Performance of potential European biological control agents of *Vincetoxicum* spp. with notes on their distribution

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Abstract

Exploratory surveys were conducted in Europe to locate potential biological control agents of *Vincetoxicum nigrum* and *Vincetoxicum rossicum* and to collect known specialist herbivores of *Vincetoxicum hirundinaria* (*Abrostola asclepiadis*, *Chrysolina a. asclepiadis*, *Eumolpus asclepiadeus* and *Euphranta connexa*). Development of the most abundant herbivores found attacking leaves, roots, and developing seeds was evaluated in the laboratory on three *Vincetoxicum* spp. Field surveys revealed that the highest diversity of herbivores was associated with the abundant and widespread *V. hirundinaria*. No new herbivores were reported from *V. hirundinaria* or *V. nigrum,* but the leaf-feeding noctuid *Hypena opulenta* was recorded for the first time attacking *V. rossicum* and *V. scandens* in Ukraine. Based upon larval survival and development and adult fecundity, the leaf feeders *A. asclepiadis, C. a. asclepiadis*, and *H. opulenta,* perform better on *V. nigrum* and *V. rossicum* than on *V. hirundinaria.* Larval performance of the root feeder *E. asclepiadeus* followed a similar pattern, but adult fecundity of this insect did not vary among host plant species. Immature development time of the pre-dispersal seed feeder *E. connexa* is similar among hosts, but larvae grow larger on *V. nigrum* and *V. hirundinaria* than on *V. rossicum*. All herbivores are promising biological control agents of *Vincetoxicum* in North America and we have prioritized host range testing with *H. opulenta* and *E. asclepiadeus*.

Introduction

Swallow-worts (*Vincetoxicum nigrum* (L.) and *V. rossicum* (Kleopow) Barbar.) are milkweeds of European origin that are invasive in central and northeastern North America (DiTommaso et al. 2005). Black swallow-wort, *V. nigrum* (synonym: *Cynanchum louiseae* L.), is native to the Mediterranean region with populations in Northern Italy, France, Portugal, Spain, and the Balearic Islands, while pale swallow-wort, *V. rossicum* (synonym: *C. rossicum* [Klepow] Borhidi), is native to Ukraine and southwestern European Russia (Pobedimova 1952; Markgraf 1972). The current distribution of *V. nigrum* in the United States extends from the northeastern states to the mid-Atlantic, west to Nebraska and at one location in California (USDA-NRCS 2010). *Vincetoxicum rossicum* ranges throughout most of the northeast (except Maine and Vermont) south to New Jersey, west into Pennsylvania, and in a few states of the mid-west (USDA NRCS 2010). In Canada, both species are scattered throughout eastern and southern areas of Ontario and southern Quebec (DiTommaso et al. 2005). Both species are considered invasive in Canada and the United States and considered noxious in Vermont and Ontario (DiTommaso et al. 2005; USDA NRCS 2010).

Most *Vincetoxicum* prefer calcareous soils, but *V. nigrum* and *V. rossicum* grow in a variety of substrata in North America. For instance, both species have been

observed growing along fencerows, in moist woodlands, grasslands, roadsides, pastures, streambanks, cliffs, and encroaching on agricultural row crops (DiTommaso et al. 2005). In Ukraine small, scattered populations of *V. rossicum* (pale swallow-wort or dog-strangling vine) grow within forested areas, typically near rivers (Pobedimova 1952). However, in North America this species invades open sites fully exposed to sun as well as forest understories (DiTommaso et al. 2005). *Vincetoxicum nigrum* (black swallow-wort) develops into dense populations in open habitats and along forest margins in North America, but in France small populations persist on open to forested slopes in dry, stony areas (Fournier 1977; DiTommaso et al. 2005; Douglass et al. 2009).

Vincetoxicum spp. (Family Apocynaceae) are long-lived, herbaceous perennial plants that produce multiple shoots from overwintering root buds each spring. Flowering begins in late spring and continues until late August. Flowers are insect- or self-pollinated and produce one to two elongate seedpods after fertilization. Each seedpod contains about 20 seeds with fibrous tufts that aid in wind dispersal. The polyembryonic seeds either germinate during late summer to fall or the following spring (DiTommaso et al. 2005). Swallow-worts are strong competitors for resources and threaten sensitive alvar communities comprised of endemic flora (DiTommaso et al. 2005). Infestations of *V. rossicum* also have pronounced negative impacts on native arthropod diversity that may decrease foraging habitat of native birds and small mammals (Ernst and Cappuccino 2005). Many of the negative environmental effects are associated with dense, monocultures, which at certain sites reach densities of 1000 stems/m^2 (Sheeley 1992). Swallow-worts are highly resistant to cultural and herbicide methods of control (Lawlor and Raynal 2002; Averill et al. 2008) and continual expansion of populations in North America has created interest in the development of a biological control program because current methods are costly and ineffective (Tewksbury et al. 2002).

