



Predicting the outcome of potential novel associations: interactions between the invasive *Vincetoxicum rossicum* and native western *Chrysochus* beetles

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Abstract Understanding the potential outcomes of interactions between native insects and invasive plants is important for predicting the magnitude of effects caused by an invader in its new environment. Here, we investigate the ability of the native western leaf beetle, *Chrysochus cobaltinus*, and a hybrid of North American *Chrysochus* species, (hybrid of eastern *C. auratus* and western *C. cobaltinus*) to initiate a novel association with introduced pale swallow-wort (*Vincetoxicum rossicum*) (Apocynaceae). This European vine is invasive in eastern North America but has not yet been encountered by *C. cobaltinus* in the field. Lab tests demonstrate that *C. cobaltinus* can feed on introduced *V. rossicum* foliage, and that they are not locally-specialized to hosts from which they were collected. Thus, adult *C. cobaltinus* may use *V. rossicum* as a transient host when encountered in the field. *Chrysochus* hybrids were unable to feed on introduced *V.*

rossicum (similar to their *C. auratus* parents) but did feed on native North American *Asclepias* spp. (similar to their *C. cobaltinus* parents). Hybridization and subsequent gene introgression may explain both decreased feeding by western *C. cobaltinus* and increased feeding by eastern *C. auratus* on native *Asclepias* spp. in this region, but does not appear to affect feeding on *V. rossicum*. We predict the potential novel association between native *C. cobaltinus* and invasive *V. rossicum* will have a positive or neutral outcome for the beetles, but is unlikely to slow the spread of the vine in North America unless further adaptation occurs.

Keywords Novel association · Novel host · Invasive species · Biological control · Hybridization · Insect–plant interaction · Enemy release · Adaptation

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Introduction

When a non-native plant invades a habitat, novel associations may occur between that plant and a multitude of native insects. The majority of these novel interactions are thought to have negative effects on the native insects (Schirmel et al. 2016). For example, invasive swallow-worts in Canada and the northeastern U.S. act as oviposition sinks for native monarch butterflies, *Danaus plexippus* (L.) (DiTommaso and Losey 2003; Mattila and Otis 2003; Casagrande and Dacey 2007). Increasing evidence

suggests; however, that some novel interactions with invasive species can have positive effects on insects, providing them with a new abundant food supply or habitat refuge, which in turn allows them to expand their geographic range (Graves and Shapiro 2003; Schlaepfer et al. 2005; Agosta 2006; Rodriguez 2006; Carroll 2007; Carlsson et al. 2009). The ability to predict, or encourage, novel interactions of this type could represent an extremely valuable tool for the management and control of invasive weeds. Here we investigate the potential for novel associations between North American *Chrysochus* beetles, in particular *Chrysochus cobaltinus* LeConte (Col.: Chrysomelidae), and the invasive swallow-wort *Vincetoxicum rossicum* (Kleopow) Barbar (Apocynaceae; syn. *Cynanchum rossicum* (Kleopow) Borhidi). We subject *C. cobaltinus* to feeding and host-specificity tests, similar to those that would be conducted on candidate classical biological control agents.

In the context of this study, the term ‘novel association’ refers to a shift in host plant use by a native herbivorous insect, i.e. from a native plant species to an introduced one, such that it: (1) forms immediately following initial interaction *or* after a short adaptive period of a few generations; (2) permits either feeding, oviposition, and/or development by the native insect on the introduced plant; and (3) persists to the extent that further adaptation for use of the novel resource may occur on the part of the insect.

There are a number of factors that may increase the likelihood of a novel association forming between a native insect and a non-native plant. First, novel associations with invasive plants are more likely to occur in native ectophagous herbivores (Lawton and Schroder 1977), especially those closely-related to herbivores from the introduced plant’s country of origin (Futuyma and Mitter 1996). Such insects commonly demonstrate a broad host range (Bertheau et al. 2010), have high genetic diversity (Frankham 2005), and/or naturally feed on native plants carrying similar traits or sharing genetics with the introduced plant (Futuyma and Mitter 1996; Jobin et al. 1996; Agrawal and Kotanen 2003; Dalin and Bjorkman 2006; Pearse et al. 2013). Second, hybridization of insect herbivores [including gene introgression through backcrossing with parental species (Rhymer and Simberloff 1996)] can also lead to novel host use (Scriber 2002; Schwarz et al. 2005). These traits in and

of themselves, however, can only help predict the occurrence of novel associations, not the outcome. In fact, current models provide little information on whether novel associations will be positive, negative or neutral (McEvoy 2002; Pearse and Altermatt 2013; Pearse et al. 2013) even though such outcomes have broad ecological implications. Key attributes of species that form successful novel associations and the processes that drive them need to be explored in order to better predict the impact of species invasions in the field. Such baseline data on the evolution of novel associations will also improve predictive models for biological control and inform the management of invasive species globally.

Vincetoxicum rossicum, commonly known as pale swallow-wort in the U.S. or dog-strangling vine (DSV) in Canada, is an invasive exotic plant from Europe that threatens native plant biodiversity in forest ecosystems across eastern North America (Fig. 1) (Sheeley and Raynal 1996). To date, there has been very limited native insect herbivory observed on this plant in North America (Sheeley and Raynal 1996; Ernst and Cappuccino 2005; Milbrath and Biazzo 2012). No native *Vincetoxicum* species are known from North America (Tewksbury et al. 2002), thus removing the possibility that natural enemies can move from native plants in the same genus. Classical biological control is considered one of the few options available for long-term management of this weed, due to the rising costs of mechanical and chemical control and the lack of climatic barriers for its expansion (Lawlor and Raynal 2002; Tewksbury et al. 2002; Averill et al. 2008; Douglass et al. 2011; Sanderson and Antunes 2013).

Several candidate biological control agents have been identified from the plant’s region of origin in eastern Europe, including the beetle, *Chrysochus asclepiadeus* (Pallas) (Coleoptera: Chrysomelidae), which can cause significant plant mortality to *V. rossicum* due to its combined root-feeding larval and leaf-feeding adult stages (Weed et al. 2011a, b). Host-range testing however, showed that in laboratory tests this European beetle was also able to feed and develop on native North American milkweeds (*Asclepias* spp.), which made it an unlikely candidate for introduction (Gassmann et al. 2011, 2012; Sforza 2011). The leaf-feeding European moth, *Hypena opulenta* (Christoph) (Lepidoptera: Erebididae) has been screened and permitted for release in Canada in 2013 and in the U.S. in

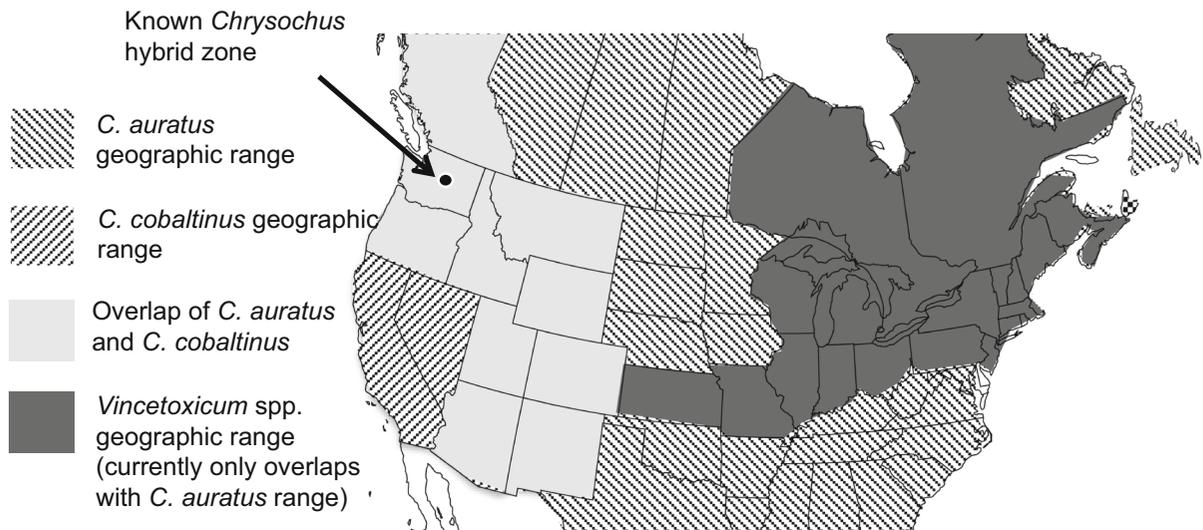


Fig. 1 The current geographic distribution of *Chrysochus* and invasive *Vincetoxicum rossicum* across the North American continent (Hatch 1953; Sheeley and Raynal 1996; Dobler and Farrell 1999; Peterson et al. 2001)

2017 (USDA-APHIS 2018). The moth has been shown to reduce above-ground plant biomass and seed set in the laboratory (Weed and Casagrande 2010; Milbrath and Biazzo 2012), but has yet to show impact on overall plant mortality in the field (R. S. Bourchier, unpubl. data). In order to further augment plant mortality and limit westward expansion of swallow-wort, new biocontrol agents are being investigated. In this regard, two native North American *Chrysochus* species are of particular interest for conservation biological control. The larvae and adults of these species feed on the roots and leaves respectively of plants in the same plant family as *V. rossicum*. Additionally, both species are closely related to the rejected classical biological control agent, *C. asclepiadeus*.

