

Initial Response by a Native Beetle, *Chrysochus auratus* (Coleoptera: Chrysomelidae), to a Novel Introduced Host-Plant, *Vincetoxicum rossicum* (Gentianales: Apocynaceae)

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Abstract

Native insects can form novel associations with introduced invasive plants and use them as a food source. The recent introduction into eastern North America of a nonnative European vine, *Vincetoxicum rossicum* (Kleopow) Barbar., allows us to examine the initial response of a native chrysomelid beetle, *Chrysochus auratus* F., that feeds on native plants in the same family as *V. rossicum* (Apocynaceae). We tested *C. auratus* on *V. rossicum* and closely related or co-occurring native plants (*Apocynum* spp., *Asclepias* spp., and *Solidago canadensis* L.) using all life stages of the beetle in lab, garden, and field experiments. Experiments measured feeding (presence or absence and amount), survival, oviposition, and whether previous exposure to *V. rossicum* in the lab or field affected adult beetle feeding. Beetles fed significantly less on *V. rossicum* than on native *Apocynum* hosts. Adult beetles engaged in exploratory feeding on leaves of *V. rossicum* and survived up to 10 d. Females oviposited on *V. rossicum*, eggs hatched, and larvae fed initially on the roots; however, no larvae survived beyond second instar. Beetles collected from *Apocynum cannabinum* L. field sites intermixed with *V. rossicum* were less likely to feed on this novel nonnative host than those collected from colonies further from and less likely to be exposed to *V. rossicum* (>5 km). Our experimental work indicates that *V. rossicum* may act as an oviposition sink for *C. auratus* and that this native beetle has not adapted to survive on this recently introduced novel host plant.

Key words: *Vincetoxicum rossicum*, *Chrysochus auratus*, oviposition sink, biological control, novel association

The invasion of new habitats by nonnative plants can lead to dramatic ecological changes including altered nutrient cycling (Vitousek et al. 1997, Simberloff 2011), modification of trophic interactions (Norbury et al. 2013), facilitation of secondary invasions (Simberloff and Von Holle 1999, Richardson et al. 2000), and degradation of ecosystem function (Simberloff and Von Holle 1999). One mechanism that accounts for these ecological impacts is enemy release (Keane and Crawley 2002), whereby an invasive plant escapes the natural enemies from its source habitat and native herbivorous insects avoid feeding on the unrecognized or unpalatable host plants, thus enabling them to spread unchecked in their new habitat. Such escape, however, may be temporary, as accumulating evidence shows native herbivores will move onto these introduced species and form what is termed a new or novel association (Janzen 1985, Agosta 2006, Carroll 2007, Sunny et al. 2015), often after an adaptive lag period (Janzen 1985). Once formed, these novel associations may serve to regulate the introduced species and lower its populations so that long-term disruption in the new habitat is minimized (Schlaepfer et al. 2005, Agosta 2006).

Regardless of the mechanism, a number of factors influence whether a novel association will form after an introduced species arrives. Insects that feed on plants that are closely related phylogenetically are more likely to include the novel host in their diet than those that feed on distantly related plants (Futuyma and Mitter 1996, Agrawal and Kotanen 2003, Bertheau et al. 2010, Pearse et al. 2013). Closely related species demonstrate similar ecological niches (Freckleton et al. 2002, Wiens and Graham 2005); therefore, native insect herbivores in the same genus as the natural enemies of a nonnative plant may be best-suited to feed on the novel host. Typically, the length of time insects are exposed to a novel plant (whether in the lab or field) increases their likelihood of using it as a host (Dethier 1982, Papaj and Prokopy 1989, Lankau et al. 2004, Santana and Zucoloto 2011). Thus, when investigating the potential of a native herbivorous insect to form a novel association with a recently introduced plant, factors such as the phylogenetic relationship between the introduced (potential) host plant and resident native (current) host plants, the insect's relatedness to natural enemies of the nonnative plant in its home-range, and

Table 1. Field collection sites for *Chrysochus auratus* adults in Ontario, Canada, and eastern Washington State, USA

Site		Location (DD)	Year(s) collected	Host plant ^d	Nearest <i>Vincetoxicum</i> spp.
Washington State, USA	Badger Road, Richland	46.191944, -119.355556	2013, 2015	2	>500 km
	Hybrid Site, Mabton	46.245556, -120.110278	2013, 2015	2	>500 km
Ontario, Canada	Farm Field, Guelph	43.527778, -80.322778	2011, 2012, 2015	2 (1)	<5 km
	Farm Field, Copetown	43.224051, -80.055077	2011, 2012	1 (2)	<5 km
	Koffler Scientific Reserve, King City	44.035556, -79.540833	2011	1	NA ^b
	Cootes Drive, Dundas	43.266308, -79.941197	2011	2 (1)	Intermixed
	High Park, Toronto	43.648866, -79.462608	2012	1 (2)	Intermixed

^a 1, *Apocynum androsaemifolium*; 2, *Apocynum cannabinum*. Numbers in brackets refer to host plants present at the same site but in fewer numbers (<20 stems) that had evidence of feeding by *C. auratus*.

^b Beetles from this site were not included in the field exposure test due to the small size of this colony.

the length of time the native insect has been exposed to the nonnative plant should all be considered.

The recognition of a nonnative plant as a host can in some cases be to the detriment of a native herbivore's fitness. By providing resources similar to the native host plant but with a lower degree of fitness (Schlaepfer et al. 2005), nonnative plants can act as oviposition sinks (if the host is less preferred) or evolutionary traps (if the host is more preferred; Battin 2004, Sunny et al. 2015). *Vincetoxicum rossicum* (Kleopow) Barbar. (Apocynaceae; syn. *Cynanchum rossicum* (Kleopow) Borhidi), commonly known as pale swallow-wort or dog-strangling vine (DSV), is a recently introduced perennial herbaceous vine and a successful invader of terrestrial habitats throughout eastern North America. The first records of *V. rossicum* in North America date from the late 1800s, although it was not until 1973 that it was first described as "weedy" in Ontario, Canada (Pringle 1973, Sheeley and Raynal 1996). To date, few insect herbivores have been found to feed on this plant, and of those that do, none cause sufficient damage to limit its growth or spread (Sheeley and Raynal 1996, Lawlor 2000, Ernst and Cappuccino 2005, Milbrath 2010, Milbrath and Biazzo 2012). As a member of the well-defended family, Apocynaceae, this plant contains latex (Liede 1996) and a number of secondary toxic plant compounds (Weston et al. 2005, Douglass et al. 2011). 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. However, insects from other native plants within the same family have responded to *Vincetoxicum*; i.e., the monarch butterfly (*Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) recognizes *V. rossicum* as an oviposition site (DiTommaso and Losey 2003, Casagrande and Dacey 2007). Unfortunately, this host recognition is to the detriment of *D. plexippus*, as its larvae are unable to complete development on *V. rossicum*, making the novel plant an oviposition sink for the butterfly (DiTommaso and Losey 2003, Casagrande and Dacey 2007). It might be expected then that other native insect Apocynaceae-specialists may similarly recognize nonnative *V. rossicum* as a host, but be unable to complete development on it.

