Effects of leaf and root herbivory by potential insect biological control agents on the performance of invasive *Vincetoxicum* spp.

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A R T I C L E   I N F O

A B S T R A C T

The European leaf-feeding moth *Abrostola asclepiadis* and root-feeding beetle *Eumolpus asclepiadeus* are promising biological control agents for two European swallow-worts (*Vincetoxicum rossicum* and *Vincetoxicum nigrum*) in North America, however, their impact on plant performance is uncertain. Densities of each herbivore were manipulated in a common garden to determine whether leaf and root herbivory affect the performance of these plants. During the second year of the experiment, *V. rossicum* and *V. nigrum* unexpectedly became infected with the fungal pathogens *Ascochyta* sp. and *Cercospora* sp. (Ascomycota), respectively. Although pathogen infection mainly reduced shoot height and delayed reproduction, herbivore effects on plant growth were still evident. Leaf herbivory by *A. asclepiadis* had no effect on plant growth 1 year after defoliation. Root herbivory by *E. asclepiadeus* reduced shoot height and plant biomass and decreased the ability of plants to compensate for pathogen attack. Pathogen infection prevented detection of herbivore effect on reproduction. Due to its substantial impact on plant biomass, *E. asclepiadeus* should be further evaluated as a biological control agent against *Vincetoxicum* spp. populations invading open habitats in North America. Further research is needed to evaluate the impact of *A. asclepiadis* in combination with *E. asclepiadeus* and plant competition under high and low light conditions.

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1. Introduction

Evaluating the effects of differing types of herbivory on plant performance is a critical step in the development of weed biological control programs because plant response to herbivory is highly variable (Inouye, 1982; Maron, 1998; Blossey and Hunt-Joshi, 2003; Hunt-Joshi et al., 2004). The challenge during selection of agents for weed biological control is to not only identify host specific herbivores, but also find herbivores that negatively affect the population dynamics of the target weed (Sheppard, 2003; van Klinken and Raghu, 2006). Only recently have both assessments been adopted in screening for weed biological control (Briese et al., 2002; Häfliger et al., 2006; Baars et al., 2007; Gerber et al., 2007) which traditionally emphasized host specificity testing (McClay and Balciunas, 2005).

Prerelease efficacy assessments are utilized to minimize nontarget effects (Pearson and Callaway, 2003, 2005), to improve efficiency of agent selection, and to improve probability of success (Sheppard, 2003; McClay and Balciunas, 2005). They represent a considerable improvement over basing releases on the 'lottery model' (Denoth et al., 2002; Sheppard, 2003). These studies are used to determine how agent densities affect life-history parameters of the target weeds and to evaluate plant response to herbivore attack (Morin et al., 2009). They can be used either to develop efficacy rankings of prospective agents (Häfliger et al., 2006) or to parameterize plant demographic models (Davis et al., 2006).

Two European asclepiads in the genus *Vincetoxicum* (swallowworts) have invaded a variety of natural and semi-natural habitats in central and eastern North America. *Vincetoxicum nigrum* (L.) and *Vincetoxicum rossicum* (Kleopow) Barbar. are long-lived perennials that develop into dense stands in the USA and Canada threatening native biodiversity (Christensen, 1998; DiTommaso et al., 2005; Ernst and Cappuccino, 2005). A biological control program has been initiated to lessen the environmental impact of these weeds with a leaf-feeding noctuid *Abrostola asclepiadis* (Denis and Schiffermüller) and a root-feeding chrysomelid *Eumolpus asclepiadeus* Pallas under consideration. Both species attack the closely-related host, *Vincetoxicum hirundinaria* Medik., in Europe. But, recent studies have determined that the two target weeds are suitable hosts for the insects (Weed, 2010), which has created interest in these species as potential biological control agents for *Vincetoxicum* spp. in North America (Weed and Gassmann, 2007).

The effects of artificial defoliation and herbivory on *V. nigrum* and *V. rossicum* performance have been studied (McKague and Cappuccino, 2005; Milbrath, 2008; Weed and Casagrande, 2010),

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but nothing is known about the impact across years, and defoliation by *A. asclepiadis* has not been studied. The response of *V. nigrum* and *V. rossicum* to artificial defoliation varies with light intensity, with dramatic effects on plant architecture (Milbrath, 2008). Infestations growing in open habitats subjected to high light intensity are resistant to mowing and typically plants fully compensate for this damage (McKague and Cappuccino, 2005). Although both studies have importantly demonstrated plant response to this type of attack, it is possible that feeding by *A. asclepiadis* may elicit different effects on plant fitness (Agrawal et al., 1999).

The effects of root herbivory on *Vincetoxicum* spp. growth and reproduction are unknown. Root feeders have been shown to be excellent biological control agents (Blossey and Hunt-Joshi, 2003). This is likely due to a combination of effects including reduction of water and nutrient uptake, suppression of plant height, and alteration of plant competitive ability (Müller-Schärer, 1991; Nötzold et al., 1997; Sheppard et al., 2001; Briese et al., 2002; Blossey and Hunt-Joshi, 2003). Current management practices have minimal impact on the root system (Christensen, 1998; Averill et al., 2008) and root herbivory could be particularly effective in controlling these weeds because the majority of plant biomass is allocated belowground (DiTommaso et al., 2005; Milbrath, 2008).

In this study we evaluated the individual effects of above- and belowground herbivory on the performance of *V. nigrum* and *V. rossicum* in a common garden experiment. We investigated whether: (1) the impact of herbivory will increase with herbivore density in the same manner on both *Vincetoxicum* sp. and (2) root herbivory will have a greater adverse effect on *Vincetoxicum* spp. performance than leaf herbivory. The results are discussed relative to the selection of biological control agents against *Vincetoxicum* spp. in North America.

