North American species, *Noccaea fendleri* (A. Gray) Holub (see Fig. 2), was formerly known as *Thlaspi montanum* L. (Thlaspiidae), until all North American species of *Thlaspi* were recently assigned to the tribe Noccaeeae, genus *Noccaea*, to form a monophyletic group separate from the European species of *Thlaspi* (Koch and Al-Shehbaz, 2004; also see Fig. 2). Host-specificity results with *C. scrobicollis* supported this new classification.

**L. draba—C. cardariae**

Of the 58 plant species for which data on both host-specificity test results and genetic distance were available, galls were induced on 11 species and adults emerged from ten (Fig. 3A, B), including the second target weed *L. appelianum*. In contrast to *C. scrobicollis* on *A. petiolata*, neither the number of galls induced nor the number of offspring produced per *C. cardariae* female was correlated with genetic distance of test-plant species from the target weed *L. draba* (number of galls induced, $r^2 = 0.025, F_{1,57} = 1.46, P = 0.232$; number of offspring produced, $r^2 = 0.020, F_{1,57} = 1.15, P = 0.287$). Plant species that supported gall induction and the development of adults included the fairly distantly related species *Caulanthus anceps* Payson and *C. inflatus* S. Wats. (the genetic distance for both of which was extrapolated from *C. crassicaulis*, see ‘Methods and materials’ for details), *Stanleya pinnata* (Pursh) Britt. and *Lobularia maritima* (L.) Desv. (Fig. 4). While only one adult each emerged from each of the latter two species, a similar number of adults emerged from *C. anceps* and *C. inflatus* as from *L. draba* control plants (Hinz et al., 2007). In contrast, *S. viridiflora* Nutt. did not support gall induction and was minimally fed on by *C. cardariae*.

![Figure 3.](image-url) Relationship between genetic distance of test-plant species to the control (i.e. target weed) *Lepidium draba* and (A) the number of galls induced and (B) the number of offspring produced per *Ceutorhynchus cardariae* female on the respective test-plant species.
Discussion

In three multiple-choice, field-cage tests established between 2004 and 2006 with *C. cardariae*, in which several test species were exposed that had supported development under no-choice conditions, only the three target weeds, i.e. the two subspecies *L. draba* spp. *draba* and *L. draba* spp. *chalapense* and *L. appelianum* were attacked, indicating a very narrow host range for the weevil under multiple-choice conditions. However, plants that supported development under no-choice conditions constitute the physiological host range of a species, which in turn appears to be an effective criterion for identifying species potentially at risk of attack, as there is no example of an insect agent attacking a plant outside its physiological host range after release (Pemberton, 2000; van Klinken and Edwards, 2002).

In North America alone, the family Brassicaceae is represented by approximately 600 species in more than 35 endemic genera. Also in North America, there...
are at least 123 Brassicaceae species within 23 genera that are considered economically important. Our host-specificity test results showed that none of the most widespread and important economic Brassicaceae species are at risk of attack by either biological control candidate. In addition, if commercially grown Brassicaceae would be part of the normal host range of any of our agents tested, the species would have been recorded as a pest in the European literature, which is not the case (Schwarz et al., 1990). However, native North American Brassicaceae have never been previously exposed to the insects studied here, and the large number of plant species makes it difficult, if not impossible, to test them all. We were therefore hoping to use the new Brassicaceae phylogeny to select plant species for additional host-specificity tests and to extrapolate the risk of non-target attack on more distantly related genera to the respective target weeds.

The host range of C. scrobicollis, investigated for the biological control of A. petiolata, only appears to include species closely related to the target. In contrast, C. cardariae on L. draba appears to have a disjunct host range with some distantly related plants supporting development to a similar degree as the control under no-choice conditions. These results are at odds with the centrifugal phylogenetic method (Wapshere, 1974), where it is generally assumed that species closely related to the target are at greater risk of attack than species more distantly related. In the absence of detailed, explicit phylogenies, the centrifugal phylogenetic method has usually been based on traditional taxonomic classifications, which has been questioned (Briese and Walker, 2002; Kelch and McClay, 2003). However, our study was based on the latest molecular phylogeny of the Brassicaceae.

For Longitarsus jacobaeae Waterhouse, which was studied for the biological control of Senecio jacobaea L., neither adult feeding nor the fundamental larval host range of L. jacobaeae were clearly predicted by the phylogeny of the genus Senecio (U. Schaffner, unpublished data). In contrast, it was found that leaf dry matter content of Senecio species explained a significant amount of the variability in the amount of leaf area eaten by adult beetles (U. Schaffner, unpublished data). Another study found that the functional composition of herbivore assemblages on 18 shrubs was correlated with respective leaf structural traits (Peeters, 2002). More specifically, leaf trichome density and leaf surface waxes have been shown to influence host suitability or preference of insect herbivores (Eigenbrode and Espelie, 1995; Levin, 1973, and Edwards, 1982, in Peeters, 2002). Finally, secondary plant compounds play an important role in host finding, host acceptance and host suitability of insect herbivores (e.g. Renwick, 1989; Rask et al., 2000). We are currently investigating some of these potential factors to better explain the host acceptance and suitability patterns observed for C. cardariae. Because host acceptance and suitability of herbivores might be influenced by a combination of many different secondary compounds and physical characteristics (see above), we will be using a relatively new technique, i.e. metabolomics that will allow us to identify and quantify all metabolites (primary and secondary) of an organism simultaneously (Bezemer and van Dam, 2005 and refs therein).

In conclusion, it is not our intention to question the importance of phylogenetic relatedness to understanding host-choice behaviour of herbivorous insects. Indeed, since the general adoption of the centrifugal phylogenetic method for host-range testing of weed biological control candidates (Wapshere, 1974), there has not been a single significant case of an agent that attacked a non-target that was completely unanticipated or unpredicted (Pemberton, 2000). We do, however, propose that other factors influence the host choice behaviour of insect herbivores more than commonly considered. As already suggested previously (e.g. Keller, 1999; Withers; 1999; Briese, 2005), these factors need to be better understood and should be included in the selection of test plant species and in the interpretation of host-specificity test results.

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References


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