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Impact of herbivory on performance of *Vincetoxicum* spp., invasive weeds in North America

Dorothy Maguire · René Sforza · Sandy M. Smith

Abstract The alien invasive vines *Vincetoxicum rossicum* and *Vincetoxicum nigrum* (swallow-wort) are of major concern in eastern North America, where both species invade forested landscapes and threaten faunal and plant diversity. Among the few native natural enemies reported in Eurasia, the specialist chrysomelid, *Chrysochus* (*Eumolpus*) *asclepiadeus* (Coleoptera; Chrysomelidae), feeds on *Vincetoxicum* both above ground (as adults) and below ground (as larvae). The goal of our study was to assess the potential for using this beetle to manage invasive *Vincetoxicum* spp. in North America by quantifying the impact of herbivory by *C. asclepiadeus* on *Vincetoxicum* and determining whether this effect was influenced by plant density ("Allee effect"). Experimental work was carried out using a split plot design in the field in southern France. Pots of *V. nigrum* and *V. hirundinaria*, a substitute for *V. rossicum*, were planted at high (255 plants/m^2), medium (127 plants/m^2), and low (32 plants/m^2) plant densities, and received treatments of 0 (control), 2 or 4 *C. asclepiadeus* adult beetles/pot. Leaf damage, root and shoot biomass, and quantity of seeds were measured after 4 weeks of adult feeding. Densities of 2 and 4 beetles/pot caused similar damage, with significant reductions in plant biomass at low plant density. While *V. hirundinaria* increased allocation of resources to roots in response to herbivory, *V. nigrum* did not. Seed production was greatest for both species grown at low plant densities, but only *V. nigrum* produced fewer seeds in response to herbivory. Our results, based on the effects of herbivory by *C. asclepiadeus* adults, suggest that if this beetle were to be introduced into North America for the management of *Vincetoxicum* spp. such as *V. rossicum*, reductions in plant biomass and spread would be greatest if beetles were released on edges or in newly-established satellite populations at low plant densities. In the case of *V. nigrum*, beetles could be released irrespective of plant density as reproductive output and seed dispersal would be reduced similarly.

Keywords Invasive species · Herbivory · Plant fitness · Biological control · Apocynaceae

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Introduction

In Canada and the US, ca. 72 and >90 plant species, respectively, are officially designated as noxious

weeds in natural ecosystems (Mullin et al. 2000; White et al. 1993). In the last three decades, two Eurasian species of swallow-wort, *Vincetoxicum rossicum* (Kleopow) Barbar (L.) Moench and *Vincetoxicum nigrum* (L.) Moench. (Apocynaceae) have become invasive in northeastern North America and now pose a significant threat to native biodiversity. Both species are herbaceous, perennial vines related to native North American milkweeds. In NA, black swallow-wort (*V. nigrum*) ranges west from the Atlantic coast to southeastern Ontario, southern Pennsylvania through to Kansas, while pale swallow-wort (*V. rossicum*) extends discontinuously from the Great Lakes through New England and the mid-Atlantic US (Tewksbury et al. 2002). Currently, both species are abundant in the lower Great Lakes Basin of NA and are expanding their range at alarming rates; this expansion is predicted to increase further under increasing temperatures and climate change (DiTommaso et al. 2005; Weston et al. 2006). A third species, *V. hirundinaria* Medik., was introduced into the US but never established (Sheeley and Raynal 1996). Originally from the Palearctic region, the genus *Vincetoxicum* comprises ca. 70 species with centers of diversity in Eurasia (Liede 1997).

Swallow-worts have been present in NA for over a century, but have started to spread only recently. This slow establishment phase followed by exponential growth may be due to density-dependant responses (Miller et al. 2008; Cappuccino 2004; Cappuccino et al. 2002), and such responses may explain why this group has successfully invaded a variety of disturbed and undisturbed habitats (Averill et al. 2008). *Vincetoxicum rossicum* tends to be shade-tolerant, and is found further into forest understories, while *V. nigrum* tends to be limited to habitat edge and open areas (Milbrath et al. 2006) where its densities can approach 180 shoots/m^2 and 2,500 seedlings/m^2 (Smith et al. 2006). Although somewhat less in closed canopied habitats, swallow-wort densities can still reach as high as 117 shoots/m^2 and dominate ground cover up to 70% (Miller et al. 2008; DiTommaso et al. 2005). In sharp contrast, both of these species are sparse in their natural range and difficult to find (Sforza 2009).

Invasive *Vincetoxicum* spp. impact native faunal and plant species by out-competing endangered species (Tewksbury et al. 2002), potentially reducing breeding grassland bird populations (DiTommaso et al. 2005), changing arthropod community composition and diversity (Ernst and Cappuccino 2005), and inhibiting regeneration of native plant species (Miller et al. 2008). There is currently no effective method to manage populations, and biological control has been suggested as one of the few options available to provide long-term control (Miller et al. 2008). From a list of potential natural enemies in Eurasia, the chrysomelid beetle *Chrysochus* (*Eumolpus*) *asclepiadeus* Pallas (Coleoptera: Chrysomelidae) is of particular interest because it feeds on *Vincetoxicum* spp. during all developmental stages; as a larva on the roots and as an adult on the foliage. It is a specialist on *V. hirundinaria* (a species closely related to *V. rossicum*), and is found throughout its host's range in western, central and eastern Europe (Dobler et al. 1998; Tewksbury et al. 2002; Sforza 2009).