2. Materials and methods

2.1. Study organisms

Vincetoxicum (Apocynaceae) are clump-forming, perennial herbaceous plants native to Eurasia. The European species *V. nigrum* and *V. rossicum* have invaded shaded woodlands, roadsides, dry grasslands, pastures, and coastal shores in North America (DiTommaso et al., 2005). Polyembryonic seeds are dispersed by wind and germinate either during the same season or the following spring. Both species produce woody rootstalks with fibrous roots that deeply penetrate the substrate. In spring, new shoots of mature plants expand from buds on the root crown and can grow up to 3 m in one season depending on light conditions and stem density (Sheeley and Raynal, 1996; DiTommaso et al., 2005). Plants do not produce a persistent aboveground woody stem. Flowers that are insect or self-pollinated are produced at leaf axils beginning in late May and continuing until August. Plants produce multiple, elongate seedpods that mature during the summer and seed dispersal extends from August to October (DiTommaso et al., 2005).

Abrostola asclepiadis (Lepidoptera: Noctuidae) is widely distributed within Europe and recorded from Asia Minor and the Caucasus Range primarily attacking *V. hirundinaria* (Goater et al., 2003). In some parts of Europe *A. asclepiadis* is bivoltine (Goater et al., 2003) but populations in Sweden (Förare, 1995) and Switzerland (A. Weed, pers. obs.) are univoltine. Females deposit batches of eggs (1–20) on the undersides of leaves and larvae complete development in about 6 weeks in Sweden (Förare, 1995). Young larvae feed on the lower leaves during the day and night. Larger larvae feed on young foliage almost exclusively at night and typically re-

quire multiple shoots to complete their development (Förare and Engqvist, 1996).

The colony of *A. asclepiadis* used for the experiments in this study originated from 20 larvae collected in Moutier, Switzerland (N 47° 17.073′, E 07° 23.160′) in 2007. Larvae were transported to the CABI laboratory in Delémont, Switzerland and fed *V. hirundinaria* until they completed their development. Pupae were kept in 250 ml plastic cups filled with moist vermiculite and cups were stored in an outdoor shelter until the spring of 2008. In early July 2008, five to 10 mating pairs were placed into a wooden cage ($20 \times 25.5 \times 38$ cm) for oviposition. When eggs were present they were transferred from leaf surfaces to Petri dishes (9 cm diameter) lined with moistened filter paper. In preparation for treatments (described below), *A. asclepiadis* larvae were raised to the L2 stage in batches of 20 on excised leaves of *V. nigrum* or *V. rossicum* in Petri dishes.

Eumolpus asclepiadeus (Coleoptera: Chrysomelidae) occurs throughout Europe and into the Caucasus Range commonly attacking *V. hirundinaria* in dry, open habitats (Weed, 2010). Adults are active from June until September and are commonly observed feeding and mating during midday at the top of their host plant (Weed, 2010). After mating, females descend shoots and oviposit at the shoot base (A. Weed, pers. obs.). Neonate larvae immediately burrow into the soil and feed externally on the roots. Most larvae have a 2-year life cycle but some (<5%) take 1 year or up to 3 years to complete development (Weed, 2010). Pupation occurs during early spring and newly emerged adults ascend host shoots and begin feeding and mating. A colony of *E. asclepiadeus* was established during 2006 from 25 mating pairs collected in Kiev, Ukraine (N 50° 23.572′, E 30° 33.315′). The colony has been maintained annually by infesting potted *V. hirundinaria*, *V. nigrum*, and *V. rossicum* plants held at the CABI laboratory in Switzerland as described below. Larvae used in this experiment were obtained from eggs taken from approximately 40 mating pairs originating from the F_1 generation emerging in 2008.

2.2. Plant culture

In preparation for experiments seeds of *V. rossicum* originating from New York, USA and *V. nigrum* from Rhode Island, USA were sown in potting mix in a greenhouse in Switzerland during the winter of 2004. Seedlings were transplanted into 2 L pots with a growing mixture containing 1:4:30, vermiculite:sand:soil. All pots with plants were spaced 15 cm apart in raised beds, surrounded with coarse sawdust, and maintained outdoors at the CABI laboratory from 2004 to 2008. The raised beds were fully exposed to sun and irrigated as needed to ensure healthy plant growth. No preventive measures were taken to suppress antagonists because insect and pathogen attack was uncommon in previous years. At the end of each growing season, seedpods and dead shoots were removed from pots and plants were protected over winter with a layer of pine boughs which were removed in April.

2.3. Experimental design

The effect of increasing herbivore (leaf and root) density on *V. nigrum* and *V. rossicum* performance was evaluated simultaneously in a common garden at the CABI laboratory in Switzerland. Individual plants of *V. nigrum* (*n* = 64) and *V. rossicum* (*n* = 56) were randomly assigned to one of the following seven treatments: no larvae, *A. asclepiadis* densities of 1, 3, and 5 larvae per plant, and *E. asclepiadeus* densities of 5, 20, and 60 larvae per plant. Twice the number of plants was allocated to the treatment receiving no larvae (controls) so that plant biomass could be measured from half of these plants prior to larval infestation. The same set of control plants was used for both insect species because the experi-

ments were run simultaneously in the same garden. On 12 July 2008, the number of shoots, the height of the tallest shoot, and number of flowers and seedpods were measured on all plants. A two-way ANOVA confirmed that prior to infestation, maximum shoot height, a good indicator of aboveground biomass ($r^2 = 0.33$; $P = 0.0260$), was similar between plant species ($F_{1, 112} = 1.46$; $P = 0.1985$) and among treatments ($F_{6, 112} = 0.29$; $P = 0.5893$) for all plants.

Dry aboveground biomass (5.3 ± 2.7 g [mean ± SD], $n = 15$) was similar between species ($t = 0.48$, $df = 14$, $P = 0.6397$) as was maximum shoot height (42.1 ± 9.8 cm, $n = 15$) ($t = 0.41$, $df = 14$, $P = 0.6566$) prior to the establishment of density treatments in the summer of 2008. At that time root biomass of *V. rossicum* (22.4 ± 5.4 g, $n = 7$) was larger than *V. nigrum* (13.3 ± 5.7 g, $n = 8$) ($t = 3.19$, $df = 14$, $P = 0.0071$). At this dissection 80% of plants were either in flower or producing seedpods. No *V. rossicum* were flowering but these plants had significantly more seedpods compared to *V. nigrum* ($t = 2.29$, $df = 14$, $P = 0.0390$) demonstrating that *V. rossicum* completed reproduction sooner than *V. nigrum*.