Based on the long-time European co-evolutionary association between *V. rossicum* and *C. asclepiadeus*, we hypothesized that the beetle's North American relatives, the eastern *Chrysochus auratus* Fabricius (Coleoptera: Chrysomelidae) and western *Chrysochus cobaltinus*, would both likely recognize this new host plant and potentially develop a novel association with it. In the short term, our work has shown that this is unlikely to be the case for the eastern beetle (*C. auratus*), who's geographic range currently overlaps with *V. rossicum* (Fig. 1), as adults feed exclusively on *Apocynum cannabinum* L. and *Apocynum androsaemifolium* L. (Apocynaceae), and larvae cannot develop

beyond the 2nd instar on *V. rossicum* roots (deJonge et al. 2017). As yet, the interaction between the western beetle (*C. cobaltinus*) and *V. rossicum* has not been investigated, even though this species has a higher degree of genetic diversity and expresses a broader host range than its eastern relative (Dobler and Farrell 1999). The western beetle also hybridizes with the eastern species in a small zone within Washington State, USA (Fig. 1). While the F₁ hybrids have extremely low fertility and F₂ hybrids are rare (Peterson et al. 2005), there is some evidence of weak genetic introgression between the two, thereby increasing genetic variability and the likelihood that the hybrids themselves could feed or develop on novel host plants (Peterson et al. 2001, 2005; Schwarz et al. 2005). There exists clear potential for *C. cobaltinus* and/or its hybrid (with *C. auratus*) to form novel associations with invasive swallow-worts.

The long-term goal of our work is to better understand critical biological traits and processes by which the outcome of novel associations between plants and insects can be predicted. Such predictions can inform programs for conservation biological control and invasive species management. Here, we test whether native western North American leaf beetles (*C. cobaltinus* and *C. auratus*) and their hybrids can feed and/or develop on the invasive swallow-wort, *V. rossicum*, as a necessary first step in forming a novel association. We employ conventional

host-specificity methods used for testing classical biological control agents to assess adult feeding and host-use on both native and non-native plants. In this case, we focus on whether native beetles will recognize, feed, and/or develop on the non-native host plants. Our expectation is that if *C. cobaltinus* and/or its hybrid already display significant fitness on this new plant prior to its introduction, then they will be much more likely to adapt over time to form a novel association that could contribute to the suppression of the invasive vine across North America.

Methods

Adult *Chrysochus* spp.: no-choice feeding experiments

Experiment 1: Feeding by C. cobaltinus on V. rossicum over a geographical gradient

Adult *C. cobaltinus* beetles were collected from six sites in the western U.S. during 2013: three sites in California along a north–south gradient, and three sites in central Washington State, two of which were within the *Chrysochus* hybrid zone (Table 1). Throughout their geographical range, beetles were collected from native host plants in the family Apocynaceae, including: *Asclepias eriocarpa* Benth.; *Asclepias speciosa* Torr.; *Apocynum cannabinum*; and *Asclepias fascicularis* Decne (Table 1). Adult beetles were placed in 4-L (12 cm × 12 cm × 26 cm) clear plastic containers with mesh lids and provided foliage collected from their host plants at the site. Beetles were held in ambient lab conditions (16:8 L:D, 20–25 °C) prior to testing.

We conducted no-choice feeding trials to assess whether *C. cobaltinus* would feed on *V. rossicum*, and how this feeding varied in beetles collected across their native range. No-choice feeding trials using the beetles host plant, *Ap. cannabinum*, which is found throughout the beetle's geographic range, were conducted concurrently as a control. Beetles collected from each site were evenly distributed between the two test species. Individual beetles were placed into 11 cm petri dishes with a moist filter paper and one leaf of the test species. Each leaf was scanned to measure surface area (mm²) prior to beetle placement using ImageJ software v.1.47 (Bethesda, MD). Petri

dishes were set up under ambient lab conditions (20–25 °C) with a photoperiod of 16:8 L:D; the placement of the dishes ensured that no leaves of the same plant species were adjacent to each other to account for possible variation in the lab environment. To reduce any error due to water loss and reduction in surface area, leaves were removed after 2 days and feeding galleries were traced onto a scan of the leaf to record the amount of leaf surface area (mm²) lost or fed upon.

Experiment 2: Comparison of feeding by hybrid beetles and the two parent Chrysochus species

Hybrid *Chrysochus* adults, along with adults of both parental species (*C. auratus* and *C. cobaltinus*), were collected from a single location in Mabton, WA (Table 1), at the center of the *Chrysochus* hybrid zone (Peterson et al. 2001). The distributions of the eastern and western *Chrysochus* beetles overlap in up to nine states and one province in North America (Fig. 1); however, *Chrysochus* hybrids have only been identified in this one small region in the Yakima River Valley (Peterson et al. 2001), limiting the number of *Chrysochus* hybrid beetles available for collection. A total of 120 beetles (45 *C. auratus*, 45 *C. cobaltinus*, and 30 hybrids) were collected during 2013. Species were separated on-site based on their colour (Fig. 2), and the identification of each individual was confirmed at the end of the experiment by measuring the length:width ratio of the 8th flagellomere using a digital microscope, as reported by Peterson et al. (2001). Beetles were stored as described above prior to testing.

In order to compare the feeding behaviour of hybrid beetles with that of the two parent species, we conducted no-choice feeding trials on leaves of *V. rossicum*, *Ap. cannabinum*, and *As. speciosa*. *Apocynum cannabinum* is the field host of both *C. cobaltinus* and *C. auratus* within the hybrid zone, and so was included as a control. *Asclepias speciosa* was included in the experiment to test levels of feeding on the *Asclepias* genus. *Chrysochus cobaltinus* adults are known to feed on several *Asclepias* spp., while *C. auratus* do not (deJonge et al. 2017). Each beetle species, as well as hybrids, were distributed evenly between the plant species, and feeding trials were conducted using the procedure described in experiment one.

Table 1 Collection locations for North American *Chrysochus* spp. (eastern *C. auratus*, western *C. cobaltinus*, and their hybrids from Washington state), and their native field host plants

Region	Site	Beetles collected	Decimal degrees (DD)	Field host plant	Experiment
California	UC Davis Hastings Research Centre, CA	<i>C. cobaltinus</i>	36.362724, – 121.565709	<i>As. speciosa</i>	1
	Pine Mountain Club, CA	<i>C. cobaltinus</i>	34.853610, – 119.149212	<i>As. eriocarpa</i> , <i>As. fascicularis</i>	1
	Yosemite National Park, CA	<i>C. cobaltinus</i>	37.739354, – 119.595166	<i>Ap. cannabinum</i> , <i>As. speciosa</i>	1
WA hybrid zone	Mabton, WA	<i>C. cobaltinus</i> , <i>C. auratus</i> , and <i>Chrysochus</i> hybrids	46.245556, – 120.110278	<i>Ap. cannabinum</i>	1, 2, 3
	Granger, WA	<i>C. cobaltinus</i>	46.320556, – 120.225833	<i>Ap. cannabinum</i>	1, 2
WA outside hybrid zone Ontario	Ellensburg, WA	<i>C. cobaltinus</i>	46.945833, – 120.517778	<i>Ap. cannabinum</i>	1, 3, 4, 5, 6
	Guelph, ON	<i>C. auratus</i>	43.527778, – 80.322778	<i>Ap. cannabinum</i>	3
	Dundas, ON	<i>C. auratus</i>	43.266308, – 79.941197	<i>Ap. cannabinum</i>	3