Chrysochus auratus F. (Coleoptera: Chrysomelidae) is a phytophagous insect native to eastern North America found feeding only on *Apocynum androsaemifolium* L. and *Apocynum cannabinum* L., native plants in the family Apocynaceae (Weiss and West 1921, Williams 1992, Dobler and Farrell 1999). Adults feed on the leaves and female beetles lay eggs on leaves and stems (Hatch 1953, Arnett 1968, Dobler and Farrell 1999), which hatch and larvae drop to the ground to feed on the roots. The insects overwinter as pupae or

prepupae in the soil (Weiss and West 1921). We hypothesized that this species has the potential to form a novel host-association with *V. rossicum* in North America because: 1) it is specialized to feed on well-defended lactiferous plants within the same family (Weiss and West 1921, Williams 1992, Dobler and Farrell 1999); 2) it is closely related phylogenetically to *Chrysochus asclepiadeus* (Pallas), the vine's most-damaging herbivore in its original habitat in Europe (Weed et al. 2011); and 3) it is found in similar habitats as introduced *V. rossicum*.

Here we examined the novel interaction of a native insect herbivore, *C. auratus*, on the introduced vine *V. rossicum* to assess whether this beetle has the potential to become a biological control agent for this recent invader. Specifically, we measured the response variables of adult feeding, survivorship, and ovipositional preference and success of larval hatching and development of *C. auratus* on *V. rossicum* compared with that on its native *Apocynum* spp. hosts. Key variables of beetle age, sex, and prior exposure to *V. rossicum* (manipulated in the lab) were used as covariates for the response variables measured. We also assessed the importance of preexposure to *V. rossicum* for acceptance of the novel host by comparing beetle populations from Ontario, Canada (where *V. rossicum* is present), with colonies from both Ontario and Washington State, USA, where native *Chrysochus* had never encountered this introduced plant.

Materials and Methods

Beetle and Plant Collection

Adult *C. auratus* were collected in the field from *Apocynum androsaemifolium* or *Apocynum cannabinum* in southwestern Ontario and eastern Washington (Table 1 for collection date and locations). Upon collection, beetles were placed in 4-liter (12 by 12 by 26 cm) clear plastic containers with mesh lids and provided with foliage collected from the same sites. Beetles were held under these conditions at a photoperiod of 16:8 (L:D) h and ambient temperature (20–22 °C) until tested in the lab, greenhouse, and garden.

Adult Feeding

Experiments to assess the effect of age, sex, and exposure to *V. rossicum* on the feeding of adult beetles were conducted while controlling for temperature, collection site, and date. Regular small field collections were used for these experiments because *C. auratus* could not be mass-reared. For testing in the lab, adult beetles were placed singly in 11-cm petri dishes with a moist filter paper and a leaf from a single test-plant species per petri dish. Plants were collected as young plants in the field from the Royal Botanical Gardens in

Hamilton, ON, and grown in a greenhouse in 4-liter pots with Sungro Sunshine Mix #1 potting soil (Agawam, MA). Each leaf used for testing was selected from the middle stratum of the plant to maintain consistency in leaf size and scanned prior to placement using ImageJ software v.1.47 (Bethesda, MD) to determine the surface area (mm²). Petri dishes were placed on the test bench so as to ensure no dish with the same treatment was adjacent to another and to account for possible variation in the lab environment. All dishes were kept at ambient lab conditions (20–22 °C) with a photoperiod of 16:8 (L:D) h. To reduce error due to surface area reduction from water loss, leaves were removed after 2 d and feeding galleries were traced with initial scan overlaid to record the amount of leaf surface area (mm²) removed. All leaves were reviewed visually to confirm presence or absence of feeding. All adult feeding tests described below followed this protocol.

Age and Sex

As age and sex have been shown to influence adult beetle feeding (Jaenike 1990), we compared the adult feeding results from beetles that had recently emerged from the soil (within 24 h) and had not yet initiated feeding with older beetles collected at least 1 wk later in the field that were actively feeding. To obtain recently emerged *C. auratus* beetles, sites were surveyed daily from the beginning of June (before the beetles have emerged) through to mid-June 2012. Sites were searched thoroughly in order to collect all newly emerged beetles present on *Apocynum* spp. in each patch at each date. No evidence of adult *C. auratus* feeding was observed on *Apocynum* plants during these collections. All 60 recently emerged beetles were collected from four surveyed sites (Dundas, Copetown, Guelph, and Toronto [Table 1]) between 16 and 23 June 2012. Adults were distributed evenly between petri dishes containing leaves of *Ap. androsaemifolium* and *V. rossicum* (30 beetles per plant species). These same sites were visited at least one week later to collect 120 older beetles, the last of which were collected on 8 July 2012. Older beetles were also distributed evenly on *Ap. androsaemifolium* and *V. rossicum*. Older beetles are identified as those that had already initiated feeding on *Apocynum* spp. whereas in contrast, younger, newly emerged beetles observed in the greenhouse and the field remain relatively stationary and do not initiate feeding, mating, or oviposition immediately (i.e., naïve or with very little exposure to *Apocynum* spp.; personal observations, R. deJonge). To determine whether sex has an effect on feeding presence or absence with *V. rossicum* and amount on native hosts, *Apocynum* spp., we compared the adult feeding test results of 33 females and 47 males (80 beetles total) collected from *Ap. cannabinum* in Richland, WA, in 2013 (Table 1). Forty beetles (15 female and 25 male) were fed leaves of *V. rossicum*; the remaining 40 (18 female and 22 males) were fed *Ap. cannabinum* as a control. All beetles were measured from the front of the head to the tip of the elytron to the nearest 0.01 mm using electronic calipers. Beetles were sexed following the procedure by dissection. For all other tests, beetles were sexed using external morphology.