On 17 July 2008, plants were infested with *A. asclepiadis* (L2 stage) by transferring larvae directly onto the leaves, and *E. asclepiadeus* (L1 stage) by transferring larvae to the shoot base using a fine-tip brush. Mesh bags (2 mm mesh) were used to confine *A. asclepiadis* to those plants receiving larvae but all plants were individually covered regardless of treatment. Bags were secured tightly to plant pots with elastic bands and supported by wires ($\sim$80 cm) inserted into the growing medium. Bagged plants were randomly arranged within the raised bed (2×6 m) 15 cm apart. Mesh bags were removed from all plants after all *A. asclepiadis* larvae completed development (28 August).

2.4. Pathogen and herbivore effects on reproduction prior to plant harvest in Spring 2009

On 11 June 2009 – the spring following larval transfer, plants displayed minor symptoms of pathogen infection (shoot tip damage, lesions, and lower leaf drop) that were not previously encountered. At this time the presence of reproductive parts and apical shoot damage were recorded from all plants to determine whether reproduction was delayed by herbivory and disease. Differences in the proportion of plants flowering and fruiting due to shoot tip damage and herbivore treatment were analyzed separately for each herbivore using multinomial logistic regression. Plant species, herbivore density (*A. asclepiadis* or *E. asclepiadeus*), and shoot tip damage (yes or no) were included as factors in the analyses.

2.5. Root herbivory (E. asclepiadeus) treatments (Summer 2009)

All plants were dissected approximately 1 year following herbivore infestation (13 July 2009) to record all plant growth measurements and determine the number of larvae within the soil of plants infested with *E. asclepiadeus*. In the soil of many of these pots roots were severed from the plant by *E. asclepiadeus* feeding (in one case the severed root mass was over 80% of the total root biomass). Only roots that were connected to the plant were included in the final root biomass measurement because severed roots did not produce new plants in another study (unpublished data). The number of *E. asclepiadeus* larvae that were recovered was compared among densities and plant species using a generalized linear model (Poisson error structure) to assess the actual densities achieved.

2.6. Herbivore effects on plant performance (Summer 2009)

The shoot height, number and length of axillary branches, and the number of flowers and seedpods were recorded from each shoot of every plant at plant dissection (13 July 2009). Only seed-

pods and flower buds wider than 1 mm were counted. Additionally, the number of diseased and healthy leaves (including those on axillary shoots) was recorded to calculate the proportion of diseased leaves per plant. Apical meristem damage to shoots was also noted. Leaf and shoot samples displaying the common disease symptoms were taken from *V. rossicum* and *V. nigrum* plants to isolate pathogens for identification. These samples were dried, weighed, and then shipped to CABI Europe, UK (Surrey, UK). Aboveground biomass was separated from the roots following measurements and all biomass was cleaned and dried to constant weight at 80 °C. The dry biomass of aboveground parts (leaves, shoots, and seedpods) and roots were weighed and seeds were counted from each pod.

Herbivore effects on plant growth variables measured at harvest (biomass, difference in main shoot number between years, shoot height, and axillary growth) were analyzed separately for each herbivore using ANCOVA. In the analyses plant species, herbivore density (*A. asclepiadis* or *E. asclepiadeus*), and their interaction were set as the main effects and pathogen severity (proportion of diseased leaves) as the covariate. The number of shoots was included as another covariate in the analyses comparing mean shoot height. Herbivore effects on the proportion of plants flowering and producing seedpods were analyzed using multinomial logistic regression with plant species, herbivore density (*A. asclepiadis* or *E. asclepiadeus*), and proportion of diseased leaves as factors. Because all plant variables at harvest may have been affected by the interaction between pathogen severity and treatments these interactions were initially included into all models but they were removed from the final models when not significant ($P > 0.05$).

2.7. Statistical analyses

Two plants infested with *E. asclepiadeus* were excluded from all analyses because larvae and root damage were not observed on these plants at dissection. Data were transformed as necessary to meet assumptions of statistical methods. Tukey's HSD test was used to compare means whenever significant main effects were observed in all analyses. All analyses were performed using JMP Version 8.0.2 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Leaf herbivory (A. asclepiadis) treatments (Summer 2008)

Three of 63 (5%) *A. asclepiadis* pupae were recovered from *V. rossicum* (all from plants that were infested with a single larva) after mesh bags were removed on 28 August 2008. Thirty-one pupae were recovered from *V. nigrum* (19%) across all densities. Many dead larvae in the L4 and L5 stages were noted. Most plants were completely defoliated at all infestation densities and damage was similar between plant species.

3.2. Pathogen and herbivore effects on reproduction prior to plant harvest (Spring 2009)

Shoot tip damage was observed on about 20% of all plants approximately 1 month prior to plant harvest (11 June 2009). Shoot tip damage delayed reproduction in both experiments but the effects were not independent of herbivore density and plant species. In the leaf herbivory trial, the proportion of plants flowering ($\chi^2 = 11.03$, $df = 1$, $P = 0.0009$, multinomial logistic regression) and bearing seedpods ($\chi^2 = 5.67$, $df = 1$, $P = 0.0083$, multinomial logistic regression) varied between plant species at each level of shoot tip damage. Only 15% and 18% of damaged *V. nigrum* and *V. rossicum* plants were reproducing, respectively. In contrast, all

Table 1
Effects of leaf (*A. asclepiadis*) and root herbivory (*E. asclepiadeus*) on growth of *Vincetoxicum* spp. Degrees of freedom and *F*-ratios from ANCOVA. Values with * indicate significance at $P < 0.05$ and ** at $P < 0.001$. Model effects with blank *F*-ratios were not included in the model for that particular growth variable.