To determine host plant acceptance by beetles in this genus, adult beetles collected from the sites in the province of Ontario (ON), Canada, and the USA states of California (CA) and Washington (WA) were tested for feeding on cut leaves of invasive *V. rossicum*, *Ap. cannabinum*, and nearby *Asclepias* spp. (all in Apocynaceae). Locations of beetle collection are ordered by region

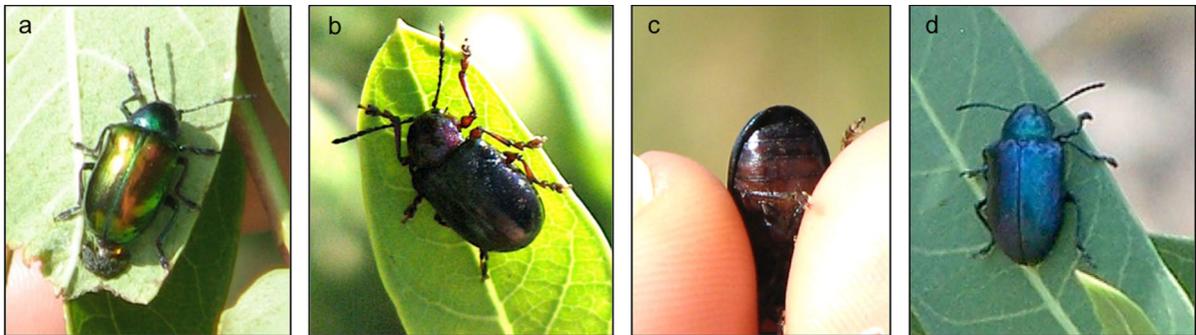


Fig. 2 North American adult *Chrysochus* beetles can be distinguished by colour: **a** *Chrysochus auratus*, both dorsal and ventral sides are iridescent green to gold, often with red tones (ovipositing female); **b** *Chrysochus* hybrid (dorsal), lacking iridescence, typically purple, though some have

intermediate colour of parents (Peterson et al. 2001); **c** *Chrysochus* hybrid (ventral), abdominal sterna are typically dull brown; and **d** *Chrysochus cobaltinus*, both dorsal and ventral sides are iridescent metallic blue

Experiment 3: Investigating the potential for gene introgression in the *Chrysochus* hybrid zone

To explore the potential effects of gene introgression on the feeding behaviour of north American *Chrysochus* beetles, we compared no-choice feeding trial results between *C. cobaltinus* and *C. auratus* collected

from sites within and outside the Washington *Chrysochus* hybrid zone. In 2013, the no-choice feeding procedure was conducted with 60 *C. cobaltinus* beetles collected from outside of the hybrid zone (Ellensburg, WA) and 45 *C. cobaltinus* beetles collected from the centre of the hybrid zone (Mabton, WA). Beetles were distributed evenly between petri

dishes containing one leaf of either *V. rossicum*, *Ap. cannabinum*, or *As. speciosa* as in experiment two. In 2015, the procedure was conducted with 36 *C. auratus* beetles collected from outside of the hybrid zone (Dundas and Guelph, ON, Canada) and 55 *C. auratus* beetles collected from the center of the hybrid zone. In this case, beetles were evenly distributed between petri dishes containing one leaf of either *V. rossicum*, *Ap. cannabinum*, or *As. eriocarpa*. We chose to use *As. eriocarpa*, a milkweed not found in Washington state or Ontario, Canada, to ensure that all *C. auratus* beetles in this experiment were equally naïve to the *Asclepias* species tested.

Adult *Chrysochus cobaltinus*: greenhouse study

Experiment 4: Survival, feeding, and oviposition of C. cobaltinus adults on V. rossicum

To determine whether *C. cobaltinus* adults were able to survive, oviposit on, and cause damage to live *V. rossicum* plants, a no-choice test with adults on potted plants within a greenhouse was conducted. A set of four beetles (two male, two female) collected from Ellensburg, WA were placed on each of nine *Ap. cannabinum* and nine *V. rossicum* plants in August 2013. Pots were tightly netted and placed on a greenhouse bench with no plants of the same species directly adjacent to each other. The beetles were monitored daily; any dead beetles were replaced with beetles of the same sex from the same collection site/date. Egg masses were counted daily, and the test was ended after 12 days. At the end of the test, egg masses were collected and placed singly in microcentrifuge tubes to monitor larval hatching. Adult beetles were frozen and later dissected to confirm sex and count the number of eggs retained by females. The effects of beetles on plant growth/health were determined by measuring plant height before and after the test. Additionally, at the end of the test, plants were dried and weighed. Dry weight was compared with control plants on which no beetles were placed.

Chrysochus cobaltinus larvae: feeding and development

Experiment 5: Laboratory feeding trials of C. cobaltinus larvae

To determine whether early-instar larvae can feed on the roots of *V. rossicum*, a no-choice lab experiment with *C. cobaltinus* larvae was conducted in petri dishes, using root segments of *V. rossicum*, *Ap. cannabinum*, and *As. syriaca*. Adult *C. cobaltinus* beetles were collected from *Ap. cannabinum* plants in Ellensburg, WA, and, therefore, *Ap. cannabinum* roots were used as a control. *Asclepias syriaca* was included as an alternative novel host plant in the event that larval feeding was not observed on *V. rossicum*. Including a second novel host plant, in the same family (Apocynaceae) as the known host, allowed us to determine whether avoidance of *V. rossicum* was because of fidelity to its native hosts, or whether the roots of *V. rossicum* in particular were unpalatable to the larvae. Beetles were kept in clear plastic, vented, 4-L breeding chambers with host foliage; egg masses from these adults were collected daily, and stored singly in microcentrifuge tubes until hatched. On 11 August 2015, 27 recently-hatched 1st-instar larvae were placed in groups of nine per petri dish, on cut root segments (8–10 mm in length) of each of the test species. Each week, larvae were given freshly-cut roots and were counted to determine their longevity and survival. Dead larvae and head capsules were preserved in 75% ethanol for later measurement using a digital microscope (Dino-Lite AM413TA) and image processing software [ImageJ software (v.1.47)].

Experiment 6: Greenhouse study of C. cobaltinus larval development on V. rossicum

To determine whether *C. cobaltinus* larvae can complete development on *V. rossicum* roots, a no-choice test was conducted using potted plants. On 3 August 2015, 20 pots of *Ap. cannabinum* and *V. rossicum* each received 1st-instar larvae hatched from eggs laid by adults collected from Ellensburg, WA as above. Two larval densities (11 and 16 larvae) were tested to assess their effects on plant biomass with 10 replicates per plant species. Larvae were placed on the soil at the base of each plant stem. An additional 20 pots of each plant species were used as controls to

monitor the growth of plants without larvae in order to determine whether plant biomass of either species was reduced by larval feeding. Plants were grown in 3.5L-pots in a greenhouse, and screened both top and bottom with netting to prevent beetle escape. Plants were placed on a greenhouse bench in a wooden garden box filled with sawdust up to the pot rims to provide insulation. The pots were dissected after 85 days (allowing for sufficient time for beetles to develop into late-instar larvae/pupae). All live larvae in the soil were counted, and head capsules measured. Roots were cleaned, dried at 50 °C for 10 days, and then the dry weight was recorded.

Statistical analysis

For experiment one, G tests were used to compare the presence of feeding between the different geographical sites and host plants for all adult, no-choice feeding tests conducted in the lab. The G test test was used rather than the more common χ^2 test as it is based on a multinomial distribution and is robust for smaller sample sizes (Gotelli and Ellison 2004). We used Kruskal–Wallis tests to compare feeding amounts among geographical regions (grouped as California, Washington hybrid zone, Washington outside of hybrid zone). Separate Kruskal–Wallis and Mann–Whitney U tests were conducted on the data from California and the hybrid zone respectively. These tests were run to detect any differences in the amount of feeding accruing among sites within the same region.

For experiment two, we used G tests to compare the presence of feeding by hybrid beetles and the two parent species on *V. rossicum*, *Ap. cannabinum* and *As. speciosa*. Kruskal–Wallis tests were used to compare the quantity of leaf material removed by the three beetle species among the three plant species. Mann–Whitney U tests were used for pairwise comparisons.

For experiment three, we used Mann–Whitney U tests to compare *C. cobaltinus* and *C. auratus* feeding inside and outside of the *Chrysochus* hybrid zone.