Lab Exposure

To determine whether lab exposure to *V. rossicum* increased adult feeding, the presence or absence and extent of feeding were examined by comparing beetles exposed to the vine with those that had not been exposed over a 2-d period. In June 2013, 70 beetles were collected from *Ap. androsaemifolium* plants at the Copetown site and tested using the standard adult feeding procedure detailed above with leaves from the following leaf species: *Apocynum*

androsaemifolium, *Ap. cannabinum*, *Asclepias eriocarpa* Benth., *Asclepias fascicularis* Dcne., *Asclepias speciosa* Torr., *Solidago canadensis* L., and *V. rossicum* (10 beetles per plant species). In the event we did not observe feeding on *V. rossicum*, we added *Asclepias* spp. to determine whether this result was due to host fidelity to *Apocynum* spp., or whether *V. rossicum* leaves in particular are unpalatable to *C. auratus* adults. In the event we observed a high amount of feeding on *V. rossicum*, we added *S. canadensis* (an unrelated common plant often found in the field with *Apocynum* spp. where *C. auratus* is present) to determine whether this result was due to a lack of phylogenetic specificity in exploratory feeding demonstrated by *C. auratus* adults. Immediately after the initial procedure was completed, the same beetles were subjected to the identical procedure, with each beetle receiving the same leaf species as they had 2 d before. The results of the two procedures were compared to each other to determine whether mean feeding had changed for beetles following a 2-d exposure to the same leaf species.

Field Exposure

To determine whether exposure to *V. rossicum* (as measured by proximity to *V. rossicum* in the field) affected feeding, beetles were collected from *Apocynum* spp. at sites at varying distances from the introduced vine in both Ontario, Canada, and Washington State, USA. Specifically, we collected beetles from sites intermixed with *V. rossicum* (i.e., the “exposed group”) (from Toronto, ON (17), and Dundas, ON (49)), and those without *V. rossicum* nearby (i.e., the “unexposed group”): (Copetown, ON (13), Guelph, ON (22)), which are both 5 km distant from *V. rossicum*, and (Mabton, WA (25), and Richland, WA (40)), which are both >500 km distant from *V. rossicum* (Table 1). Distance to closest *V. rossicum* was determined using an invasive plant database (EDDMapS 2016), and personal observation (R. deJonge). Leaves of native hosts *Ap. cannabinum* were tested concurrently as a control (Toronto (19), Dundas (59), Copetown (12), Guelph (16), Mabton (27), and Richland (42)). In all cases, beetles were returned to the lab for testing using the standard adult feeding protocol with cut leaves in petri dishes.

Statistical Analysis

G-tests were used to compare the presence or absence of feeding between—1) recently emerged and older adult beetles, 2) males and females, and 3) different collection sites or distances from *V. rossicum* in the field. The G-test was used rather than the more common χ^2 test, as it is based on a multinomial distribution and is robust to smaller sample sizes (Gotelli, 2004). McNemar’s test was used to compare feeding presence or absence on all leaf species pooled by genera before and after 2-d exposure to plant leaves.

ANOVAs were used with Tukey’s HSD (Tukey 1953, Kramer 1956) when comparing the amount of feeding by—1) recently emerged and older adult beetles and 2) between the collection sites. To compare feeding amounts by male or female beetles and between feeding on *Ap. cannabinum* and *V. rossicum*, *t*-tests were conducted. All statistical analyses in this study were carried out using R software, version 3.2.2 (R Core Team, 2015) except for the G-tests, which were calculated using the G-test calculator (McDonald 2014).

Adult Survival, Oviposition, and Egg Ecllosion

No-Choice Survival Test

During the first week of July 2011, shortly after initial *C. auratus* emergence had been observed, 120 adult beetles were collected from *Apocynum* spp. at four sites in Ontario: Copetown, Guelph,

Dundas, and King City (Table 1) and subjected to a no-choice test. Mated pairs were allocated evenly between single potted plants of either *Ap. androsaemifolium* ($n=10$), *Ap. cannabinum* (10), *Vincetoxicum nigrum* (L.) Moench (a second introduced, *Vincetoxicum* species but less common in Ontario) (20) or *V. rossicum* (20). Pots were tightly netted and placed on a greenhouse bench with no plants of the same species directly adjacent to each other. Beetles were monitored daily to determine survival. The procedure was stopped 1 wk after the last beetle on a *Vincetoxicum* plant died, at which time only those beetles on *Apocynum* spp. remained alive.

Choice Oviposition Test

To determine whether beetle oviposition was highly host-specific (only on *Apocynum* spp.) or whether females would also use plants from other genera within Apocynaceae for oviposition, and if so, the ranking of their oviposition preference, a choice test using tightly netted pairs of potted plants in a common garden was set up. In July 2012, 60 mated beetle pairs were collected from *Ap. androsaemifolium* plants at the Copetown site (Table 1) and placed on paired plants in a common garden within a single tightly netted pot containing either: 1) *Ap. androsaemifolium* and *V. rossicum*; 2) *Ap. androsaemifolium* and *Asclepias incarnata* L. (Apocynaceae); or 3) *Ap. androsaemifolium* and *Ap. androsaemifolium* (20 replicates of each plant-pair). In the event that no females oviposited on *V. rossicum* plants, we added *As. incarnata*, a North American native Apocynaceae that is not typically used as a host by *C. auratus*, in order to determine whether *C. auratus* females are highly specific to *Apocynum* spp., or whether *V. rossicum* itself is not preferred as an oviposition site. As female preference for oviposition sites was being measured, not absolute fecundity, we chose not to count individual eggs within each egg mass due to lack of high variation observed. All egg masses laid on plants and nonplant substrates during the 14-d procedure were collected and counted. Egg masses were removed from leaves and stems then stored separately in microcentrifuge tubes in the lab, where they were monitored daily for 6 wk to record hatching.

Statistical Analysis

No-choice adult survival on *Apocynum* spp. and *Vincetoxicum* spp. was compared using an ANOVA with a post hoc Tukey's test. In the choice oviposition test, a type II ANOVA (*Anova* function (car package, Fox and Weisberg 2016)), which uses Wald χ^2 tests to generate *P*-values, was used to determine whether the number of egg masses laid on each plant species was significantly different from each other. A type II ANOVA was used, as it allows the testing of each of the two main effects: 1) plant species and 2) the random effect of total number of egg masses laid by the female in each pair, after testing for the other main effect. The total number of egg masses laid by the female in each pair was included in the model because the beetles would often lay egg masses on the plant and on nonplant substrates like the screen and pot and this parameter captures that response. Total egg masses laid by each pair was set as a random effect with the *lmer* function (lme4 package (Bates et al. 2016)). As multiple ANOVAs were calculated in order to identify differences between all three species, a Bonferroni-corrected alpha was used.