Experiment	Source of variation	df	Aboveground biomass (g)	Root biomass (g)	Mean shoot height (cm)	Max. shoot height (cm)	Increase in shoot prod. (2008–09)	No. axillary shoots	Axillary growth (cm)
Leaf herbivory									
Abrostola asclepiadis	Plant species (Pl)	1	0.32	4.43*	2.49	1.08	0.01	15.82**	24.88**
	Herbivore density (D)	3	1.03	1.85	2.09	0.43	2.14	0.20	0.06
	Pl × D	3	0.45	1.56	1.10	1.70	0.57	1.03	1.13
	Pathogen	1	1.94	2.25	4.70*	2.22	0.10	0.93	0.93
	No. shoots	1			8.06**				
Root herbivory									
Eumolpus asclepiadeus	Plant species (Pl)	1	4.63*	16.92**	2.99	5.68*	0.03	6.26*	11.48*
	Herbivore density (D)	3	6.85**	16.73**	2.02	3.36*	1.27	0.21	1.3
	Pl × D	3	1.12	0.91	0.55	1.63	0.11	1.72	2.37
	Pathogen (Path)	1	0.59	0.07	0.01	0.05	0.03	1.38	0.57
	No. shoots	1			3.25**				
	D × Path	3			19.72**				
	Pl × Path	1						4.42*	

healthy plants were reproducing. Additionally, the proportion of plants flowering ($\chi^2 = 10.56$, $df = 3$, $P = 0.0144$, multinomial logistic regression) and ripening pods ($\chi^2 = 8.32$, $df = 3$, $P = 0.0397$, multinomial logistic regression) varied between plant species and *A. asclepiadis* density. In general, the proportion of plants reproducing decreased with increasing leaf feeder density on *V. rossicum* but was similar among densities on *V. nigrum*.

Shoot tip damage affected plant reproduction similarly in the root herbivore experiment. A greater proportion of healthy *V. nigrum* plants was flowering (81%) and ripening pods (81%) compared to all *V. rossicum* (flowering: 57%, seedpods: 46%) and damaged *V. nigrum* plants (flowering: 19%, seedpods: 19%) (flowering: $\chi^2 = 7.07$, $df = 3$, $P = 0.00078$, seedpods: $\chi^2 = 4.28$, $df = 3$, $P = 0.0386$, multinomial logistic regression). Flowering of *V. rossicum* decreased with root feeder density, but was similar among densities on *V. nigrum* (flowering: $\chi^2 = 8.10$; $df = 3$, $P = 0.0439$, multinomial logistic regression). However, a similar decrease in seedpod production with *E. asclepiadeus* density occurred on both plant species (seedpods: $\chi^2 = 12.23$; $df = 3$, $P = 0.0066$, multinomial logistic regression). The proportion of plants with seedpods for zero, five, 20, and 60 larval densities was 47%, 60%, 27%, and 13%, respectively.

3.3. Root herbivory (E. asclepiadeus) treatments (Summer 2009)

The number of *E. asclepiadeus* larvae recovered at harvest on 13 July 2009 confirmed that the treatments resulted in increasing larval densities ($\chi^2 = 25.56$; $df = 39$; $P < 0.0001$, GLM) that were similar between plant species ($\chi^2 = 3.21$; $df = 39$; $P = 0.2004$, GLM). However, there was about 50% mortality across treatments. On average, 3.1 ± 0.4, 7.1 ± 2.6, and 27.2 ± 9.3 larvae (mean ± 2SE, $n = 14$ or 15) were recovered from the plants originally infested with five, 20, and 60 larvae, respectively. Similar numbers of larvae were recovered between the five and 20 larval density treatments, but significantly more larvae were recovered from plants infested with 60 larvae.

3.4. Herbivore effects on plant performance (Summer 2009)

3.4.1. Incidence of fungal pathogens
A single fungal pathogen was isolated from leaf samples of each *Vincetoxicum* sp. Leaves of *V. nigrum* were infected with *Cercospora* sp. and *V. rossicum* with *Ascochyta* sp. (Ascomycota). A greater proportion of *V. nigrum* leaves per plant (86%) were diseased than *V. rossicum* (60%) ($\chi^2 = 33.2$; $df = 1$; $P < 0.0001$, Mann–Whitney *U*-test). The effects of pathogen infection on plant growth are discussed in relation to herbivore impact below.

3.4.2. Biomass
Total aboveground biomass was similar between plant species in each experiment and was not affected by the density of the leaf feeder *A. asclepiadis* (Table 1 and Fig. 1a). In contrast, attack by 60 *E. asclepiadeus* root-feeding larvae per plant reduced aboveground biomass compared to controls (Table 1 and Fig. 1b). Root biomass of *V. rossicum* was greater than that of *V. nigrum* (Table 1). Leaf herbivory had no effect on root biomass (Fig. 1c), but root biomass decreased with *E. asclepiadeus* larval density on both plant species (Fig. 1d). Sixty larvae per plant caused significant reductions in root biomass relative to control plants (Fig. 1d). Pathogen severity did not affect plant biomass (Table 1).

3.4.3. Shoot height
In general, *V. rossicum* plants produced taller shoots than *V. nigrum* (Fig. 2a and b), but the difference was only significant in the root herbivory experiment (Table 1). Mean shoot height was similar between plant species (Table 1 and Fig. 2c and d). Leaf herbivory by *A. asclepiadis* had no effect on maximum or mean shoot height (Table 1 and Fig. 2a and c). Root herbivory did not affect mean shoot height (Fig. 2d), but 60 larvae per plant reduced maximum shoot height compared to plants infested with zero and five larvae (Fig. 2b).

Pathogen severity did not significantly affect maximum shoot height in either analysis of herbivore impact, but negatively affected mean shoot height in both experiments (Table 1, Fig. 3, and Fig. 4a). Mean shoot height decreased with pathogen severity on both plant species in the leaf herbivory experiment (Fig. 3). The effect of increasing pathogen severity on mean shoot height differed among root feeder densities (Fig. 4a). For example, mean shoot height and the proportion of diseased leaves were inversely correlated at zero, five, and 60 larvae per plant; however, this relationship was positive in plants infested with 20 larvae (Fig. 4a).

3.4.4. Main and axillary shoot production
Main shoot production of control *V. nigrum* plants increased by 2.1 ± 1.9 shoots ($n = 8$, mean ± 2SE) and *V. rossicum* by 1.7 ± 2.0 ($n = 7$) from 2008 to 2009. Herbivory and pathogen severity did not affect main shoot production between years (Table 1), but, on average, shoot production of plants infested with 60 root-feeding larvae remained unchanged between years (0.2 ± 0.8 shoots, $n = 15$).