For experiment four, *t* tests were used to compare final dry weight of plants treated with four adult *C. cobaltinus* beetles with untreated control plants. Growth (height) of plants with and without beetles was compared using ANOVA with repeated measures (pre-height and post-height). ANOVA was also used to compare the number of beetles that died on *Ap.*

cannabinum and *V. rossicum* during the test. Both the mean numbers of egg masses laid and retained by female *C. cobaltinus* placed on *V. rossicum* or *Ap. cannabinum* plants were compared using *t* tests.

For experiment five, no statistical analyses could be conducted due to the limited number of early-instar larvae of *C. cobaltinus* available. Descriptive statistics are, however, presented in the results section.

For experiment six, mean dry root weights of potted *V. rossicum* and *Ap. cannabinum* plants with *C. cobaltinus* larvae were compared with untreated control plants using an ANOVA with a post hoc Tukey's HSD. Throughout this study non-parametric analyses were conducted using SPSS version 25 (IBM, Armonk, USA). Parametric analyses were performed using R software version 3.2.4 (Very Secure Dishes) (R Development Core Team 2016). G tests were carried out using the G test calculator (McDonald 2014).

Results

Adult *Chrysochus* spp.: no-choice feeding experiments

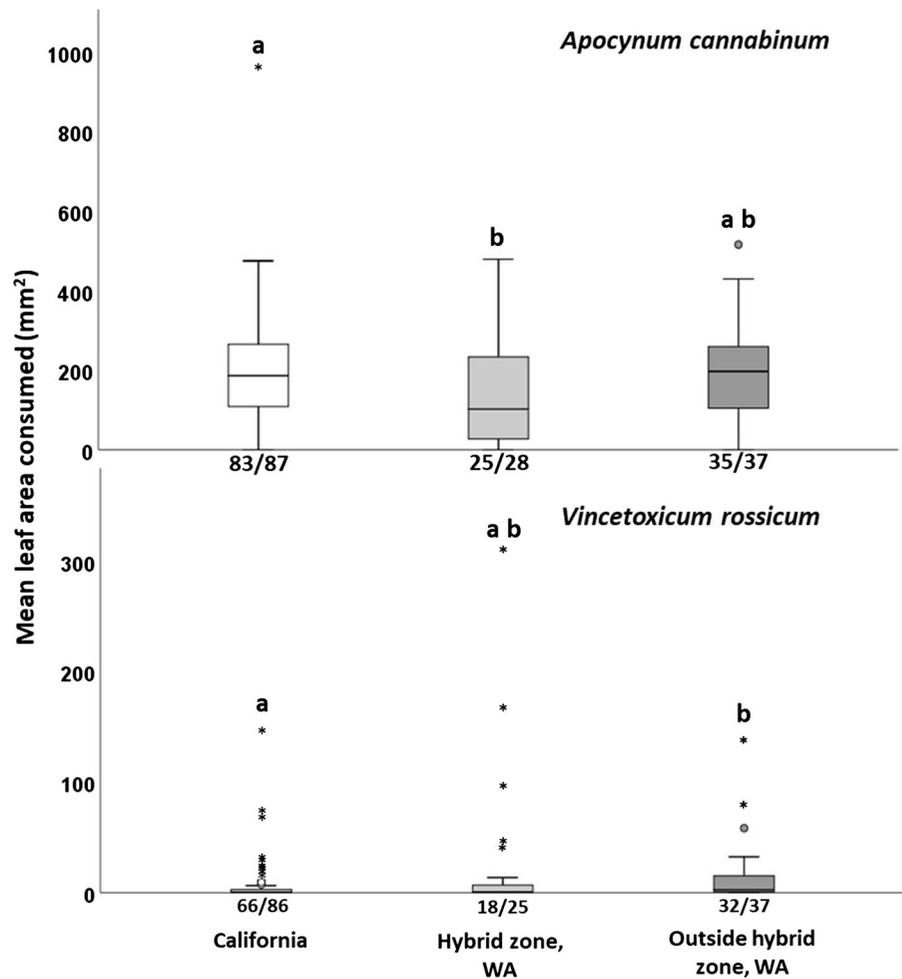
Experiment 1: Feeding by C. cobaltinus on V. rossicum over a geographical gradient

Adult *C. cobaltinus* beetles from all sites fed on *V. rossicum* and there was no difference among the sites in the presence/absence of feeding on *V. rossicum* ($G_2 = 2.297$, $P = 0.317$) nor *Ap. cannabinum* ($G_2 = 1.257$, $P = 0.533$). *Chrysochus cobaltinus* adults from all sites fed more often on *Ap. cannabinum* than they did on *V. rossicum* ($G_1 = 16.449$, $P < 0.001$) (Fig. S1).

No differences in the overall quantity of feeding (on both *V. rossicum* and *Ap. cannabinum*) were observed among sites within California ($N = 174$, $z = 5.988$, $df = 2$, $P = 0.051$), or the hybrid zone ($N = 53$, $z = 295.0$, $df = 1$, $P = 0.519$) (Fig. S1). Beetles from WA outside the hybrid zone were all collected from a single site. The absence of among-site differences in feeding, within each region, meant that site could be excluded as a factor from among region tests.

The geographical location where beetles were collected (California sites, Washington hybrid zone, and Washington outside of hybrid zone) had a

Fig. 3 Mean leaf area of *Apocynum cannabinum* and *Vincetoxicum rossicum* fed on by *Chrysochus cobaltinus* beetles collected in California and Washington State during 2013. Beetles are grouped into California (3 sites), Washington inside the Hybrid Zone, WA (1 sites), and Washington outside the Hybrid Zone, WA (2 sites). Box plots are arranged from south to north, and represent a 5 number summary of the data (minimum, first quartile, median, third quartile, and maximum). Open circles represent outliers ($1.5 \times$ interquartile range (IQR)). Stars represent extreme values ($3 \times$ IQR). Letters indicate significant differences. Numbers below the bars represent the number of beetles that fed versus the number of beetles tested



significant impact on feeding, both on *V. rossicum* ($N = 149$, $z = 8.559$, $df = 2$, $P = 0.014$) and *Ap. cannabinum* ($N = 152$, $z = 6.788$, $df = 2$, $P = 0.034$). Beetles from Washington, outside of the *Chrysochus* hybrid zone, removed significantly more leaf material from *V. rossicum* (13.351 ± 4.429 mm²) than beetles from California (6.279 ± 1.957 mm²) ($N = 124$, $z = -2.864$, $P = 0.013$). No differences in *V. rossicum* feeding were observed between beetles from the hybrid zone and beetles from California ($N = 112$, $z = -0.165$, $P = 1.000$), or between beetles from inside and outside the hybrid zone in Washington ($N = 62$, $z = -2.027$, $P = 0.128$). Beetles collected from California removed significantly more leaf material from native host *Ap. cannabinum* (200.582 ± 15.303 mm²) than beetles from the hybrid zone (131.688 ± 23.717 mm²) ($N = 115$, $z = 2.459$,

$P = 0.042$). No differences in feeding on *Ap. cannabinum* were observed between beetles from the hybrid zone and Washington outside the hybrid zone ($N = 65$, $z = -2.263$, $P = 0.071$), or between Washington outside the hybrid zone and California ($N = 124$, $z = -0.166$, $P = 1.000$) (Fig. 3).

Experiment 2: Comparison of feeding by hybrid beetles and the two parent *Chrysochus* species

The ability to distinguish between the different adult *Chrysochus* beetle species and their hybrids using colour was confirmed by the flagella length:width ratios as initially presented in Peterson et al. (2001). All 45 beetles identified using morphology as *C. auratus* had flagella l:w ratios larger than 1.546 (1.733 ± 0.021 mm), while all 45 *C. cobaltinus*

beetles had flagella l:w ratios smaller than 1.472 (1.249 ± 0.026 mm), and all 30 hybrid beetles had flagella l:w ratios intermediate of both parent species (1.487 ± 0.030 mm).

The presence of feeding by the *Chrysochus* hybrids was not significantly different from either parent species on the novel non-native host *V. rossicum* ($G_2 = 2.963$, $P = 0.227$) or the native host from which the beetles had been collected, *Ap. cannabinum* ($G_2 = 4.639$, $P = 0.098$). Hybrid beetles fed on *As. speciosa* significantly more often than *C. auratus* parents ($G_1 = 13.104$, $P < 0.001$) but fed as often on *As. speciosa* as *C. cobaltinus* parents ($G_1 = 0.089$, $P = 0.766$).