Larval Feeding and Development

Early Larval Feeding and Development on Excised Roots

Adult beetles were placed in 4-liter clear plastic containers (12 by 12 by 26 cm) containing host plant foliage (*Apocynum* spp.). Egg

masses laid on the foliage were removed from stems and leaves every 3 d and placed separately in microcentrifuge tubes where they were monitored daily for hatching. Following hatching, 120 first-instar larvae from Richland, Washington, 190 from Mabton, Washington, and 180 from Dundas, Ontario, were placed in 10 petri dishes in the lab in groups of 12, 19, and 18, respectively, each containing cut root segments (8–10 mm in length) of either *Ap. cannabinum* or *V. rossicum* (five petri dishes of each root species). To maximize data collection, we used all available larvae for each site instead of an equal number because pretesting suggested variances between sites were relatively uniform. Each week the larvae were given freshly cut roots, and dead larvae and shed head capsules were collected and preserved in 75% ethanol. To determine the feeding instar or instar at death, head capsules were measured using a digital microscope (Dino-Lite AM413TA) and image processing software (ImageJ). Head capsules were oriented with the mouth parts at the bottom and the distance at the widest point between sides was measured for head capsule width (Delbac et al. 2010). ANOVA was used to compare larval survival and head capsule widths between collection sites and root species.

Late Larval Development on Potted Plants

In July of 2012, 15 potted plants each of *V. rossicum* and *Ap. androsaemifolium* (as a control) were placed on a greenhouse bench and received 20 first-instar larvae from the Copetown site (Table 1). Larvae were produced using the adult rearing and egg mass collection procedure described above. Larvae were placed at the base of each plant stem and the plants were grown in 4-liter pots with potting soil (Sungro Sunshine Mix #1). A screen (mesh size = 0.5 mm) at the base of each pot and tightly secured netting over the above-ground plant matter prevented escape of the larvae. Pots were held in the greenhouse under ambient light conditions (temperature ranging 16–34 °C with an average of 24 °C cooled by cold-water air vents). The pots were dissected after 85 d (allowing for sufficient time for beetles to develop into late-instar larvae or pupae). All live larvae in the soil were counted and head capsules measured as described above.

Results

Adult Feeding

Age and Sex

Feeding by *C. auratus* adults on *V. rossicum* leaves was characterized only by nibbling or exploratory feeding (<5 bites per leaf). When comparing the presence or absence of feeding, older beetles fed significantly more often than recently emerged (24 h) beetles on *V. rossicum* ($G_1 = 7.992$, $P < 0.050$), thus they were used for all subsequent adult feeding tests. There was no significant difference between age ($F_{1,82} = 0.264$; $P = 0.608$), or collection site ($F_{3,80} = 1.319$; $P = 0.274$) for the amount of beetle feeding on *Ap. androsaemifolium* leaves. Females were larger ($t_{111.63} = 2.719$; $P < 0.010$) and fed in greater amounts on *Ap. cannabinum* ($t_{22.65} = 4.123$; $P < 0.001$) than males. There was no difference between sexes for either presence or absence of feeding on *V. rossicum* ($G_1 = 0.404$, $P = 0.525$) nor amount of feeding on the nonnative plant ($t_{14.03} = 0.964$; $P = 0.353$).

Lab Exposure

There was no significant increase or decrease in the presence of feeding by *C. auratus* adults on *V. rossicum* following short-term (2-day) exposure to cut leaves of the same species, with 2% feeding initially and 1% following exposure ($n=10$; $\chi^2 = 0$, $df = 1$, $P = 1$). In addition, no increase or decrease in the presence of feeding was observed on native *Apocynum* spp. hosts with 86% feeding initially

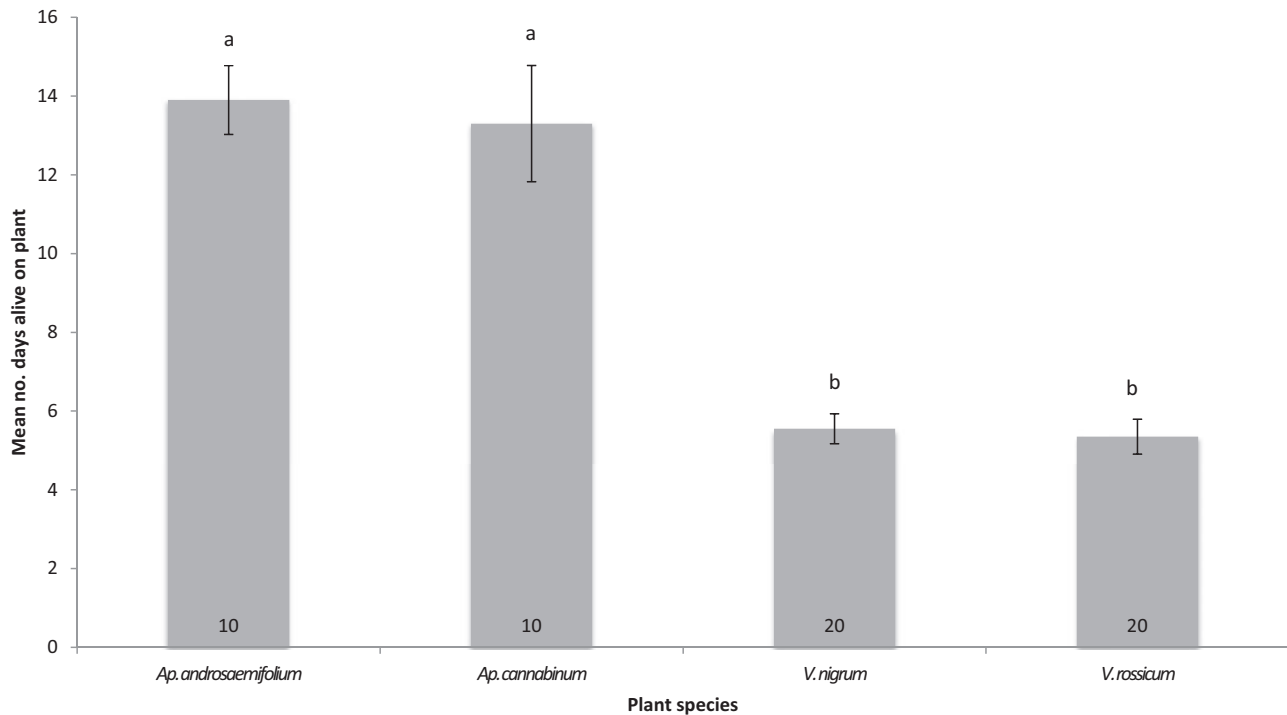


Fig. 1. Mean (\pm SE) number of days adult *Chrysochus auratus* lived when caged on potted plants of either *Apocynum androsaemifolium*, *Ap. cannabinum*, *Vincetoxicum nigrum*, or *V. rossicum* in greenhouse experiments, 2011. Bars with the same letter are not significantly different from each other. Numbers within bars reflect sample size (number of potted plants). Test was stopped 1 wk after the last beetle on *Vincetoxicum* spp. died, at which time only beetles on *Apocynum* spp. remained alive.

and 76% following exposure ($n=30$; $\chi^2 = 1.333$, $df = 1$, $P=0.248$), nor *Asclepias* spp. (native plants in the same family, Apocynaceae, yet not used as hosts) with 80% feeding initially and 83% following exposure ($n=30$; $\chi^2 = 0$, $df = 1$, $P=1$) or the common native weed *S. canadensis* (Asteraceae) ($\chi^2 = 0$, $df = 1$, $P=1$), on which only one beetle fed prior to exposure and none following exposure to *S. canadensis* ($n=10$).