Axillary growth was found on 63% of plants, but the number of axillary shoots per plant was not affected by leaf or root herbivory (Table 1). In the leaf herbivory experiment, the number of axillary shoots was greater on *V. nigrum* (2.2 ± 0.4, $n = 30$) (mean ± 2SE) than *V. rossicum* (0.4 ± 0.1, $n = 28$) with no apparent effect due to
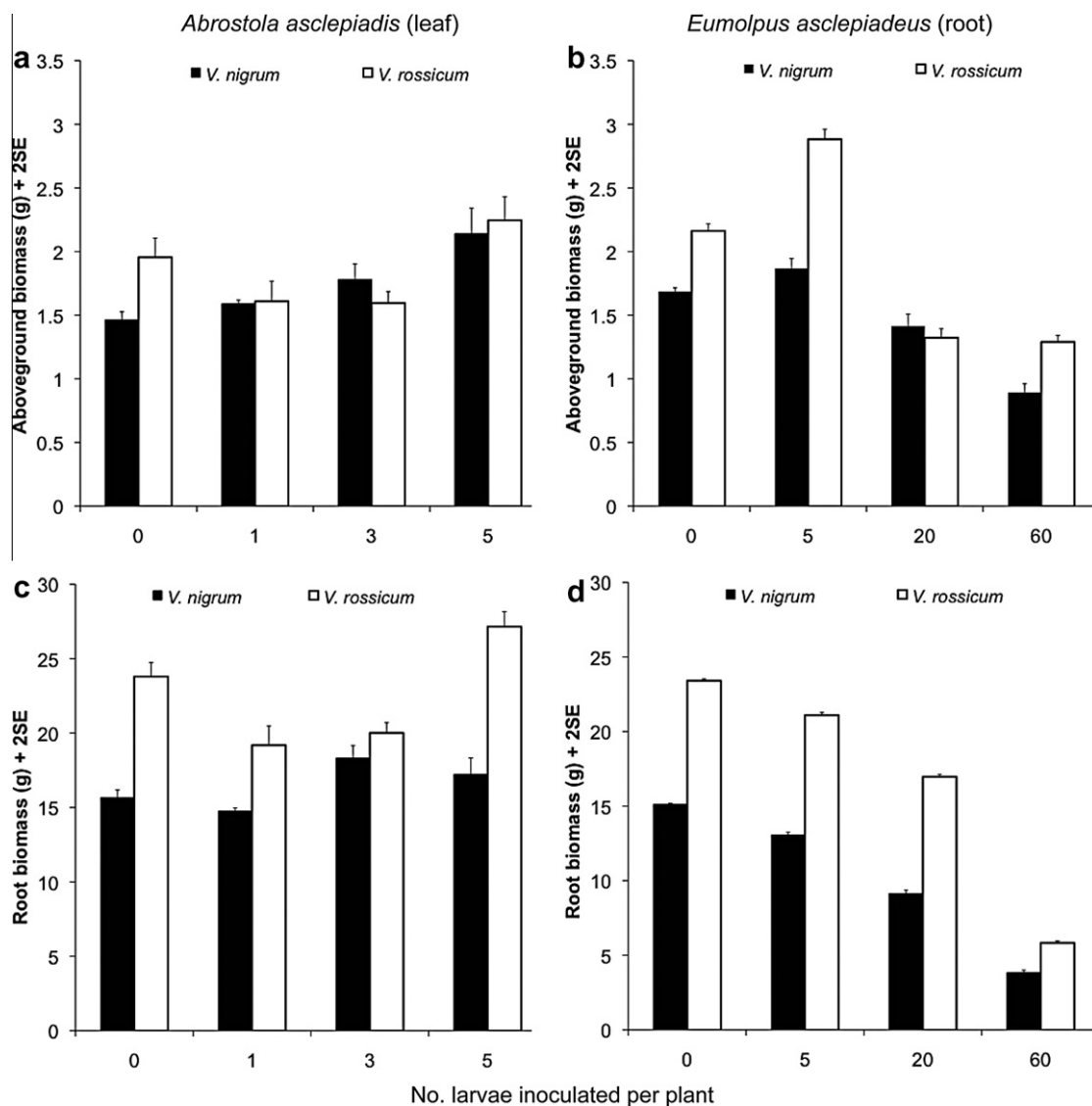
Fig. 1. The effect of *A. asclepiadis* and *E. asclepiadeus* density on aboveground (a and b) and root (c and d) biomass of *V. nigrum* and *V. rossicum*. Values are adjusted means from the ANCOVA.

pathogen severity (Table 1). A similar trend occurred between plant species in the root herbivory experiment, however, pathogen severity was positively correlated with axillary shoot production of *V. nigrum*, but not *V. rossicum* (Fig. 4b). *Vincetoxicum nigrum* also produced a greater amount of axillary growth compared to *V. rossicum* in both experiments, which was unrelated to pathogen damage (Table 1). Moreover, neither type of herbivory affected the amount of axillary growth (Table 1) but the plant by root herbivore density interaction approached significance (removed from final model). Axillary shoot growth of *V. nigrum* plants infested with 60 larvae was on average 1.5 ± 0.3 cm ($n = 8$) compared to 16.0 ± 0.5 cm ($n = 8$) of control plants. This trend was not observed on *V. rossicum*, which produced very little axillary shoot growth (0.9 ± 0.2 cm, $n = 28$) across all density treatments.

3.4.5. Reproduction

The proportion of *V. nigrum* plants flowering at harvest (leaf herbivory: 58%, root herbivory: 43%) was greater than *V. rossicum* (leaf herbivory: 17%, root herbivory: 4%) (Table 2). Leaf and root herbivory did not affect flowering in the overall analysis (Table 2). However, the difference in flowering between plant species most likely prevented detection of an effect due to root herbivory

(Table 2). For example, 88% of *V. nigrum* receiving zero larvae were in flower compared 14% and 0% receiving 20 and 60 larvae, respectively. No control *V. rossicum* plants were flowering compared to 14% and 0% receiving 20 and 60 larvae, respectively. Pathogen severity did not affect flowering in either experiment (Table 2).