The quantity of leaf material removed from *V. rossicum* ($N = 40$, $z = 7.880$, $df = 2$, $P = 0.019$) and *As. speciosa* ($N = 40$, $z = 21.544$, $df = 2$, $P < 0.001$) varied significantly among the three beetle species. No significant differences in the quantity of feeding were observed among beetle species on *Ap. cannabinum* ($N = 40$, $z = 2.448$, $df = 2$, $P \leq 0.294$).

Chrysochus cobaltinus fed on *V. rossicum* in significantly greater amounts (45.714 ± 22.635 mm²) than did *C. auratus* (0.117 ± 0.061 mm²) ($N = 30$, $z = -2.552$, $P = 0.032$). No difference was observed in the amount of *V. rossicum* leaf material removed by hybrid beetles and *C. auratus* ($N = 25$, $z = -0.094$, $P = 1.000$), or *C. cobaltinus* ($N = 25$, $z = -2.188$, $P = 0.086$).

Both *C. cobaltinus* (33.682 ± 10.426 mm²) ($N = 30$, $z = -4.094$, $P \leq 0.001$) and hybrid beetles (41.603 ± 18.669 mm²) ($N = 25$, $z = -3.787$, $P \leq 0.001$) fed significantly more on *As. speciosa* than *C. auratus* (0.322 ± 0.192 mm²). No difference in feeding on *As. speciosa* was observed between hybrid beetles and *C. cobaltinus* ($N = 25$, $z = 0.125$, $P = 1.000$) (Fig. 4).

Experiment 3: Investigating potential for gene introgression in the *Chrysochus* hybrid zone

Chrysochus cobaltinus beetles from outside the hybrid zone (Ellensburg, WA), and from the center of the hybrid zone (Mabton, WA), showed no difference in feeding amounts on either *V. rossicum* ($N = 35$, $z = -0.402$, $P = 0.705$) or the native host plant *Ap. cannabinum* ($N = 35$, $z = -1.334$, $P = 0.191$). Beetles outside of the hybrid zone did however

demonstrate a higher amount of feeding on *As. speciosa* (147.580 ± 21.725 mm²) compared to those from the center of the zone (33.682 ± 10.426 mm²) ($N = 35$, $z = -3.867$, $P < 0.001$) (Fig. 5). For *C. cobaltinus* beetles, no difference in the quantity of feeding was observed between *Ap. cannabinum* and *As. speciosa* ($N = 70$, $z = -1.004$, $P = 0.315$), and this remained the case when beetles inside ($N = 30$, $z = -1.639$, $P = 0.106$) and outside ($N = 40$, $z = -0.108$, $P = 0.925$) of the hybrid zone were analyzed separately.

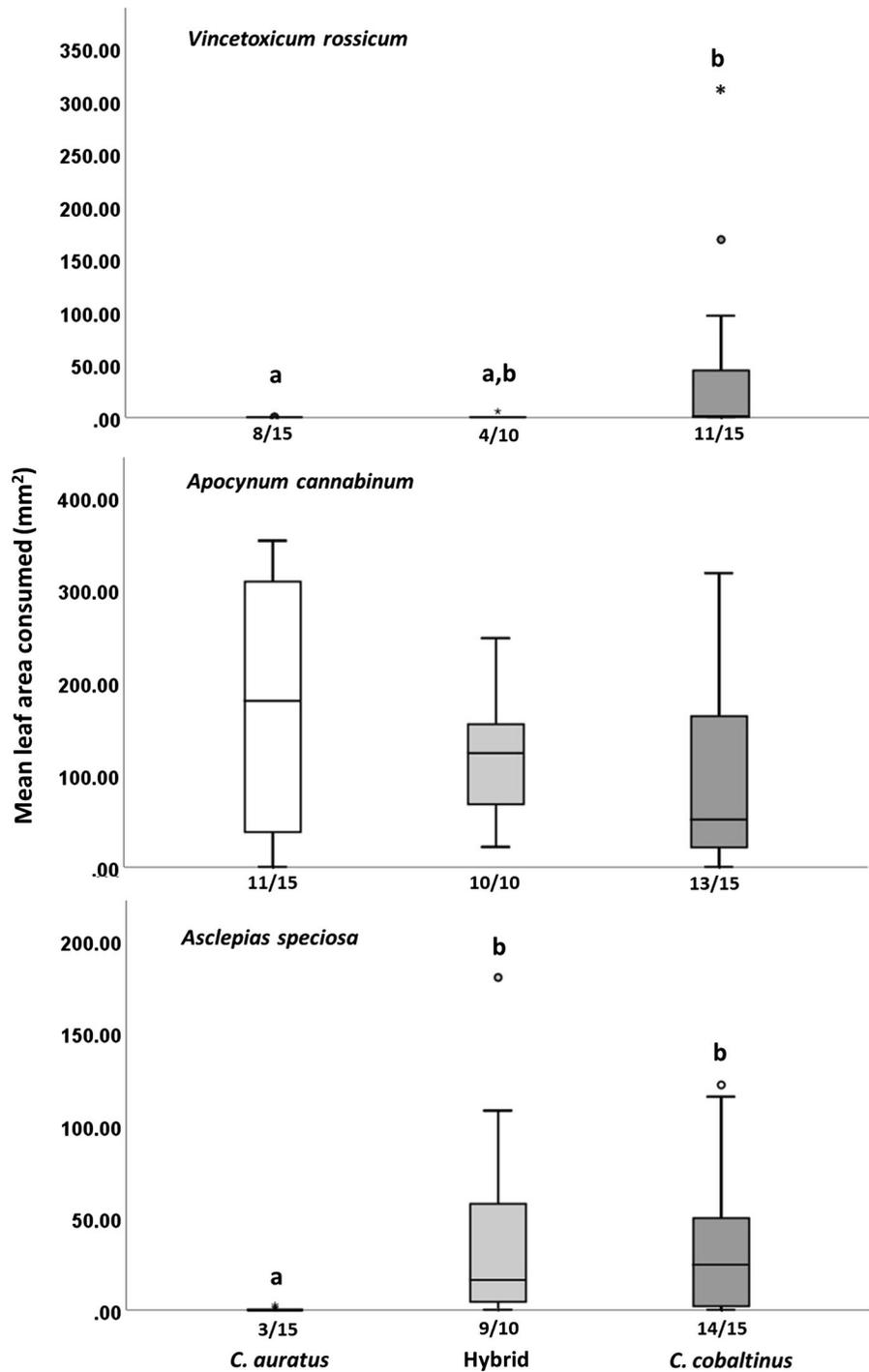
In the no-choice lab feeding tests with *C. auratus* collected from within and outside the hybrid zone, there was no difference in the amount of feeding on *V. rossicum* between adults within or outside the *Chrysochus* hybrid zone ($N = 32$, $z = -0.440$, $P = 0.803$). We also observed no difference in leaf material removed from *Ap. cannabinum* ($N = 32$, $z = -0.467$, $P = 0.659$) or *As. eriocarpa* ($N = 30$, $z = -0.314$, $P = 0.659$) by *C. auratus* collected from inside or outside of the *Chrysochus* hybrid zone.

Adult *Chrysochus cobaltinus*: greenhouse study

Experiment 4: Survival, feeding, and oviposition of *C. cobaltinus* adults on *V. rossicum*

In the no-choice greenhouse experiment on live potted plants, the dry weight of *V. rossicum* plants treated with four adult *C. cobaltinus* beetles was the same as that of the untreated (no beetle) control plants ($t_{15.305} = 0.3743$, $P = 0.713$). Mean growth of *V. rossicum* plants (post-test height–pre-test height) with beetles present (23.33 ± 4.72 mm) was 17% less than plants without beetles (27.94 ± 5.02 mm), however this was not statistically significant ($F_{1,48} = 0.321$, $P = 0.586$). More beetles died on *V. rossicum* (4.777 ± 0.434) than on *Ap. cannabinum* (1.666 ± 0.471) over the 12-day test ($F_{1,16} = 23.579$, $P < 0.001$) (Fig. 6). Female *C. cobaltinus* laid egg masses on *V. rossicum* (2.55 ± 0.63), but in far lower numbers than on *Ap. cannabinum* (31.44 ± 6.44) ($t_{8.06} = 4.078$, $P < 0.010$) (Fig. 6). Females on *V. rossicum* had more eggs remaining in their ovaries at the end of the test (8.66 ± 1.04) than females on *Ap. cannabinum* (6.55 ± 1.06), but this difference was not significant ($t_{33.992} = -1.417$, $P = 0.165$).