Three main factors may limit natural populations of *Vincetoxicum* spp. in their native range of Eurasia: interspecific competition, intraspecific competition, and herbivory; we focus here on the latter two. Recent work by Cappuccino (2004) suggests that intraspecific competition may actually have a positive effect on plant fitness in *Vincetoxicum* spp.; she found that overall performance in *V. rossicum* was greatest in patches of high plant density with both biomass and seed set significantly higher in patches of 81 plants/m^2 versus either 1 or 9 plants/m^2. Thus, *Vincetoxicum* appears to exhibit an Allee effect, and if true, this may help to explain its success as an invasive plant and to direct management strategies (Taylor and Hastings 2005). *Vincetoxicum* spp. establishing in NA would be released from natural enemies and this would allow high densities to build up. Under an Allee effect, these high plant densities would actually increase plant fitness and this could potentially counteract the impact of new herbivores in NA, resulting in less effective control. It is clearly important then to understand how plant density may interact with herbivory to influence plant fitness in *Vincetoxicum* spp. if we are to develop effective management strategies for these invasives.

Our study investigates the physiological response of two *Vincetoxicum* species, *V. nigrum* and *V. hirundinaria,* to herbivory by a native natural enemy, the chrysomelid beetle *Chrysochus asclepiadeus*. Here, we examine the impact of adult beetles alone as studies on the larval stage are on-going. Our objectives were to: (1) determine how beetle density

affected plant fitness in terms of total biomass, root:shoot biomass, and reproductive output; (2) evaluate whether positive density dependence (e.g. an Allee effect) exists in *Vincetoxicum* spp. over a range of plant densities and under the pressure of herbivory; and (3) measure the effect of herbivory on both *Vincetoxicum* species.

Materials and methods

Study site

The study was conducted at the United States Department of Agriculture's European Biological Control Laboratory (EBCL) in the vicinity of Montpellier, France (Fig. 1). An experimental garden was set up in the field outside the facility. In January 2009, an area ca. 10 m by 20 m was ploughed and surrounded by a 12 V-electric fence to prevent disturbance by large mammals. Holes ca. 30 cm in diameter and 15–20 cm deep were dug with an auger in February 2009 to fit potted plants.

Species

Vincetoxicum nigrum and *V. hirundinaria* were chosen for our study because both species occur naturally in southern France (Fig. 1). The latter was considered a good substitute for *V. rossicum* (the NA

Fig. 1 Site locations in France where the Apocynaceae plants, *Vincetoxicum nigrum* (*filled circle*) and *V. hirundinaria* (*asterisk*), and the chrysomelid beetle *Chrysochus asclepiadeus* (*plus sign*) were field collected in 2009. The map also includes the approximated native ranges of *V. nigrum* (*solid line*), *V. hirundinaria* (*dotted line*), and *V. rossicum* (*dashed line*)

invasive) because: (1) preliminary phylogenetic evaluation of both *V. rossicum* and *V. hirundinaria* in their native and introduced ranges showed identical genetic sequences of 1,300 bp in length for the two chloroplastic intergenic regions between the trnS (GCU) and trnG (UCC) genes and the trnG and trnR (UCU) genes (MC Bon, *pers. com.*); (2) plant protection regulations prevented *V. rossicum* from being transplanted in the field in France outside of its natural eastern European range; and (3) the European range of *V. hirundinaria* encompasses both that of *V. rossicum* and *V. nigrum*.

Vincetoxicum nigrum rootstalks were collected on 20 November 2008 from a natural population ca. 3 km from EBCL at an elevation of 100 m. *Vincetoxicum hirundinaria* rootstalks were collected on 23 November 2008 from the southern slope of Mont Ventoux (Vaucluse, France) at an elevation of ca. 1,400 m. Plants were collected from two locations because of availability; differences in elevation were not considered important because both species are distributed naturally from sea level to 2,000 m.

The chrysomelid beetle, *Chrysochus asclepiadeus* (Pallas), was selected as the herbivorous natural enemy because it is a specialist on *V. hirundinaria* and impacts both above and belowground biomass. Freshly emerged adults of *C. asclepiadeus* (1–5 days old) were collected from local *V. hirundinaria* in the field on 8 June 2009 from northeastern France (Jura). Approximately 2,000 individuals were collected and stored in paper tubes (100 beetles per tube) 7 cm in diameter and 12 cm long with leaves from their host plant for transportation back to the lab on 9 June 2009. Beetles were fed on leaves from the plant species they were to be released on (either *V. hirundinaria* or *V. nigrum*) in larger cages (20 × 20 × 7 cm) at room temperature (20–25°C).