When comparing the presence or absence of feeding on *V. rossicum* by beetles at sites where this nonnative plant was intermixed with their host plants, *Apocynum* spp. (Dundas, ON, and Toronto, ON) to those found at sites where *V. rossicum* was not known to be present within at least 5 km (Copetown, ON, Guelph, ON) and 500 km (Mabton, WA, and Richland, WA), we first determined there was no significant difference in the presence or absence of feeding by beetles either 5 km or 500 km distant from *V. rossicum* ($G_1 = 0.111$, $P=0.739$). The beetles from intermixed sites (“exposed”) were much less likely to feed on *V. rossicum* than those from sites where beetles were unexposed to *V. rossicum* (from sites 5 km to 500 km away; $G_1 = 7.950$, $P < 0.010$). There was no difference between presence of feeding on native hosts *Ap. cannabinum* either 5 km or 500 km distant from *V. rossicum* ($G_1 = 1.090$, $P=0.296$), nor between sites exposed or unexposed ($G_1 = 0.28$, $P=0.866$). Overall, beetles from all sites fed significantly more often ($G_1 = 153.351$, $P < 0.001$) and in higher amounts ($t_{108.08} = 13.185$, $P < 0.0001$) on *Ap. cannabinum* than on *V. rossicum*.

Adult Survival, Oviposition, and Egg Eclosion

No-Choice Survival Test

Adult survival on potted plants in the no-choice test was not significantly affected by collection site ($F_{3,56} = 0.976$, $P=0.411$) or date in July on which they were collected ($F_{5,54} = 0.793$, $P=0.559$).

Adult beetles survived significantly longer on *Apocynum* spp. (13.60 ± 0.837 d), than on *Vincetoxicum* spp. (5.45 ± 0.288 ; mean \pm SE; $F_{3,56} = 42.010$, $P < 0.001$), with adults on *Apocynum* spp. living over twice as long as those on the *Vincetoxicum* spp. (max. 10 d on *V. rossicum*; Fig. 1). As the experiment was stopped while *C. auratus* beetles remained alive on *Apocynum* spp., these survival data are truncated, and do not reflect the maximum life-length of these beetles.

Choice Oviposition Test

Female beetles laid significantly fewer egg masses on *V. rossicum* than they did on *Ap. androsaemifolium* ($\chi^2 = 28.257$, $df=1$, $P < 0.001$) or *Asclepias incarnata* ($\chi^2 = 15.118$, $df=1$, $P < 0.001$; Fig. 2). There was no significant difference between the number of larvae hatching per mass laid on the three plant species ($F_{2,6.96} = 2.145$, $P=0.118$).

Larval Feeding and Development

Early Larval Feeding and Development on Excised Roots

Larvae from all three sites (Richland, WA, Mabton, WA, and Dundas, ON) fed on both *Ap. cannabinum* and *V. rossicum* but survived significantly longer on *Ap. cannabinum* ($F_{1,957} = 23.595$; $P < 0.001$), with no larvae living on *V. rossicum* beyond week seven of the procedure (Fig. 3). Of larvae given *V. rossicum* roots ($n=245$), only 20.0% were able to undergo one molt, with no larvae molting a second time. No head capsules were found for larvae feeding on *V. rossicum* following the 5th week of the procedure. Larvae feeding on *Ap. cannabinum* molted a maximum of two times during this same time period, with 19.6% ($n=245$) undergoing the first molt and 10.41% of these going on to shed a second head capsule. The longest larval survival on *Ap. cannabinum* was 31 wk.

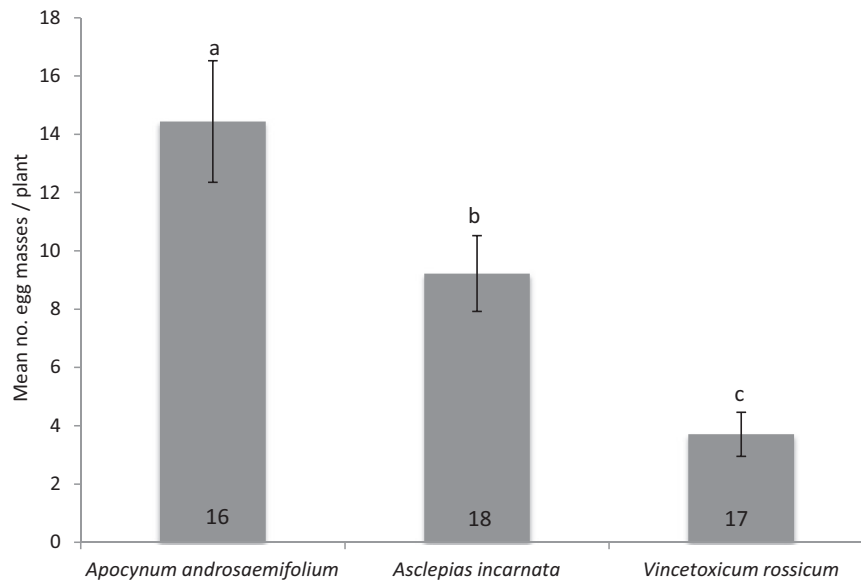


Fig. 2. Mean (\pm SE) number of *Chrysochus auratus* egg masses laid by beetle pairs on each plant species in a common garden experiment, 2012. Bars with the same letter are not significantly different from each other. Numbers within bars reflect sample size (number of potted plants) for each species. Although 20 tested pairs were used for each plant treatment, only beetle pairs later confirmed through dissection to be male–female were used for statistical tests.