The proportion of plants with seedpods was similar between species (leaf herbivory: 29%, root herbivory: 23%) and among leaf feeder densities (30%) (Table 2). Pathogen infection decreased seedpod production of *V. nigrum* in the leaf herbivory experiment but the relationship was reversed on *V. rossicum*. Seedpod production varied with root feeder density (Table 2), but the proportion of plants with seedpods was not lower than controls at any density of *E. asclepiadeus*.

4. Discussion

Root herbivory by *E. asclepiadeus* was more detrimental to *Vincetoxicum* spp. performance than leaf herbivory by *A. asclepiadis*. Root herbivory negatively affected entire plant biomass of both *Vincetoxicum* species as shown for other root feeders attacking herbaceous plants (Sheppard et al., 2001; Briese et al., 2002; Gerber
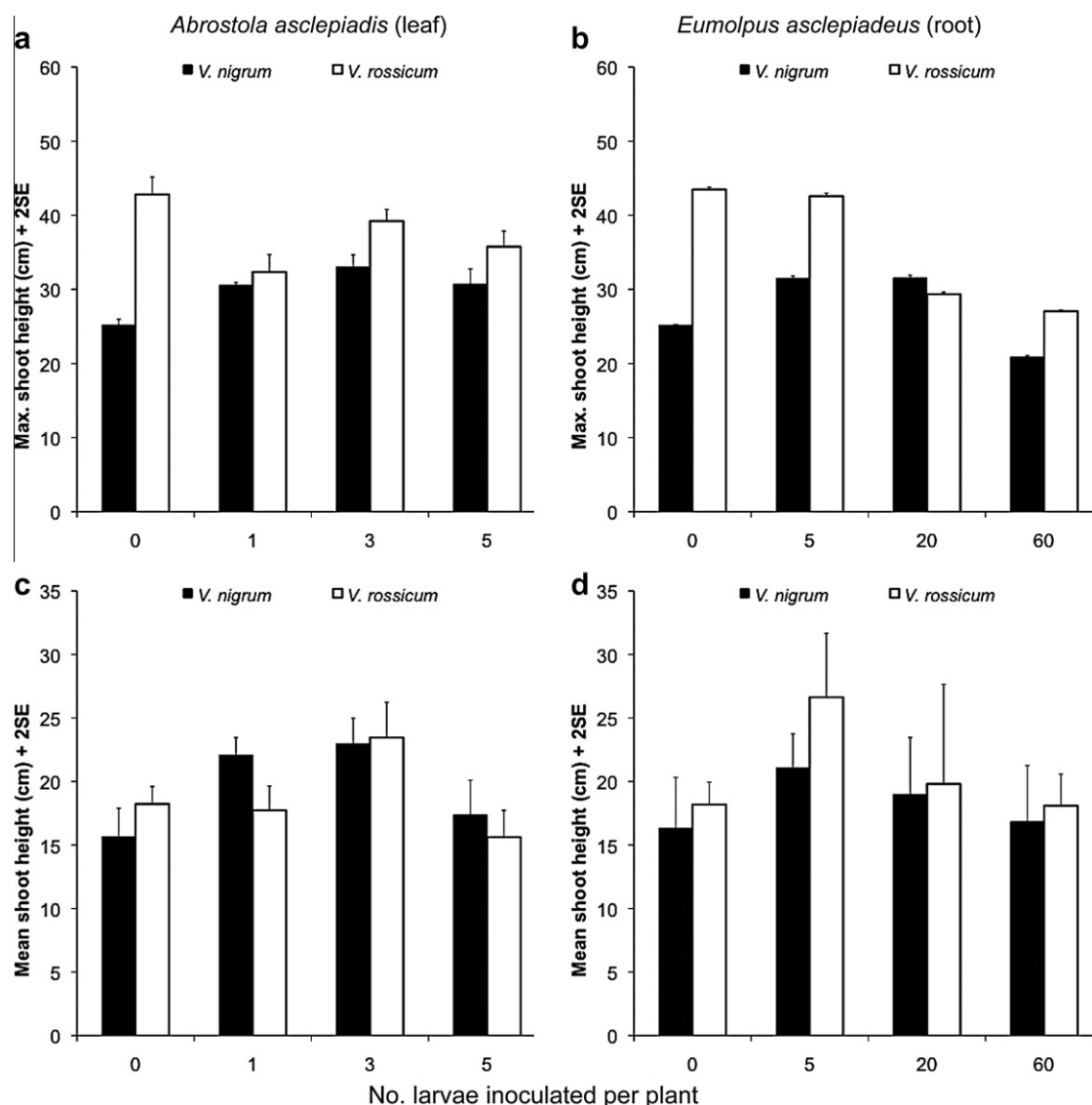
Fig. 2. The effect of *A. asclepiadis* and *E. asclepiadeus* density on maximum (a and b) and mean (c and d) shoot height of *V. nigrum* and *V. rossicum*. Values are adjusted means from the ANCOVA.

et al., 2007), but it did not kill plants. This is in contrast to two root-crown weevils which caused over 40% mortality to the biennial *Alliaria petiolata* (M. Bieb.) Cavara (Gerber et al., 2007) and the annual *Echium plantagineum* L. (Sheppard et al., 2001). The *Vincetoxicum* spp. plants used in this experiment were about 4 years old and because they are long-lived perennials, they may be more resilient to high levels of root attack compared to annuals or biennials. At the end of the experiment six plants infested with 60 *E. asclepiadeus* showed noticeable signs of shoot dieback and only had a small portion of the root crown remaining. These plants may have eventually died if the experimental period was longer.

Although *A. asclepiadis* completely defoliated plants at all densities, this did not affect plant growth, which may have been due to the timing of attack (Maschinski and Whitham, 1989) or because *Vincetoxicum* spp. compensate for biomass loss between years (Maron and Crone, 2006). Caterpillars of *A. asclepiadis* are commonly collected from local *V. hirundinaria* populations throughout July (Weed and Gassmann, 2007) so the timing of our treatments coincided with the typical period of larval activity. However, predicting the long-term effects of aboveground herbivory is difficult due to variable environmental conditions affecting the intensity of herbivory and plant response to attack (Maschinski and Whi-

tham, 1989; Julien and Chan, 1992). It is possible that *A. asclepiadis* had no impact on plant biomass because plants were grown under optimal conditions with no plant competition.