Experimental design

The experiment was set up as a split plot design, where plant species was the whole plot factor, and the within plot factors were plant and beetle density. The field was split into two sections containing blocks of one of the two plant species: 14 blocks of *V. hirundinaria* in one section, and 7 blocks of *V. nigrum* in the other. Each block (1.3 m × 1.3 m) contained 9 equally-spaced pots (20 cm diameter × 25 cm deep) ca. 35 cm apart.
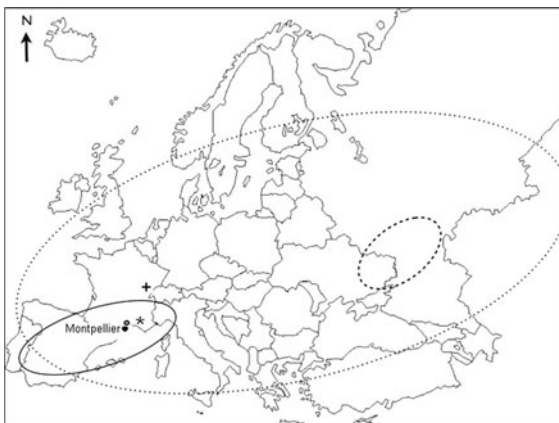
On 3 March 2009, rootstalks collected from the field were standardized by weight and planted in pots with commercial potting soil at low (32 plants/m^2 = 1 rootstalk/pot), medium (127 plants/m^2 = 4 rootstalks/pot), and high (255 plants/m^2 = 8 rootstalks/pot) densities. Humin Substrat soil (pH: 5.5–6.5; with added fertilizer N:P:K = 14:16:18), Neuhaus, Germany, was used for the potting soil. Three pots of each density were randomly assigned to positions within the blocks. These plant densities reflected the observed range for invasive NA populations of *Vincetoxicum* spp. Densities were also chosen to reflect a range below and above that density identified by Cappuccino (2004) showing an Allee effect in *Vincetoxicum* spp. (i.e. 81 plants/m^2). On 11 June 2009, cages were installed around each pot to protect the plants from other damage and to keep fixed beetle densities on their designated host plants. Cages consisted of a metal frame ca. 60 cm tall placed inside the pots with mesh over top and fastened at the base with a plastic strap. The caged plants were allowed to grow in the field with regular watering throughout the experiment.

On 15 June 2009, equal numbers of male and female beetles were released into the pots in the field at densities of 2 and 4 individuals per pot; an additional group of pots received no beetles as controls. The beetle densities were chosen based on their availability as well as their observed feeding impact in the lab; more than 4 beetles on low density plants resulted in rapid and complete defoliation. Because the goal was to maintain feeding throughout the 4-week experiment, a maximum of 4 beetles was released into a pot. We used a 1:1 (M:F) sex ratio to: (1) prevent a bias in differential feeding activity; (2) ensure larvae for continued studies on root herbivory; and (3) accurately reflect the sex ratio in natural field populations (R. Sforza, unpublished data). Each beetle density was applied within a block so that each of the three pots corresponding to one of three plant densities (high, medium or low) was randomly assigned either 0 (control), 2 or 4 beetles in a 2-factorial design. Beetles remained on the plants for 4 weeks (15 June–15 July 2009) and their status was monitored every second day throughout the experiment. Dead beetles were removed from pots and replaced immediately with live beetles from the lab of the same age and sex to ensure plants received equivalent treatments.

Data collection

Data were collected on potted plants before the beetle treatments began. On two occasions (29 April and 3 June 2009) measurements were taken of the presence and number of seed pods, and presence and amount of damage by *C. asclepiadeus*. The same data were collected on 16 July 2009 at the end of the experiment after beetles had been removed. The amount of above-ground damage was measured as the percentage defoliation (in terms of leaf area) per pot on a scale from 1 to 5: 1 = 0%, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, 5 = 100%. Pots with seed pods were recorded throughout the duration of the experiment. Pods remaining on the plant at the end of the experiment were removed and dissected to determine seed count.

Plants were removed from the field on 16 July 2009. All 7 blocks of *V. nigrum* and a randomly selected 7 out of the 14 available blocks of *V. hirundinaria* were disassembled (7 blocks of *V. hirundinaria* remained in the field for studies on larval impact). Only data from the 14 total blocks removed are included in the present study. The samples (total plant material per pot) were cleaned of soil with water and stored individually in paper bags for weighing. Root and shoot weights were recorded before and after samples were dried at 70°C for 24 h in an oven.

Statistical analysis

All data analyses were performed using R Version 2.9.1 (R Development Core Team 2005). The impact of beetles on defoliation and plant fitness in terms of total dry mass and root:shoot biomass was analyzed using a 3-factorial analysis of variance (ANOVA) with interaction for a split-plot design followed by a post-hoc test (Tukey HSD). The same was done for the impact of beetles on root:shoot biomass measured in terms of damage level when damage level was analyzed as an independent variable. All data adhered to the assumptions of parametric statistics, which include independence, normality, and equal variances. In the case where Tukey's HSD test was too strict to explain significant factorial interactions (i.e. when the impact of beetles on dry mass/pot was tested), a Bonferroni test of multiple comparisons was used to account for simultaneous inference and adjust the significance level.

The effect of plant and beetle treatments on plant reproductive success was measured as a single binary response variable (presence or absence of seeds per pot) because so few pots produced seeds. Data were analyzed with logistic regression analysis. A full factorial general linear model was created for the presence/absence of seeds as a function of species, plant density and beetle treatment. A stepwise model selection by Akaike's Information Criterion (AIC) was then performed to reduce the full factorial model to the most parsimonious model. The final model was accepted if it had a weighted AIC value (W_i) within 10% of the most explanatory model (model with the largest W_i), or was within the "confidence set" of candidate models (Royall 1997). The weighted AIC value was calculated as:

$$W_i = \exp(-\Delta_i/2)/\Sigma_{(r=1,M)} \exp(-\Delta_r/2)$$

where W_i = weighted AIC, M = number of models, Δ_i = AIC_i – AIC min, and r = any given model from 1 to M. The final model included all factors and interactions between factors that significantly explained trends in the data.