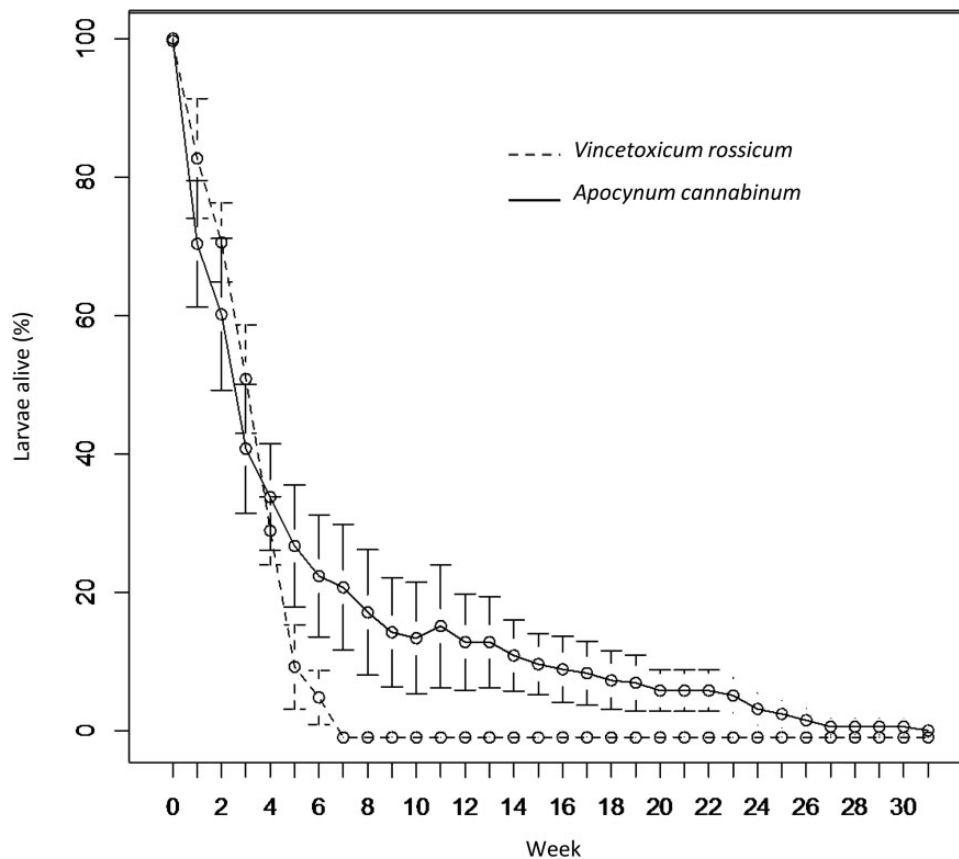


Fig. 3. Percentage of *Chrysochus auratus* larvae found alive each week on excised root segments of either *Apocynum cannabinum* (native host) or *Vincetoxicum rossicum* (nonnative host) in lab experiments, 2015 (means \pm 95% confidence intervals; $n = 245$).

During the first 4 wk of the procedure (when larvae were present on both root species) there was no significant difference between the shed head capsule widths of larvae fed *V. rossicum* or *Ap. cannabinum* ($F_{1,66} = 1.794$; $P = 0.185$). Throughout the procedure there

was no difference in shed head capsule widths of larvae collected from the different sites and fed *V. rossicum* in the laboratory ($F_{2,31} = 3.026$; $P = 0.063$). However, there was between-site variation in the width of head capsules from larvae fed *Ap. cannabinum*. Larvae

from the Dundas, ON, site were smaller than both Richland, WA ($P < 0.050$), and Mabton, WA ($P < 0.050$), sites (Tukey's HSD), when comparing head capsule width of dead larvae measured throughout the duration of the procedure.

Late Larval Development on Potted Plants

No *C. auratus* larvae were recovered from any of the pots containing *V. rossicum* in this no-choice test. Larvae given *Ap. androsaemifolium* ($n = 300$) had a survival rate of 9.7%, with an average of $8.3 \pm 1.4\%$ (mean \pm SE) per pot. Larvae or pupae were found in eight of the 15 pots with *Ap. androsaemifolium* 85 d after larvae placement.

Discussion

Chrysochus auratus initially accepts the nonnative *Vincetoxicum rossicum* vine for adult and larval feeding and oviposition in the lab, but is unable to feed beyond nibbling or complete larval development on this novel host, suggesting that it is not a viable host and may act as an oviposition sink, very similar to that observed for other specialists on plants in the Apocynaceae such as monarch butterflies (DiTommaso and Losey 2003, Mattila and Otis 2003, Casagrande and Dacey 2007). It is likely that *C. auratus* responds to flavonoid glycosides in *V. rossicum* that are common within the Apocynaceae (Haribal and Renwick 1998). The low incidence of feeding by later instar larvae and adults may be due to compounds found in *V. rossicum* that deter feeding, as was observed for other insect species (Mogg et al. 2008). The implication of the novel chemistry of *V. rossicum* preventing herbivory has been made in earlier studies (Cappuccino and Arnason 2006). North American herbivores may need to adapt to the unique chemistry of *V. rossicum* in order to sustain feeding and complete development. Overall, *V. rossicum* seems to provide feeding cues for adult beetles (unlike the native common weed, *Solidago canadensis*), initial feeding cues for motile first-instar larvae, and ovipositional cues for female beetles; however, its leaves do not permit feeding beyond the initial exploratory stage, and its roots are unable to support complete larval development, likely due to its chemical compounds.

Beetle age, but not sex, predicted feeding by adult beetles on *V. rossicum*, with younger (<24 h) beetles feeding less frequently than those at least a week older. This is in-line with predictions made in optimality models for host specialization, which predict that older beetles are more likely to accept poor quality hosts when compared with younger adults of the same species (Jaenike 1990). Female *C. auratus* fed on native *Apocynum* hosts in greater amounts than the smaller males. However, there was no difference between the sexes and their feeding presence or absence and amounts on nonnative *V. rossicum*. If feeding had been greater by the larger females on *V. rossicum* as compared to males (i.e. the amount of feeding would be directly tied to food requirements of the individual beetles), this would suggest that *C. auratus* accepts *V. rossicum* as a host, albeit less preferred. The lack of difference in feeding on *V. rossicum* between sexes instead suggests that *C. auratus* does not accept the nonnative vine as a food source.