Fig. 4 Mean feeding by adult *Chrysochus auratus*, *C. cobaltinus*, and their hybrids on cut leaves of test plant species. All beetles were collected from the center of the hybrid zone (Mabton, WA) in 2013. Numbers below the bars are the number of beetles that fed versus the number of beetles tested for each site. Box plots represent a 5 number summary of the data (minimum, first quartile, median, third quartile, and maximum). Open circles represent outliers ($1.5 \times$ interquartile range (IQR). Stars represent extreme values ($3 \times$ IQR). Letters indicate significant differences. Numbers below the bars represent the number of beetles that fed versus the number of beetles tested



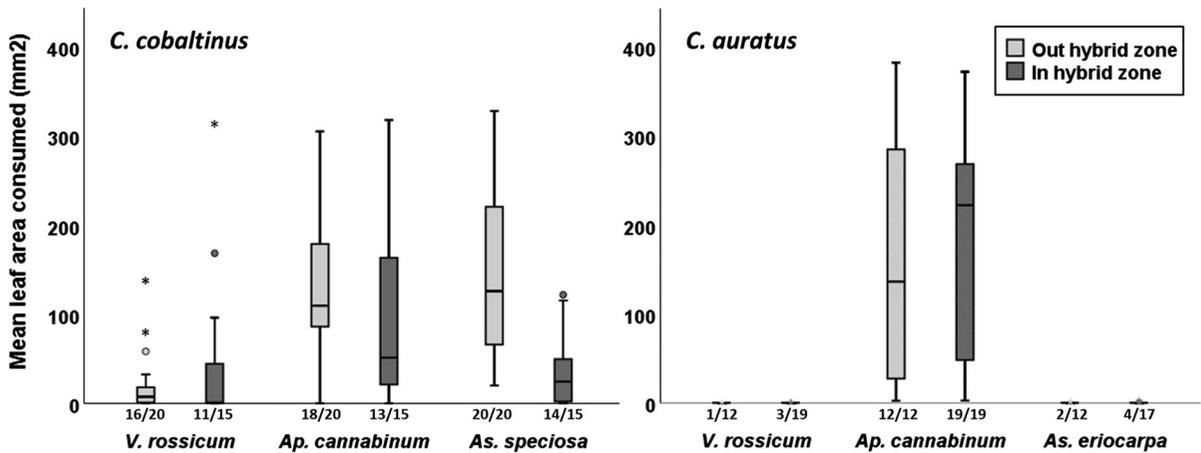


Fig. 5 Mean feeding on *Vincetoxicum rossicum*, *Apocynum cannabinum*, and *Asclepias speciosa*, by adult *Chrysochus cobaltinus* collected from inside and outside of the *C. auratus* × *C. cobaltinus* hybrid zone. Numbers below the bars are the number of beetles that fed versus the number of beetles tested for each site. Box plots represent a 5 number summary of

the data (minimum, first quartile, median, third quartile, and maximum). Open circles represent outliers (1.5 × interquartile range (IQR)). Stars represent extreme values (3 × IQR). Letters indicate significant differences. Numbers below the bars represent the number of beetles that fed versus the number of beetles tested

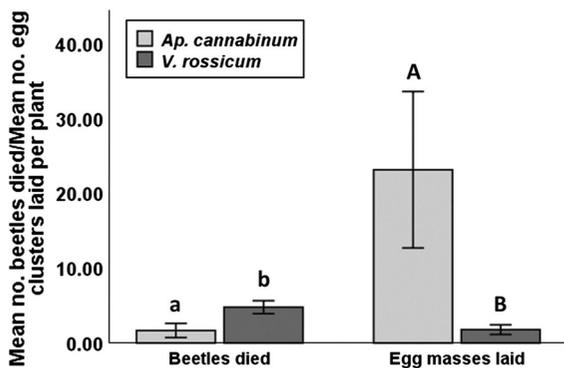


Fig. 6 Mean number of *Chrysochus cobaltinus* beetles dying on potted *Vincetoxicum rossicum* and *Apocynum cannabinum* plants during experiment 4. Mean number of egg masses laid by females during experiment 4. Letters indicate significant differences. Error bars represent standard error

Chrysochus cobaltinus larvae: feeding and development

Experiment 5: Laboratory feeding trials of C. cobaltinus larvae

Early-instar larvae of *C. cobaltinus* fed and completed at least one moult on the roots of all three plant species (*V. rossicum*, *Ap. cannabinum*, and *As. syriaca*) during the lab test in petri dishes. Larvae survived the longest on *Ap. cannabinum* (40.555 ± 7.694 days), followed

by *As. syriaca* (23.111 ± 10.386 days). Larvae survived for the shortest time on *V. rossicum* (22.778 ± 8.225 days). Head capsule measurements confirmed that only larvae feeding on *As. syriaca* completed a second moult during the test.

Experiment 6: Greenhouse study of C. cobaltinus larval development on *V. rossicum*

During the no-choice greenhouse trials with potted plants, no *C. cobaltinus* larvae were found on the roots or within any of the pots containing *V. rossicum*, while developing larvae or pupae were found in four of the 20 pots containing *Ap. cannabinum*. There was no significant difference between the dry weight of *V. rossicum* roots treated with 11 or 16 larvae, compared to control roots (no larvae) (F_{2,37} = 0.997, P = 0.378). Roots from *Ap. cannabinum* plants treated with 16 larvae (5.219 ± 0.622 g) weighed significantly less than those from the control group (2.341 ± 0.592 g) (F_{2,37} = 4.589, P < 0.050) (Fig. 7).

Discussion

Our results suggest that the western leaf beetle, *C. cobaltinus*, is able to form a novel association with the

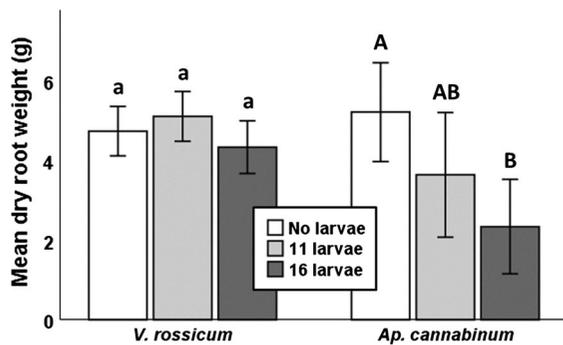


Fig. 7 Mean dry root weights of potted *Vincetoxicum rossicum* and *Apocynum cannabinum* plants after an 85 day exposure to 0, 11, and 16 *Chrysochus cobaltinus* larvae. Letters indicate significant differences. Error bars represent standard error

invasive vine, *V. rossicum*. Although *C. cobaltinus* beetles fed in higher quantities, and survived longer, on their native host plant, *Ap. cannabinum*, they were able to recognize *V. rossicum* as a potential host plant and feed on it for up to 12 days (the full length of the no-choice feeding trial in the greenhouse). The mean feeding on *V. rossicum* by *C. cobaltinus* at all sites in this study ($14.835 \pm 3.662 \text{ mm}^2$) was significantly higher than that of the eastern beetle, *C. auratus*, measured during a previous study ($0.644 \pm 0.218 \text{ mm}^2$) (deJonge et al. 2017). The difference between these two species is biologically significant as *C. auratus* adults never fed on *V. rossicum* beyond exploratory bites, whereas *C. cobaltinus* caused visible feeding damage on leaves and stems. The feeding demonstrated by *C. cobaltinus* is an important first step in forming a novel association with the vine, and may enable this native beetle to use introduced *V. rossicum* as an intermittent host for adult feeding when the vine eventually spreads into the region. As the range of *V. rossicum* continues to expand westward, feeding by *C. cobaltinus* on the vine is likely to increase due to higher rates of exposure and greater opportunity for adaptation to this novel host plant (Bezemer et al. 2014).

Although *C. cobaltinus* is known to be less host-specific than the eastern beetle, *C. auratus* (deJonge et al. 2017), previous studies have suggested that *C. cobaltinus* tends to be locally-specialized on a single host (Dobler and Farrell 1999). Our results, however, refute this idea. First, during experiment one, at the Yosemite, CA site, we collected *C. cobaltinus* adults from both *C. cannabinum* and *As. speciosa*, and we

regularly observed beetles flying between the two native plant species. Second, during experiment three, *C. cobaltinus* beetles collected from Ellensburg WA fed in equal amounts on two host species, *Ap. cannabinum* and *As. speciosa*, despite having been collected exclusively from the former. Our previous work has also shown that *C. cobaltinus* adults often feed extensively on native Apocynaceae to which they have not previously been exposed (deJonge et al. 2017). This ability to use multiple host plants in the field, and to feed on novel Apocynaceae plants, increases the likelihood that *C. cobaltinus* will form novel associations with *V. rossicum*, even if other acceptable native host plants are available.