Results

Impact of beetles on plants: defoliation

Both *V. hirundinaria* and *V. nigrum* were defoliated by beetle treatments to the same degree ($P = 0.7515$; n = 14) (Fig. 2). As expected, the control treatment (0 beetles) showed no damage. There was significant interaction between beetle density and plant density ($P < 0.0001$) as plant damage increased significantly for medium ($P = 0.0028$) and high ($P = 0.0028$) but not for low ($P = 0.8228$) plant densities as beetle density increased from 2 to 4 beetles per pot. Overall, the amount of damage per beetle was not additive i.e. doubling the number of beetles released on plants did not double corresponding damage.

Impact on plant fitness: biomass and resource allocation

Total Dry Mass: *V. hirundinaria* and *V. nigrum* responded similarly ($P = 0.0602$; n = 14) in total dry biomass across the three plant densities as a
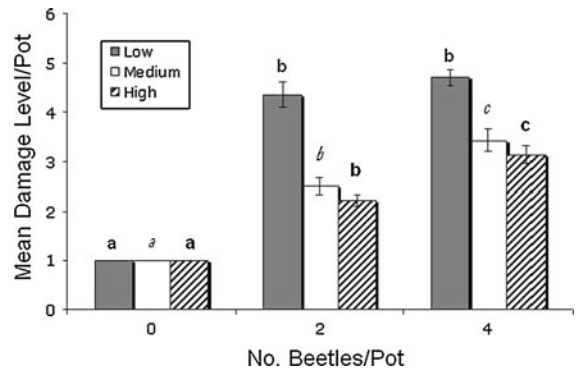

Fig. 2 The observed damage per pot for *Vincetoxicum hirundinaria* and *V. nigrum* (pooled) at low (32 plants/m^2) (*gray*), medium (127 plants/m^2) (*white*), and high (255 plants/m^2) (*dashed*) pot densities, plotted against beetle density per pot (n = 14). Values are presented as means ± SE. Damage levels were recorded as percentage defoliation per pot (*1* 0%, *2* 25%, *3* 50%, *4* 75%, *5* 100%). An ANOVA performed using R (v. 2.9.1) revealed there was a significant plant density effect ($P < 0.0001$), beetle density effect ($P < 0.0001$), and interaction between plant density and beetle density ($P < 0.0001$). *Differing letters* above bars represent statistical significance at the $P < 0.05$ level between beetle densities within a particular plant density; low (*a-normal*), medium (*a-italics*) and high (*a-bold*)

function of beetle density. As expected, there was a significant plant density effect with low-density pot biomass < medium pot density biomass < high pot density biomass ($P < 0.0001$) (Fig. 3). Of all possible interaction terms, only that for plant density and beetle density was significant ($P = 0.0343$). Within each plant density, there was no significant difference in biomass between beetle densities at medium and high plant densities, but at low plant densities any defoliation by beetles significantly decreased total biomass ($P = 0.0029$; n = 14) (Fig. 3). For both species, the biomass in low-density pots was similar between 2 and 4 beetles per pot and different from the control.