Chrysochus auratus beetles found on their host plants intermixed with *V. rossicum* in the field appeared to avoid feeding on this introduced vine when compared to those collected from sites with no known *V. rossicum*. This held true for beetles collected greater than 5 km (Ontario) and 500 km (Washington State) from *V. rossicum*. The hypothesis that gene introgression due to hybridization with a western sibling species, *Chrysochus cobaltinus* LeConte (which

sustains feeding on *V. rossicum* (deJonge et al. unpublished data)) caused an increase in feeding, could be explored if only beetles from Washington State (500 km), and not Ontario (5 km), had fed on *V. rossicum* in significantly higher amounts. As we saw no difference in feeding levels by beetles independent of continental location, we instead propose two possible explanations for the observation of close-proximity beetles (from sites intermixed with *V. rossicum*) feeding less on the novel host. First, either individual beetles from sites closest to *V. rossicum* had learned to avoid unacceptable hosts, as has been observed in locusts on an unpalatable forb, *Senecio vulgaris* (L.) (Blaney et al. 1985) or second, beetle populations in these locations may have adapted over decades of exposure to avoid *V. rossicum*. Early adaptation to avoid an unpalatable host within decades had been seen in a leaf mining fly, *Amauromyza flavifrons* (Meigen) on sugar beets (Uesugi 2008), and more recently with the European corn borer (*Ostrinia nubilalis* (Hubner)) on maize (Orsucci et al. 2016). The lack of increase or decrease in presence of feeding by adult *C. auratus* on *V. rossicum* following two days of exposure suggests that the reduction in feeding on *V. rossicum* by adults intermixed with the vine in the field may be an adaptation to avoid *V. rossicum*; however, longer term lab exposure studies are necessary, as this shorter length of time may not be sufficient to test for changes in adult feeding.

Our study is one of the first to examine the potential relationship between a native herbivorous insect and an invasive nonnative plant before actual feeding and use have been observed in the field (see also Dalosto et al. 2015, Pfammatter et al. 2015). Investigating such novel associations before they are altered through longer-term interaction is important, as it describes a baseline from which to compare any future relationship between these species. By studying the herbivore–host association before it is observed in the field, we can better determine whether any possible association between these two species is due to adaptation following some specified lag period (i.e., the current time period), and if so, the time taken, or in contrast, whether the herbivore and host plant required no adaptation to form an association or “ecological fit” (Agosta 2006, Harvey et al. 2010). Use of *V. rossicum* by *C. auratus* from the initial point of contact would support the latter mechanism.

The investigation of novel associations before they occur in the field may also help to identify potential native biological control agents to help limit the spread of invasive species. If we can confirm the potential for a native herbivore to assist in controlling an invasive plant, then its augmentation and relocation would be supported, much like that undertaken with the native weevil, *Eubrychiopsis lecontei* (Dietz) to control Eurasian watermilfoil (Sheldon and Creed 2003). Lastly, the investigation of such novel associations before they are actually observed in the field can help identify species of concern that may be affected directly when an invasive arrives and becomes an oviposition sink or ecological trap. Dalosto et al. (2015) used just such a strategy to protect native species at risk by helping make recommendations for the management and control of an introduced crayfish in South America. By studying this herbivore–host relationships before it actually occurs in the field, we have been able to add valuable information on how novel associations form, as well as improve our understanding as to the potential threat and risk of invasion for at least one native beetle.