Genetic introgression describes the flow of genes from one species, into the gene pool of another, through repeated backcrossing between hybrid individuals and their parent species (Peterson et al. 2001, 2005). This phenomenon may be one mechanism behind some of the observed differences in host use within and outside the Washington hybrid zone. For example, we observed a decrease in feeding on *As. speciosa* by *C. cobaltinus* within the hybrid zone (Fig. 4). Feeding by *C. cobaltinus* on *V. rossicum* was also reduced in the hybrid zone compared with that observed at California sites (Fig. 3). These results suggest that the ecological host-range of hybrid beetles may be somewhat restricted compared to that of *C. cobaltinus*, perhaps as a result of gene introgression from the more host-specific *C. auratus*. *Chrysochus auratus* does not normally feed or develop on *Asclepias* spp. in the field, whereas *C. cobaltinus* uses *Asclepias* spp. as a host in addition to *Apocynum* spp. (Dobler and Farrell 1999). It is unlikely; therefore, that *Chrysochus* hybrids will ever feed on *V. rossicum* in amounts necessary to form a novel association. We did, however, observe large variation in the level of feeding by *C. cobaltinus* on *V. rossicum* within the Washington hybrid zone. For example, one beetle collected from the Washington hybrid zone fed on over 300 mm^2 of *V. rossicum* leaf during no-choice tests. Under the same conditions, average feeding on *Ap. cannabinum* was only $187.75 \pm 10.93 \text{ mm}^2$. *Chrysochus* hybridization may be one mechanism behind the high variability in adult feeding for this region as hybridization is known to add genetic diversity and increase the use of novel hosts in other systems (Seehausen 2004; Abbott et al. 2013).

Our study shows regional differentiation in feeding by a native beetle (*C. cobaltinus*) on a non-host plant (*V. rossicum*) before any opportunity for adaptation has occurred. It is likely, therefore, that genetic diversity may play an important role in host plant choice and the acceptance of novel hosts. Further genetic study is warranted, as identification of specific alleles associated with increased feeding ability by *Chrysochus* on *V. rossicum* would allow us to better predict those geographical regions where native western beetles might have a greater impact on *V. rossicum* infestations.

Chrysochus beetles have the potential to be excellent biological control agents as they feed on both the roots and the foliage of their host plants during their larval and adult stages respectively. According to the multiple stress hypothesis, most plants require more than one stressor in order to be effectively suppressed (Harris 1991). Our results, however, indicate that the larvae of *C. cobaltinus* survive for only a short time on *V. rossicum*, and feeding on the roots is minimal. While there remains potential for *C. cobaltinus* to be a useful tool in slowing the spread of *V. rossicum* in the western U.S., it appears that its impact on the invasive plant will, at least initially, be limited to defoliation by adult beetles.

Several of the life history traits associated with *C. cobaltinus* beetles (high genetic diversity, ectophagous, broad feeder on plants closely related to *V. rossicum*) suggest that a novel association between this native beetle and the invasive *V. rossicum* is highly likely. Without conducting host-specificity tests, however, the potential outcomes of such an association would be difficult to predict. The host-specificity testing that we conducted points to an initial neutral or positive outcome for the western *C. cobaltinus* beetles as *V. rossicum* may provide an additional food source for adults, while ovipositional errors on the vine are limited. In contrast, if these tests had demonstrated a high level of *C. cobaltinus* oviposition on *V. rossicum*, combined with no larval survival, we would predict a novel association with an initially negative outcome for the beetles, similar to that which has been observed between *V. rossicum* and monarch butterflies (DiTommaso and Losey 2003; Mattila and Otis 2003; Casagrande and Dacey 2007).

Our study highlights the importance of conducting host-specificity testing, similar to that done in biological control research, in order to predict the potential

outcomes of a novel association. Although novel associations with invasive plants often result in negative outcomes for native insects (Schirmel et al. 2016), this should not be assumed. Some novel associations can result in a positive outcome for the native insect (Sheldon and Creed 2003; Cogni 2010), while other outcomes may transition with continued exposure and subsequent adaptation to an invasive plant (Keeler and Chew 2008; Dai et al. 2014). The study of novel associations, with clear consideration for their potential outcomes, can inform and improve invasive species management.

Assessing the likely outcomes of novel associations on a landscape scale requires consideration of several additional factors. First, different genotypes of an insect species may vary in their ability to adapt and/or accept a novel host (García-Robledo and Horvitz 2012; Messina and Durham 2013). Studies predicting the outcomes of novel associations should, therefore, include insects from a number of different populations. Where possible, insects should also be reared in the laboratory on a common host for several generations before testing, to control for previous host plant exposure and for potential maternal effects. In the present study, tests were performed over a large geographical area, and the facilities and time required for rearing insects were not available. Care was taken, however, to control for previous host plant exposure. The *Asclepias* spp. tested in experiments two, three, and five, for example, were chosen such that all or none of the beetles tested would have had the opportunity for previous exposure to the plant.

The study of novel associations generally focusses on existing interactions, and few studies have pre-emptively investigated the potential for such an association to form (but see Chupp and Battaglia 2014; Dalosto et al. 2015; Pfammatter et al. 2015; deJonge et al. 2017). In the field of classical biological control, however, such studies are the norm (van Lenteren et al. 2006). The goal of host-range testing in classical biological control is to identify potentially deleterious novel associations between non-target native species and the proposed biocontrol agent (van Lenteren et al. 2006). Knowledge of life-history traits, genetics, and the behaviour of both the agent and target species, all contribute to better predictions of the ecological host-range and the efficacy of a biocontrol candidate (Schaffner et al. 2018; Hinz et al. 2014; Schaffner 2001). We suggest that ecological studies

concerned with the impacts of invasive species should consider borrowing from methods typically used in classical biological control in order to better understand novel associations that may form and their potential impacts on native species. Predicting novel associations, as well as their outcomes, can be a crucial tool informing invasive species management decisions.