Root: Shoot Ratio: The responses of plants in terms of root:shoot ratio were independent of plant density ($P = 0.5758$). The highest beetle density significantly increased the root:shoot biomass of the plants ($P < 0.0001$) (Fig. 4). Both species were similar in their root:shoot dry mass ($P = 0.5261$; n = 14) when plant density was compared as a function of the number of beetles per pot. To further assess the differences in root: shoot ratio between species, the root:shoot ratio was also compared across
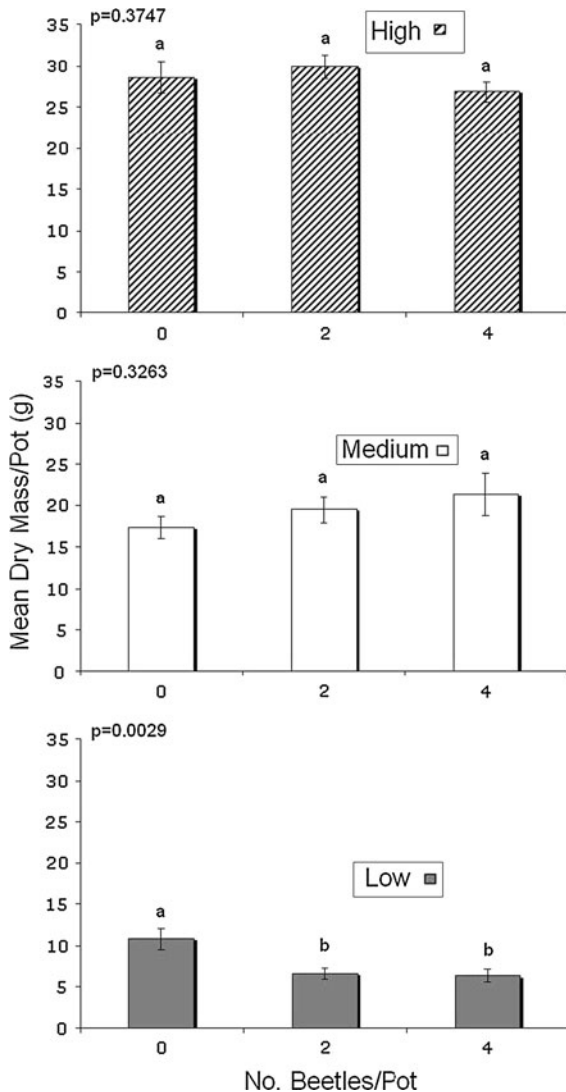
Fig. 3 Mean dry mass per pot of *Vincetoxicum hirundinaria* and *V. nigrum* (pooled) plotted against beetle density per pot for high (255 plants/m^2) (*top-dashed*), medium (127 plants/m^2) (*middle-white*), and low (32 plants/m^2) (*bottom-gray*) plant densities (n = 14). Values are presented as means ± SE. Results of a split plot ANOVA performed using R (v. 2.9.1) revealed there was a significant plant density effect ($P < 0.0001$), and interaction between plant density and beetle density ($P = 0.0343$). Significant differences in dry mass between beetle densities within each plant density are indicated by *different letters* ($P < 0.05$)
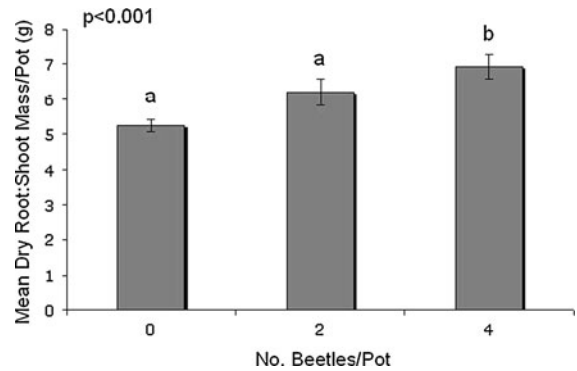

Fig. 4 Total dry root: shoot mass ratio per pot for *Vincetoxicum hirundinaria* and *V. nigrum* (pooled) plotted against beetle density per pot (n = 14). Values are presented as means ± SE. An ANOVA performed using R (v. 2.9.1) revealed there was a significant beetle density effect ($P < 0.001$). *Different letters* above *bars* represent statistical significance at the $P < 0.05$ level between beetle densities
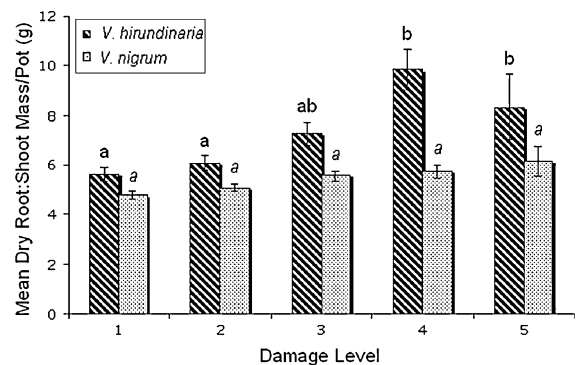

Fig. 5 Total dry root/shoot mass ratio per pot for *Vincetoxicum hirundinaria* (*dashed*) and *V. nigrum* (*dotted*) plotted against the observed damage level (n = 7). Damage levels were recorded as percentage defoliation per pot (*1* 0%, *2* 25%, *3* 50%, *4* 75%, *5* 100%).Values are presented as means ± SE. An ANOVA performed using R (v. 2.9.1) revealed there was a significant damage level effect ($P < 0.0001$), and interaction between species and damage level ($P = 0.0114$). *Different letters* above *bars* represent statistical significance at the $P < 0.05$ level between damage classes within *V. hirundinaria* (a-normal) and *V. nigrum* (a-italics)

species and plant densities as a function of defoliation level per pot. There was no significant difference in root:shoot biomass among species ($P = 0.4843$) or between plant densities ($P = 0.5109$ l; n = 14), however of all possible interactions, significant

interaction was seen for species and damage level ($P = 0.0114$) suggesting that the response to defoliation was species specific (Fig. 5). When the total root:shoot biomass was examined within each species (n = 7), the root:shoot biomass of *V. hirundinaria* increased significantly in proportion to beetle damage ($P < 0.0001$), while that for *V. nigrum* remained the same across all damage classes ($P = 0.0631$).