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References Cited

- Agosta, S. J. 2006. On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. *Oikos* 114: 556–565.
- Agarwal, A. A., and P. M. Kotanen. 2003. Herbivores and the success of exotic plants: A phylogenetically controlled experiment. *Ecol. Lett.* 6: 712–715.
- Arnett, R. 1968. The beetles of the United States (a manual for identification). The American Entomological Institute, Ann Arbor, MI.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2016. lme4: linear mixed-effects models using Eigen and S4. R Package. version 1.1-12.
- Battin, J. 2004. Bad habitats: animal ecological traps and the conservation of populations. *Soc. Conserv. Biol.* 18: 1482–1491.
- Bertheau, C., E. G. Brockerhoff, G. Roux-Morabito, F. Lieutier, and H. Jactel. 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.
- Blaney, W. M., C. Winstanley, and M.S.J. Simmonds. 1985. Food selection by locusts: an analysis of rejection behaviour. *Entomol. Exp. Appl.* 38: 35–40.
- Cappuccino, N., and J. T. Arnason. 2006. Novel chemistry of invasive exotic plants. *Biol. Lett.* 2: 189–193.
- Carroll, S. P. 2007. Brave New World: The epistatic foundations of natives adapting to invaders. *Genetica* 193–204.
- Casagrande, R. A., and J. E. Dacey. 2007. Monarch butterfly oviposition on swallow-worts (*Vincetoxicum* spp.). *Environ. Entomol.* 36: 631–636.
- Dalosto, M. M., A. V. Palaoro, C. Souty-Grosset, S.L.D.S. Bueno, T. G. Loureiro, M. P. Almerão, P. B. de Araujo, and S. Santos. 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.
- Delbac, L., P. Lecharpentier, and D. Thiery. 2010. Larval instars determination for the European Grapevine Moth (Lepidoptera: Tortricidae) based on the frequency distribution of head-capsule widths. *Crop Prot.* 29: 623–630.
- Dethier, V. 1982. Mechanism of host-plant recognition. *Entomol. Exp. Appl.* 31: 49–56.
- DiTommaso, A., and J. E. Losey. 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., and B. D. Farrell. 1999. Host use evolution in *Chrysochus* milkweed beetles: evidence from behaviour, population genetics and phylogeny. *Mol. Ecol.* 8: 1297–1307.
- Douglash, C. H., L. A. Weston, and D. Wolfe. 2011. Phytotoxicity and potential allelopathy in pale (*Cynanchum rossicum*) and black swallowwort (*C. nigrum*). *Invasive Plant Sci. Manag.* 4: 133–141.
- EDDMapS 2016. Early Detection & Distribution Mapping System. The University of Georgia - Center for Invasive Species and Ecosystem Health. (<http://www.eddmaps.org/>) (accessed 28 March 2017).
- Ernst, C. M., and N. Cappuccino. 2005. The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. *Biol. Invasions* 7: 417–425.
- Fox, J., and S. Weisberg. 2016. Car: companion to applied regression. R Packag. version 2.1-2.
- Freckleton, R. P., P. H. Harvey, and M. D. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* 160: 712–726.
- Futuyma, D. J., and C. Mitter. 1996. Insect-plant interactions: The evolution of component communities. *Philosophica Trans. Biol. Sci.* 351: 1361–1366.
- Gotelli, N. J., and A. M. Ellison. 2004. A primer on ecological statistics. Sinauer Associates, Inc., Sunderland, MA.
- Haribal, M., and J.A.A. Renwick. 1998. Identification and distribution of oviposition stimulants for monarch butterflies in hosts and nonhosts. *J. Chem. Ecol.* 24: 891–905.
- Harvey, J. A., A. Biere, T. Fortuna, L.E.M. Vet, T. Engelkes, E. Morriën, R. Gols, K. Verhoeven, H. Vogel, M. Macel, et al. 2010. Ecological fits, misfits and lotteries involving insect herbivores on the invasive plant, *Bunias orientalis*. *Biol. Invasions* 12: 3045–3059.
- Hatch, M. 1953. The beetles of the Pacific Northwest. Univ. Wash. Publ. Biol. 16: 1–340.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* 21: 243–273.
- Janzen, D. H. 1985. On ecological fitting. *Oikos* 45: 308–310.
- Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* 17: 164–170.
- Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal number of replications. *Biometrics* 12: 307–310.
- Lankau, R. A., W. E. Rogers, and E. Siemann. 2004. Constraints on the utilisation of the invasive Chinese tallow tree *Sapium sebiferum* by generalist native herbivores in coastal prairies. *Ecol. Entomol.* 29: 66–75.
- Lawlor, F. M. 2000. Herbicidal treatment of the invasive plant *Cynanchum rossicum* and experimental post control restoration of infested sites. M.S. thesis. State University of New York, Syracuse.
- Mattila, H. R., and G. W. Otis. 2003. A comparison of the host preference of monarch butterflies (*Danaus plexippus*) for milkweed (*Asclepias syriaca*) over dog-strangler vine (*Vincetoxicum rossicum*). *Netherlands Entomol. Soc.* 193–199.
- McDonald, J. H. 2014. Handbook of biological statistics. 3rd ed. Sparky house publishing, Baltimore, MD.
- Milbrath, L. R. 2010. Phytophagous arthropods of invasive swallow-wort vines (*Vincetoxicum* spp.) in New York. *Environ. Entomol.* 39: 68–78.
- Milbrath, L. R., and J. Biazzo. 2012. Development and reproduction of the foxglove aphid (Hemiptera: Aphididae) on invasive swallow-worts (*Vincetoxicum* spp.). *Environ. Entomol.* 41: 665–668.
- Mogg, C., P. Petit, N. Cappuccino, T. Durst, C. McKague, M. Foster, J. E. Yack, J. T. Arnason, and M. L. Smith. 2008. Tests of the antibiotic properties of the invasive vine *Vincetoxicum rossicum* against bacteria, fungi and insects. *Biochem. Syst. Ecol.* 36: 383–391.
- Norbury, G., A. Byrom, R. Pech, J. Smith, D. Clarke, D. Anderson, and G. Forrester. 2013. Invasive mammals and habitat modification interact to generate unforeseen outcomes for indigenous fauna. *Ecol. Appl.* 23: 1707–1721.
- Orsucci, M., P. Audiot, A. Pommier, C. Raynaud, B. Ramora, A. Zanetto, D. Bourguet, and R. Streiff. 2016. Host specialization involving attraction, avoidance and performance, in two phytophagous moth species. *J. Evol. Biol.* 29: 114–125.
- Papaj, D. R., and R. J. Prokopy. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annu. Rev. Entomol.* 34: 315–350.
- Pearse, I. S., D. J. Harris, R. Karban, and A. Sih. 2013. Predicting novel herbivore-plant interactions. *Oikos* 122: 1554–1564.
- Pfammatter, J. A., A. Krause, and K. F. Raffa. 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.
- Pringle, J. S. 1973. The spread of *Vincetoxicum* species (Asclepiadaceae) in Ontario. *Can. Field-Nat.* 87: 27–33.
- R Core Team 2015. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. (<http://www.r-project.org/>). (accessed 28 March 2017).
- Richardson, D. M., P.P.Y.S. Pysek, M. Rejmánek, M. G. Barbour, F. D. Panetta, and C. J. West. 2000. Naturalization and invasion of alien plants concepts and definitions. *Divers. Distrib.* 6: 93–107.
- Santana, A.F.K., and F. S. Zucoloto. 2011. Influence of previous experience on the preference, food utilization and performance of *Ascia monuste orseis* wild larvae (Godart) (Lepidoptera Pieridae) for three different hosts. *Neotrop. Entomol.* 40: 631–638.
- Schlaepfer, M. A., P. W. Sherman, B. Blossey, and M. C. Runge. 2005. Introduced species as evolutionary traps. *Ecol. Lett.* 8: 241–246.
- Sheeley, S. E., and D. J. Raynal. 1996. The distribution and status of species of *Vincetoxicum* in Eastern North America. *Bull. Torrey Bot. Club.* 123: 148–156.

- Sheldon, S. P., and R. P. J. Creed. 2003. The effect of a native biological control agent for Eurasian watermilfoil on six North American watermilfoils. *Aquat. Bot.* 76: 259–265.
- Simberloff, D. 2011. How common are invasion-induced ecosystem impacts? *Biol. Invasions* 13: 1255–1268.
- Simberloff, D., and B. Von Holle. 1999. Positive interactions of nonindigenous species: Invasional meltdown? *Biol. Invasions* 1: 21–32.
- Sunny, A., S. Diwakar, and G. P. Sharma. 2015. Native insects and invasive plants encounters. *Arthropod. Plant Interact.* 9: 323–331.
- Tewksbury, L., R. A. Casagrande, A. Gassmann. 2002. 16 Swallow-worts, pp. 209–216. *In*: R. Van Driesche (ed.), *Biol. Control Invasive Plants East. United States*. USDA Forest Service Publication.
- Tukey, J. 1953. The problem of multiple comparisons. unpublished manuscript reprinted in: the collected works of John W. Tukey, Volume 8, 1994 H. I. Braun (Ed.), Chapman and Hall, New York.
- Uesugi, A. 2008. Evolution of host avoidance in a leafmining fly, *Amauromyza flavifrons*. *Entomol. Exp. Appl.* 128: 398–402.
- Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7: 737–750.
- Weed, A. S., A. Gassmann, and R. A. Casagrande. 2011. Effects of leaf and root herbivory by potential insect biological control agents on the performance of invasive *Vincetoxicum* spp. *Biol. Control* 56: 50–58.
- Weiss, H. B., and E. West. 1921. Notes on the insects of the spreading dogbane, *Apocynum androsaemifolium* L. with a description of a new dogbane midge, by Dr. E. P. Felt. *Can. Entomol.* 53: 147.
- Weston, L. A., J. N. Barney, and A. DiTommaso. 2005. A review of the biology and ecology of three invasive perennials in New York state: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). *Plant Soil.* 277: 53–69.
- Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Evol. Syst.* 36: 519–539.
- Williams, C. E. 1992. Movement of the dogbane beetle, *Chrysochus auratus* (Coleoptera: Chrysomelidae), in a patchy environment. *Banisteria* 1: 8–10.