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References

- Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJE, Bierne N et al (2013) Hybridization and speciation. *J Evol Biol* 26:229–246
- Agosta SJ (2006) On ecological fitting, plant–insect associations, herbivore host shifts, and host plant selection. *Oikos* 114:556–565
- Agrawal AA, Kotanen PM (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecol Lett* 6:712–715
- Averill KM, DiTommaso A, Morris SH (2008) Response of pale swallow-wort (*Vincetoxicum rossicum*) to Triclopyr application and clipping. *Invasive Plant Sci Manag* 1:196–206
- Bertheau C, Brockerhoff EG, Roux-Morabito G, Lieutier F, Jactel H (2010) Novel insect–tree associations resulting from accidental and intentional biological “invasions”: a meta-analysis of effects on insect fitness. *Ecol Lett* 13:506–515
- Bezemer TM, Harvey JA, Cronin JT (2014) Response of native insect communities to invasive plants. *Annu Rev Entomol* 59:119–141
- Carlsson NOL, Sarnelle O, Strayer DL (2009) Native predators and exotic prey—An acquired taste? *Front Ecol Environ* 7:525–532
- Carroll SP (2007) Brave new world: the epistatic foundations of natives adapting to invaders. *Genetica* 129:193–204
- Casagrande RA, Dacey JE (2007) Monarch butterfly oviposition on swallow-worts (*Vincetoxicum* spp.). *Environ Entomol* 36:631–636
- Chupp AD, Battaglia LL (2014) Potential for host shifting in *Papilio palamedes* following invasion of laurel wilt disease. *Biol Invasions* 16:2639–2651
- Cogni R (2010) Resistance to plant invasion? A native specialist herbivore shows preference for and higher fitness on an introduced host. *Biotropica* 42:188–193
- Dai H, Lu X, Zhang J, Ding J (2014) Responses of a native beetle to novel exotic plant species with varying invasion history. *Ecol Entomol* 39:118–124
- Dalin P, Bjorkman C (2006) Chapter four: native insects colonizing introduced tree species—patterns and potential risks. In: Paine TD (ed) *Invasive forest insects, introduced forest trees and altered ecosystems*. Springer, Berlin, pp 63–77
- Dalosto MM, Palaoro AV, Souty-Grosset C, Bueno SLDS, Loureiro TG, Almerão MP et al (2015) One step ahead of the enemy: investigating aggressive interactions between invasive and native crayfish before the contact in nature. *Biol Invasions* 17:3503–3515
- deJonge RB, Bourchier RS, Smith SM (2017) Initial response by a native beetle, *Chrysochus auratus* (Coleoptera: Chrysomelidae), to a novel introduced host-plant, *Vincetoxicum rossicum* (Gentianales: Apocynaceae). *Environ Entomol* 46:617–625
- DiTommaso A, Losey JE (2003) Oviposition preference and larval performance of monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species. *Entomol Exp Appl* 108:205–209
- Dobler S, Farrell BD (1999) Host use evolution in *Chrysochus* milkweed beetles: evidence from behaviour, population genetics and phylogeny. *Mol Ecol* 8:1297–1307
- Douglass CH, Weston LA, Wolfe D (2011) Phytotoxicity and potential allelopathy in pale (*Cynanchum rossicum*) and black swallowwort (*C. nigrum*). *Invasive Plant Sci Manag* 4:133–141
- Ernst CM, Cappuccino N (2005) The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. *Biol Invasions* 7:417–425
- Frankham R (2005) Genetics and extinction. *Biol Conserv* 126:131–140
- Futuyma DJ, Mitter C (1996) Insect–plant interactions: the evolution of component communities. *Philos Trans Biol Sci* 351:1361–1366
- García-Robledo C, Horvitz CC (2012) Jack of all trades masters novel host plants: positive genetic correlations in specialist and generalist insect herbivores expanding their diets to novel hosts. *J Evol Biol* 25:38–53
- Gassmann A, Leroux AM, Bennett M, Penic M (2011) Weed biological control: quarterly report 30 June 2011. Delemont, CH
- Gassmann A, Bennett M, Penic M, Leroux AM (2012) Biological control of swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*—Annual Report 2011. Delemont, CH
- Gotelli N, Ellison A (2004) *A primer of ecological statistics*. Sinauer Associates Inc, Sunderland
- Graves SD, Shapiro AM (2003) Exotics as host plants of the California butterfly fauna. *Biol Conserv* 110:413–433
- Harris P (1991) Classical biocontrol of weeds: its definition, selection of effective agents, and administrative-political problems. *Can Entomol* 123:827–849
- Hatch M (1953) *The beetles of the Pacific Northwest*. Washington University Press, Seattle, pp 157–239

- Hinz HL, Schwarzlander M, Gassmann A, Bouchier RS (2014) Successes we may not have had: a retrospective analysis of selected weed biological control agents in the United States. *Invasive Plant Sci Manag* 7:565–579
- Jobin A, Schaffner U, Nentwig W (1996) The structure of the phytophagous insect fauna on the introduced weed *Solidago altissima* in Switzerland. *Entomol Exp Appl* 79:33–42
- Keeler MS, Chew FS (2008) Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. *Oecologia* 156:559–568
- Lawlor FM, Raynal DJ (2002) Response of swallow-wort to herbicides. *Weed Sci* 50:179–185
- Lawton JH, Schroder D (1977) Effects of plant type, size of geographical range and taxonomic isolation on number of insect species associated with British plants. *Nature* 265:137–140
- Mattila HR, Otis GW (2003) A comparison of the host preference of monarch butterflies (*Danaus plexippus*) for milkweed (*Asclepias syriaca*) over dog-strangler vine (*Vincetoxicum rossicum*). *Neth Entomol Soc* 107:193–199
- McDonald JH (2014) Handbook of biological statistics, 3rd edn. Sparky House Publishing, Baltimore
- McEvoy PB (2002) Insect–plant interactions on a planet of weeds. *Entomol Exp Appl* 104:165–179
- Messina FJ, Durham SL (2013) Adaptation to a novel host by a seed beetle (Coleoptera: Chrysomelidae: Bruchinae): effect of source population. *Environ Entomol* 42:733–742
- Milbrath LR, Biazzo J (2012) Development and reproduction of the foxglove aphid (Hemiptera: Aphididae) on invasive swallow-worts (*Vincetoxicum* spp.). *Environ Entomol* 41(3):665–668
- Pearse IS, Altermatt F (2013) Predicting novel trophic interactions in a non-native world. *Ecol Lett* 16:1088–1094
- Pearse IS, Harris DJ, Karban R, Sih A (2013) Predicting novel herbivore–plant interactions. *Oikos* 122:1554–1564
- Peterson MA, Dobler S, Holland J, Tantalio L, Locke S (2001) Behavioral, molecular, and morphological evidence for a hybrid zone between *Chrysochus auratus* and *C. cobaltinus* (Coleoptera: Chrysomelidae). *Entomol Soc Ams* 94:1–9
- Peterson MA, Monsen KJ, Pedersen H, McFarland T, Bearden J (2005) Direct and indirect analysis of the fitness of *Chrysochus* (Coleoptera: Chrysomelidae) hybrids. *Biol J Lin Soc* 84:273–286
- Pfammatter JA, Krause A, Raffa KF (2015) Evaluating predators and competitors in Wisconsin red pine forests for attraction to mountain pine beetle pheromones for anticipatory biological control. *Environ Entomol* 44:1161–1171
- R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.r-project.org>. Accessed 1 Feb 2017
- Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annu Rev Ecol Syst* 27:83–109
- Rodriguez LF (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biol Invasions* 8:927–939
- Sanderson LA, Antunes PM (2013) The exotic invasive plant *Vincetoxicum rossicum* is a strong competitor even outside its current realized climatic temperature range. *NeoBiota* 16:1–15
- Schaffner U (2001) Host range testing of insects for biological weed control: How can it be better interpreted? *Bioscience* 51:951–959
- Schaffner U, Smith L, Cristofaro M (2018) A review of open field host range testing to evaluate non-target use by herbivorous biological control candidates. *Biocontrol* 63:405–416
- Schirmel J, Bundschuh M, Entling MH, Kowarik I, Buchholz S (2016) Impacts of invasive plants on resident animals across ecosystems, taxa, and feeding types: a global assessment. *Glob Change Biol* 22:594–603
- Schlaepfer MA, Sherman PW, Blossey B, Runge MC (2005) Introduced species as evolutionary traps. *Ecol Lett* 8:241–246
- Schwarz D, Matta BM, Shakir-botteri NL, McPherson BA (2005) Host shift to an invasive plant triggers rapid animal hybrid speciation. *Nature* 436:546–549
- Scriber JM (2002) Evolution of insect–plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits. *Entomol Exp Appl* 104:217–235
- Seehausen O (2004) Hybridization and adaptive radiation. *Trends Ecol Evol* 19:198–207
- Sforza R (2011) Testing agents for the biological control of swallow worts (*Vincetoxicum* spp.) in North America Phase II USDA-ARS project 2009–2010
- Sheeley SE, Raynal DJ (1996) The distribution and status of species of *Vincetoxicum* in eastern North America. *Bull Torrey Bot Club* 123:148–156
- Sheldon SP, Creed RPJ (2003) The effect of a native biological control agent for Eurasian watermilfoil on six North American watermilfoils. *Aquat Bot* 76:259–265
- Tewksbury L, Casagrande R, Blossey B, Häfliger P, Schwarzländer M (2002) Potential for biological control of *Phragmites australis* in North America. *Biol Control* 23:191–212
- USDA-APHIS Technical Advisory Group (2018) Technical Advisory Group for biological control of weeds TAG petitions—APHIS action. http://www.aphis.usda.gov/plant_health/permits/tag/downloads/TAGPetitionAction.pdf. Accessed 20 July 2018
- van Lenteren JC, Bale JS, Bigler E, Hokkanen HMT, Loomans AM (2006) Assessing risks of releasing exotic biological control agents of arthropod pests. *Annu Rev Entomol* 51:609–634
- Weed AS, Casagrande RA (2010) Biology and larval feeding impact of *Hypena opulenta* (Christoph) (Lepidoptera: Noctuidae): a potential biological control agent for *Vincetoxicum nigrum* and *V. rossicum*. *Biol Control* 53:214–222
- Weed AS, Gassmann A, Casagrande RA (2011a) Effects of leaf and root herbivory by potential insect biological control agents on the performance of invasive *Vincetoxicum* spp. *Biol Control* 56:50–58

Weed AS, Gassmann A, Leroux AM, Casagrande RA (2011b) Performance of potential European biological control agents of *Vincetoxicum* spp. with notes on their distribution. *J Appl Entomol* 135:700–713

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