Fig. 6 The percentage of pots with seeds for *Vincetoxicum hirundinaria* (*dashed*) and *V. nigrum* (*dotted*) plotted against the number of beetles per pot for low (32 plants/m²), medium (127 plants/m²) and high (255 plants/m²) plant densities (n = 7)
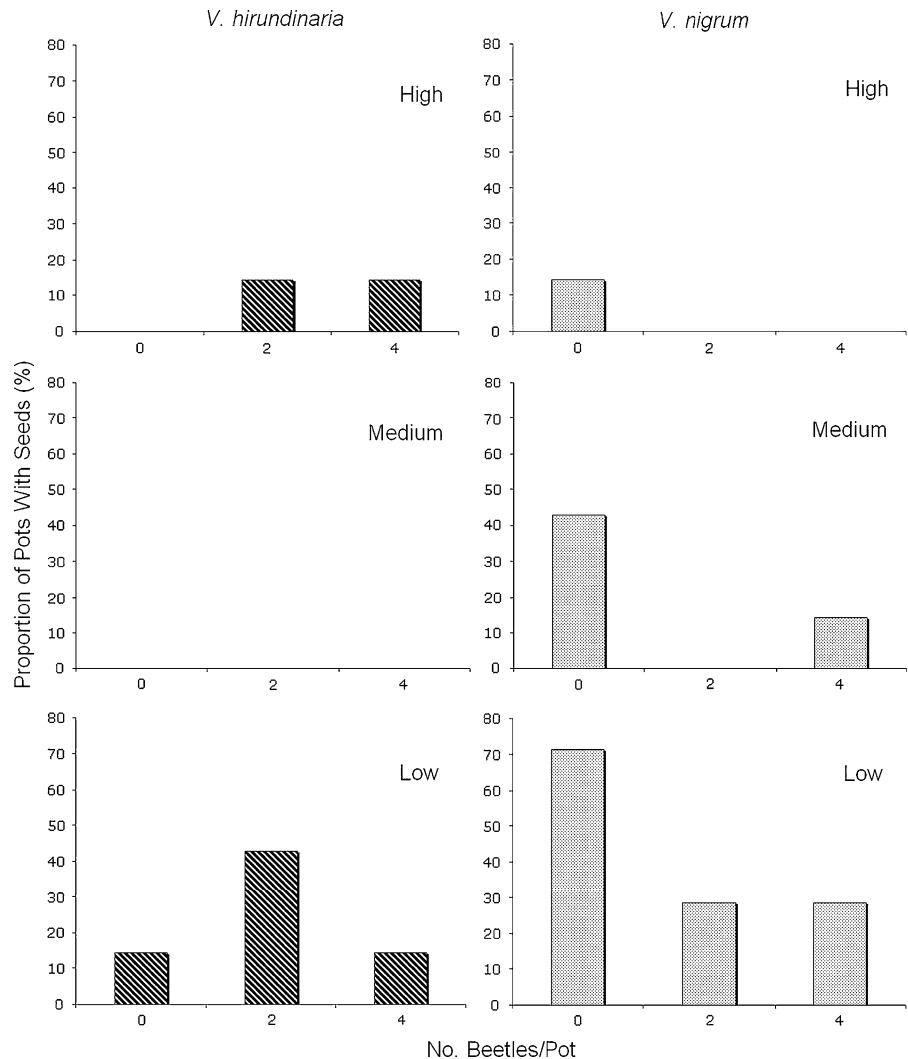
Impacts on plant fitness: seed production

The final general linear model that resulted in the best explanatory power for seed presence/absence as a function of beetle density and plant density for both species was one that included all main effects (i.e. species effects, density effects and treatment effects) along with the interactions between species and treatment and between species and density (n = 14). Because seed output (Fig. 6) varied by species, *V. hirundinaria* and *V. nigrum* were analyzed separately (n = 7). Plant density explained seed production in *V. hirundinaria* ($W_i = 0.64$) but beetle density did not ($W_i = 0.04$), and there was no significant interaction between the two ($W_i = 0.01$) (Table 1). More low density *V. hirundinaria* pots had seeds than

those at higher densities. Plant density and beetle density explained seed production in *V. nigrum* ($W_i = 0.87$) and there was no significant interaction between the two ($W_i = 0.04$). There was a significantly higher proportion of low density pots with seeds, and this was reduced significantly at higher beetle densities.

Discussion

Herbivory on *Vincetoxicum* spp. will reduce total plant biomass and therefore could possibly contribute to decreased growth and spread of this species in NA. Feeding by *C. asclepiadeus* adults caused significant defoliation to both *V. hirundinaria* and *V. nigrum*

Table 1 AIC (Akaike's information criterion) values for candidate models included in the step AIC test of seed data for (a) *Vincetoxicum hirundinaria* and (b) *V. nigrum*

Model	df	RD	AICc	Δ_i	W_i
(a)					
P × B	4	$3.46 \times e^{-10}$	27.85	8.27	0.01
P + B	4	1.23	21.08	1.50	0.30
P	**2**	**3.73**	**19.58**	**0**	**0.64**
B	2	9.17	25.02	5.44	0.04
(b)					
P × B	4	$2.59 \times e^{-10}$	31.01	6.22	0.04
P + B	**4**	**1.78**	**24.79**	**0**	**0.87**
P	2	11	30.01	5.22	0.06
B	2	12.94	31.95	7.16	0.02

Bolded values represent the models explaining a significant proportion of the data. Weighted AIC values (W_i) were considered significant if they fell within 10% of the most explanatory model

AICc, AIC value; *df* degrees of freedom, *RD* residual deviance, Δ_i relative AIC, *P* plant density, *B* beetle density

although the amount of damage was not linearly proportional to beetle density. Thus, high damage levels on the foliage of both *Vincetoxicum* spp. are possible with relatively few beetles and this will be greater when plants are at low versus high density. These results suggest that plants at higher densities could have greater fitness than plants at lower densities. If so, this could exacerbate the Allee effect observed previously in North American *Vincetoxicum* spp. (Cappuccino 2004; Taylor and Hastings 2005) and suggests that high densities of these species are able to resist damage by herbivores by increasing growth to compensate for the biomass lost due to insect feeding. This compensatory growth response is associated with increased "invasibility" of exotic plant species outside of their native range, and has also been used to explain the lag from the time of introduction to the time of establishment for some invasive species such as *Vincetoxicum* spp. (Cappuccino 2004). Such plants tend to start out as small, localized populations, and over time transition into highly invasive noxious weeds as densities increase (Taylor and Hastings 2005). The relative impact of herbivory on such species may therefore be dependent on their composition in the landscape, with sparse populations being much more affected by feeding in terms of biomass than dense ones.