Above- and belowground herbivores can alter plant architecture of their hosts by reducing shoot height or increasing the production of secondary shoots (Müller-Schärer, 1991; Hunt-Joshi et al., 2004; Gerber et al., 2007), which affects their reproductive effort (Hunt-Joshi et al., 2004) and competitive ability (Müller-Schärer, 1991; Rees and Brown, 1992; Nötzold et al., 1997). Herbivore effects on plant architecture were difficult to interpret due to pathogen infection. However, after accounting for pathogen infection, maximum shoot height of plants subjected to the high root feeder density were shorter compared to controls. This indicates that *E. asclepiadeus* feeding has the capacity to reduce shoot height, and possibly affect plant vigor (Nötzold et al., 1997). Conversely, leaf herbivory had no effect on plant height when examined against the background of pathogen infection. Although pathogen infection negatively affected shoot height it is also possible that the effects of defoliation on plant architecture may take multiple years to have measurable effects (Hunt-Joshi et al., 2004).

Many herbivores cause increases in main shoot and axillary shoot production (Meyer, 1993; Häfliger et al., 2006; Gerber
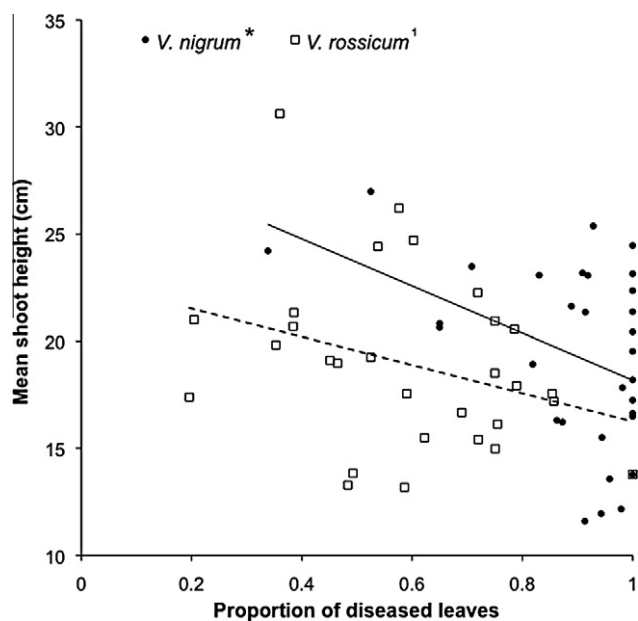
Fig. 3. Relationship between the proportion of diseased leaves and mean shoot height of *V. nigrum* (solid line) and *V. rossicum* (dashed line) in the leaf herbivory experiment. Superscripts ([1]) and * indicate that the relationship is significant at $\alpha < 0.10$ and $\alpha < 0.05$ level, respectively.

Table 2
Effects of leaf (*A. asclepiadis*) and root herbivory (*E. asclepiadeus*) on reproduction of *Vincetoxicum* spp. Degrees of freedom and χ^2 values from multinomial logistic regression. Values with * indicate significance at $P < 0.05$ and ** at $P < 0.001$. Model effects with blank χ^2 values were not included in the model for that particular reproductive variable.

Experiment	Source of Variation	df	Flowering (%)	Seedpods (%)
Leaf herbivory				
Abrostola asclepiadis	Plant species (Pl)	1	15.33**	1.53
	Herbivore density (D)	3	3.10	2.28
	Pl × D	3	6.24	6.41
	Pathogen (Path)	1	0.88	0.09
	D × Path	3		8.32*
	Pl × Path	1		14.95**
Root herbivory				
Eumolpus asclepiadeus	Plant species (Pl)	1	13.45**	2.92
	Herbivore density (D)	3	5.82	9.50*
	Pl × D	3	6.13	5.17
	Pathogen (Path)	1	0.18	1.02

et al., 2007), but neither herbivore did so in this study. The production of axillary shoots was stimulated by apical meristem damage caused by pathogen infection, which occurred on at least half of the stems on each plant. Although herbivory apparently did not affect axillary shoot growth, non-infested *V. nigrum* plants produced almost 10 times the length of axillary growth as plants infested with 60 *E. asclepiadeus* larvae. It appears that pathogen infection stimulated axillary shoot growth but root herbivory reduced the ability of *V. nigrum* plants to compensate for this damage. It was surprising that such high levels of root tissue loss due to *E. asclepiadeus* feeding did not stimulate increased production of main shoots, which is a common response to root attack (Gerber et al., 2007).