It is important to note that the levels of damage we observed on our low-density plants were higher than those in either the medium- or high-density pots. If 100% defoliation of high- and medium-density pots could have been achieved, then this would have been a better indication as to whether the effects we observed were due to increased vigor at high plant density or simply an artifact of plants at low density experiencing the highest beetle pressure. To test this, future experiments should control for the impact of beetles at different plant densities by including proportionately more beetles in the pots with higher plant densities.

Root:shoot ratio was measured to understand how *Vincetoxicum* spp. allocate resources in response to herbivory. Both species showed an increase in root:shoot mass as a result of increased beetle density compared to the controls, however, when this response was analyzed as a function of the actual observed defoliation per pot, the two had very different responses; *V. hirundinaria* increased resource allocation to its roots in a compensatory response to defoliation while *V. nigrum* did not.

The reaction of *V. hirundinaria* to herbivory is consistent with that in other plants that have adapted defense mechanisms against herbivores (Wilson et al. 2007), and this is supported by the fact that *V. hirundinaria* and *V. nigrum* differ in their evolutionary history and association with natural enemies. *V. hirundinaria* has a long established relationship with *C. asclepiadeus* whereas *V. nigrum* does not. Preliminary surveys have failed to observe any specialist herbivores feeding on *V. nigrum* in its native range, although generalists have been found in low numbers (R Sforza, unpub. data). The high "compensatory growth capacity" or ability to regenerate in response to disturbance that may be exhibited by *V. hirundinaria* could be explained by an adaptation that has arisen as a result of its long-term association with this insect (Strauss and Agrawal 1999; Kaplan et al. 2008). For example, Arredondo and Johnson (2009) examined the impact of herbivory on two closely-related plant species, one grazing-tolerant and one grazing-intolerant and found that root biomass of the grazing-tolerant species increased after defoliation while that of the grazing-intolerant species showed an inhibition of root biomass allocation. Similarly, localized populations of ragwort (*Senecio jacobaea* L.) with a history of arthropod

herbivory develop higher levels of root storage than those isolated from herbivores (van der Meijden et al. 2001). This then might support the possibility that *V. hirundinaria* has a higher level of tolerance to herbivory by *C. asclepiadeus* than *V. nigrum*.

There are several advantages associated with a tolerance to foliar damage that might have serious implications for the control of invasive *Vincetoxicum* spp. For example, increasing root biomass can improve a plant's ability to re-sprout after defoliation (Martinkova et al. 2008). Species that have the potential to reproduce vegetatively, such as *Vincetoxicum* spp., and other invasives such as alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.) are more likely to increase allocation of resources to roots in response to above-ground damage (Strauss and Agrawal 1999; Abhilasha and Joshi 2009; Jia et al. 2009). Wilson et al. (2007) describe this as a compensation mechanism by which the plant can make up for tissue/nutrient losses.

A compensatory mechanism in *V. hirundinaria* may also allow for greater resistance to below-ground damage caused by *Chrysochus* beetles in their larval stage, and this again has significant implications for understanding the ability of *Chrysochus* to reduce fitness of different swallow-wort varieties, including those that are thriving in NA. Even though our study only examined the response of *Vincetoxicum* spp. to foliar damage, other research suggests that plants with high tolerance to root damage may still allocate resources to roots (van Dam 2009; Hunter 2001). Similarly Notzold et al. (1998) found that below-ground herbivory by the weevil *Hylobius transverso-vittatus* Goeze significantly altered biomass allocation in purple loosestrife (*Lythrum salicaria* L.); attacked roots made up 50% of the total biomass while unattacked roots made up only 30–40%. We are currently investigating the impacts of larval damage on *Vincetoxicum* spp. and hopefully these results will better elucidate the differences in response of *V. nigrum*, *V. hirundinaria* and *V. rossicum* to herbivory and help design more effective control strategies for these species.

Seed production is an important measure of fitness in *Vincetoxicum* spp. The primary means of dispersal of this plant in NA is through the release of its comose seeds, which are dispersed easily by wind and predispose it to rapid spread across the landscape. For example, seeds can travel and establish satellite populations hundreds of meters from the source plant in moderate wind conditions (Cappuccino et al. 2002). Understanding factors limiting its reproductive output and dispersal ability in its natural range may contribute to developing novel controls in NA. A greater proportion of seeds were produced by *V. nigrum* than *V. hirundinaria* and this may again have to do with the differences in their evolutionary adaptations to specialist herbivores. Resource allocation to the roots of *V. hirundinaria* to increase potential regrowth after above-ground feeding damage may have occurred at a cost to reproduction (Strauss and Agrawal 1999; Jia et al. 2009; Snell and Birch 1975). One adaptive mechanism in *V. hirundinaria* might be to decrease or delay investment into seed production when there is pressure from natural enemies in order to persist after the disturbance (Agrawal 2000).

In addition to the effect of beetle density, plant density was also significant in both species. Most of the pots for both *V. hirundinaria* and *V. nigrum* that produced seeds had the lowest plant density. The decrease in reproductive output with increasing plant density observed in *V. hirundinaria,* and even more so in *V. nigrum,* contradicts the idea of a possible Allee effect. Work by Cappuccino (2004) found higher densities of *Vincetoxicum rossicum* produce more seeds than low densities. One reason for these contrasting observations regarding seed production, besides species specific differences in response (i.e. *V. rossicum* may exhibit an Allee effect in terms of seed production but *V. hirundinaria* and *V. nigrum* do not), may be that plants in low-density pots had greater access to resources and less intra-specific competition than those in high-density pots, and as a result had higher reproductive output. Other invasive plant species such as the yellow star thistle (*Centaurea solstitialis* L.) have also shown increased growth and seed production at low plant density (Garren and Strauss 2009). More significantly, another invasive plant, garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande), also has been shown to produce more seeds at lower densities; when nutrients were added to the high density samples, seed production increased further suggesting that resources were limiting with respect to seed production (Meekins and McCarthy 2000). Future studies should investigate this finding as reduced reproduction under conditions of intraspecific competition lends support for the strategy to reduce *Vincetoxicum* spp. by planting competitor species in NA.

Our study contributes to a greater understanding of the ecology of *Vincetoxicum* spp. in its native range, however, several questions remain to be answered. Two species are invasive in NA yet they tend to be treated as a single problem. Our work suggests that they could differ significantly in terms of root:shoot ratio and seed production; *V. rossicum* may also be capable of responding to disturbance quite uniquely. Future studies should examine the effects of plant density and herbivory on the fitness of *V. rossicum,* in addition to *V. hirundinaria* that we used in our study. While research on *V. hirundinaria* can provide insight into the response of *V. rossicum*, these two species likely also respond differently to herbivory and intraspecific competition in a way that will affect the success of NA control strategies.

Experiments should be carried out directly in the field rather than in pots as this may reduce confounding factors such as pot size and constrained growth. As well, investigating differences in fungal communities between NA and Europe may help to explain the stronger Allee effects in NA. For example, large amounts of arbuscular mychorrhizal fungi (AMF) were found to benefit this weed in NA in high-density *Vincetoxicum rossicum* patches. This may help confer competitive advantages to swallow-wort in high density patches over other native plant species in NA (Smith et al. 2008).

Finally, in addition to above-ground feeding as an adult, *C. asclepiadeus* impacts *Vincetoxicum* spp. by feeding on the roots as larvae. We are currently examining the relative impact of below-ground damage on *Vincetoxicum* fitness. Questions remaining to be addressed include whether above-ground feeding, below-ground feeding, or a combination of both could help maintain control over populations of *Vincetoxicum* spp. in both their natural and invasive range. Also, there may be interactions between below-ground feeding and plant density. Given the impacts already observed for above-ground herbivory on plants, it is hard to imagine how below-ground feeding damage could not also significantly reduce plant fitness.

Implications for management

Our study has important implications for management of *Vincetoxicum* spp. in NA. Clearly, significant damage can be inflicted on both species by relatively few beetles. If a biological control program involving native or introduced herbivores is initiated, areas for introduction should include population edges or newly-established satellite populations at low density to reduce biomass and spread. In the case of *V. nigrum*, there is evidence to suggest that any density population could potentially be targeted to reduce reproductive output and dispersal.

Interplanting competitive native species in low or medium density patches in NA may also help to limit the potential for seed dispersal in *Vincetoxicum* spp. Plants in our study produced fewer seeds at higher densities, and this may be due to increased competition for available resources. Limiting resource availability through planting of highly competitive native species such as goldenrod (*Solidago Canadensis* L.) or raspberry (*Rubus occidentallis* L.) may help to decrease its reproductive capacity in NA (Martinkova et al. 2008).

Our field study is the first to examine the impact of a specialist phytophagous beetle on *Vincetoxicum* spp. fitness in its native range. The results show that *C. asclepiadeus* is able to survive and feed on both *V. hirundinaria* and *V. nigrum*, and that plant fitness is reduced as a result. *Vincetoxicum nigrum* appears to be particularly susceptible to feeding by this insect, and this may be due to differences in its evolutionary relationship with herbivores. While we found evidence for an Allee effect in terms of total biomass, our seed production results were contradictory. It is possible that simple experimental issues, such as the effects of plants in pots, could account for this, or it may be that more significant ecological interactions lead to a stronger Allee effect in the invasive rather than the native range (i.e. the invasive's ability to shade out competing plant species, or its ability to form unique associations with different fungal communities). In either case, our results highlight the importance of ecological interactions between intraspecific competition and herbivory when designing control programs for *Vincetoxicum* spp. in NA. The increasing spread of *Vincetoxicum* spp. beyond degraded landscapes into northeastern temperate forests threatens native species, ecosystems, as well as forest regeneration. Development of effective management strategies will require greater understanding of population regulation in the natural range of this invasive as well as the interactions between the plant, its natural enemies and its competitors.

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