Pathogen infection in this experiment also complicated the evaluation of herbivore impact on *Vincetoxicum* spp. reproduction. Apical shoot tip damage delayed flowering and seedpod production by either directly killing expanding flower buds or causing plants to allocate resources to secondary shoot elongation rather than reproduction. This delay was most dramatic for *V. nigrum* where very few control plants had seedpods at harvest. Despite pathogen infection
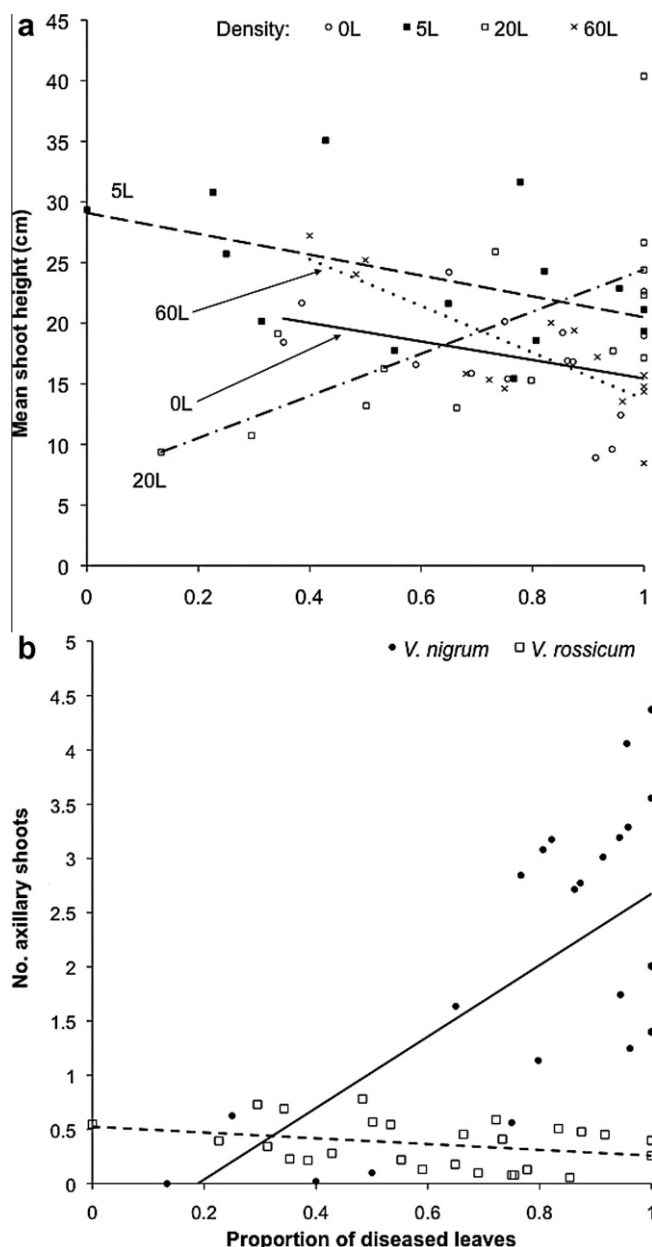
Fig. 4. Relationship between the proportion of diseased leaves and (a) mean shoot height at each density of the root feeder *E. asclepiadeus* and (b) number of axillary shoots of *V. nigrum* (solid line) and *V. rossicum* (dashed line). Superscripts ([1]) and * indicate that the relationship is significant at $\alpha < 0.10$ and $\alpha < 0.05$ level, respectively.

complicating the analysis of herbivore impact on reproduction, seedpod production of both plant species generally decreased with increasing root feeder but not leaf feeder density.

The widespread presence of two plant pathogens in this experiment was not the result of caging the plants in 2008 because many nearby plants used for insect rearing were also infected. During the 4-year growing period prior to larval infestation plants were only rarely attacked by pathogens and never to the extent observed during 2009. It is possible that minor pathogen symptoms have gone unnoticed in the past and favorable climatic conditions in the spring of 2009 facilitated pathogen infection. Although these pathogens significantly affected plant architecture and reproduction, we believe that herbivore effects on plant performance were not entirely obscured by fungal pathogen attack because pathogen severity minimally affected plant biomass.

An important question is how these experimental results relate to the ability of these herbivores to suppress *V. nigrum* and *V. rossicum* under field conditions. Neither herbivore evaluated in this study attacks the target weeds in the native range (Weed and Gassmann, 2007) so field impact cannot be directly measured. In Scandinavia, complete defoliation of *V. hirundinaria* by *A. asclepiadis* is infrequent (Förare, 1995) and this insect has minimal impact on population growth of *V. hirundinaria* (Leimu and Lehtilä, 2006). However, high natural enemy pressure (Förare, 1995) and common larval mortality due to drought (Förare and Engqvist, 1996) may minimize its impact in Scandinavia. Recent studies indicate that repeated removal of shoot biomass is needed to lessen the impact of *Vincetoxicum* spp. growing under full sun conditions in North America (McKague and Cappuccino, 2005; Milbrath, 2008). However, *A. asclepiadis* is expected to produce one generation per year where current *Vincetoxicum* spp. infestations are located in North America. Furthermore, the period of *A. asclepiadis* larval activity in Europe begins when plants are large and reproducing (Förare, 1995; A. Weed, pers. obs.). Thus, based on the phenology of attack and minimal impact observed in this study, these traits may lead one to believe that *A. asclepiadis* is of little value as a biological control agent. However, because *Vincetoxicum* spp. are long-lived plants a year-long experiment was probably too short to accurately quantify any long-term impact of *A. asclepiadis*. This insect may be highly damaging on populations growing under low light conditions where compensation to biomass loss is negligible (Milbrath, 2008). Even in full sunlight, herbivory by this moth could lessen the competitive ability of *Vincetoxicum* spp. in a diverse community setting.

Little is known about the population dynamics of *E. asclepiadeus*. At any given time overlapping generations of larvae are feeding belowground. It appears that high levels of root herbivory (>30 larvae per plant) are required to reduce the performance of mature plants and the effects of root herbivory are generally similar between both plant species. However, under similar environmental conditions in North America *E. asclepiadeus* may have a greater impact on *V. nigrum*, which allocates a smaller proportion of its biomass belowground compared to *V. rossicum* (Milbrath, 2008; this study). Further research is needed to evaluate what biotic and abiotic factors affect female dispersal and oviposition and the larval developmental period that can take from 1 to 3 years (Weed, 2010). This information will provide a better indication of expected larval density and enhance our ability to predict population-level impact by this insect (Briese et al., 2002).

Root herbivory by *E. asclepiadeus* is expected to have a greater impact on plant fitness than leaf herbivory under full sun conditions because root herbivory should limit water and nutrient absorption ultimately decreasing overall plant vigor (Blossey and Hunt-Joshi, 2003). Mowing aboveground plant tissue (McKague and Cappuccino, 2005) and artificial defoliation (Milbrath, 2008) only delay reproduction of *Vincetoxicum* spp. plants growing under high light intensity conditions. However, we cannot exclude the possibility that annual defoliation will have cumulative negative effects on *Vincetoxicum* spp. reproduction especially in mixed plant communities. Further research should evaluate the combined effect of each type of herbivory on *V. nigrum* and *V. rossicum* growth, preferably across multiple years. Additionally, factorial studies manipulating levels of *A. asclepiadis* larval damage, resource availability, and plant competition are needed to fully evaluate the potential impact of this moth.

Acknowledgments

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