The lack of herbivore pressure on *Vincetoxicum* in North America suggests that classical biological control might prove to be a suitable strategy for managing swallow-wort infestations (Sheeley 1992; Christensen 1998; Lawlor 2000; Tewksbury et al. 2004; Ernst and Cappuccino 2005; Milbrath 2010). To our knowledge, no reliable reports are available of herbivores attacking *V. nigrum* and *V. rossicum* in Europe (table 1). This is reflected in the investigation by Tewksbury et al. (2002), who only list herbivores reported from *V. hirundinaria* Medik. as potential

swallow-wort biological control agents (table 1). Included in this list are the leaf-feeding beetles, *Eumolpus* (*Chrysochus*) *asclepiadeus* Pallas and *Chrysolina* (*Chrysomela*) *aurichalcea asclepiadis* (Villa) (syn: *C. aurichalcea bohemica*) (Mohr 1966; Vig 1997; Dobler and Farrell 1999; Bieńkowski 2001), the noctuid moth *Abrostola asclepiadis* (Denis and Schiffermüller) (Förare 1995), the gall midges *Contarinia vincetoxici* Kieffer and *Co. asclepiadis* (Giraud) (Widenfalk et al. 2002), and the seed-feeding *Euphranta connexa* (Fabricius) (Solbreck and Sillén-Tullberg 1986a,b). The weevil *Otiorhynchus pinastri* Herbst (Dieckmann 1980; Germann 2004) and the seed-feeding bug *Lygaeus equestris* (L.) (Kugelberg 1977; Tullberg et al. 2000) are also listed by Tewksbury et al. (2002), but both species are generalists. The authors did not mention the lygaeid *Tropidothorax leucopterus* Goeze, which feeds on the leaves and seeds of *V. hirundinaria* and is considered monophagous (Tullberg et al. 2000).

The development of a biological control program against *Vincetoxicum* species requires careful evaluation of herbivore performance and host acceptance. First, an assessment of herbivore performance on both target weeds is necessary because herbivore development and reproduction may differ on each host (Mattson 1980; Scriber and Slansky 1981; Awmack and Leather 2002; Agosta 2008). These results will help in prioritizing agents by determining the relative suitability of the target weeds to herbivore development. Second, understanding factors affecting herbivore distribution in the native range could be helpful for predicting their distribution in the introduced range. This is especially so when considering the release of agents against multiple targets that persist within a variety of habitat types. Native range surveys are proven means to assess habitat associations (Gassmann and Schroeder 1995; Nowierski et al. 2002), competitive interactions (Blossey 1995), and host range (Gassmann et al. 2008) of potential biological control agents.

In this study, field surveys were conducted in the native European range of *V. nigrum* and *V. rossicum* to identify specialist herbivores that may be suitable biological control agents. Populations of *V. hirundinaria* were also surveyed to collect associated specialist herbivores (table 1) as biological control agents for *V. nigrum* and *V. rossicum*. Following surveys, the development and reproduction of the most abundant herbivores collected from *V. nigrum* and *V. rossicum* and the herbivores of *V. hirundinaria* were tested on three *Vincetoxicum* sp. Results of this study are discussed in relation to the ability of these herbivores

Table 1 Herbivores reported attacking *Vincetoxicum* in Eurasia

Plant species	Plant Distribution	Order	Family	Species	Plant part attacked	Reference
Vincetoxicum hirundinaria Medik.	Eurasia	Coleoptera	Chrysomelidae	*Eumolpus* (*Chrysochus*) *asclepiadeus* Pallas	Leaves/roots	Mohr 1966; Vig 1997; Dobler and Farrell 1999
				Chrysolina (*Chrysomela*) *a. asclepiadis* (Villa)	Leaves	Mohr 1966; Bieńkowski 2001
			Curculionidae	*Otiorhynchus pinastri* Herbst[a]	Leaves	Dieckmann 1980; Germann 2004
		Diptera	Cecidomyiidae	*Contarinia asclepiadis* (Giraud)	Seedpods	Widenfalk et al. 2002
				Contarinia vincetoxici Kieffer	Seedpods	Widenfalk et al. 2002
			Tephritidae	*Euphranta connexa* (Fabricius)	Seedpods	Solbreck and Sillén-Tullberg 1986a,b; Solbreck 2000
		Heteroptera	Lygaeidae	*Lygaeus equestris* (L.)[a]	Seedpods	Kugelberg 1977; Tullberg et al. 2000
				Tropidothorax leucopterus Goeze	Leaves/ seedpods	Tullberg et al. 2000
		Lepidoptera	Geometridae	*Scopula* spp.[a]	Leaves	Hausmann 2004
			Noctuidae	*Abrostola asclepiadis* (Denis & Schiff.)	Leaves	Förare 1995
			Tortricidae	*Sparganothis pilleriana* (Denis & Schiff.)[a]	Leaves	Gustafsson 1983
Vincetoxicum nigrum (L.)	Western Europe		Gelechiidae	*Nothris congressariella* Bruand.[a]	Leaves	DiTommaso et al. 2005
Vincetoxicum rossicum (Kleopow) Barbar.	Eastern Europe, Asian Russia			None previously reported		
Vincetoxicum tmoleum Boiss.	Asia (Israel)	Lepidoptera	Noctuidae	*Hypena munitalis* Mann	Leaves	Kravchenko et al. 2006

[a]Suspected to be polyphagous.

to perform as biological control agents against invasive swallow-wort populations in North America.

Methods

Field surveys

Surveys were conducted in Western, Central, and Eastern Europe during the early summer of 2006 to locate populations of *V. nigrum* and *V. rossicum* – the two weeds targeted for biological control - and *V. hirundinaria*. Survey sites were selected with the assistance of local scientists. Additional sites were selected based on examination of insect specimens held at research collections. Thus, site selection was not random. The plant species present and habitat type were recorded from individual sites located at

least 1.5 km apart. We broadly characterized habitat type based on the amount of sun exposure as open, forest edge, and forest interior. Populations located at the forest edge were located under the forest canopy but within 5 m of open habitat. Some sites of *V. hirundinaria* were scored into more than one category because plants extended from the forest edge into an open habitat. Plant abundance at each site was either counted or estimated when the population was greater than 100 individuals.

The number of sites visited within each country varied because collecting trips generally lasted only a few days although the survey in Ukraine lasted two weeks. *Vincetoxicum hirundinaria* was the most commonly surveyed species with eight populations located in Switzerland, four in Germany, and six in Ukraine. Three populations of *V. nigrum* were

surveyed in France and three of *V. rossicum* in Ukraine. *Vincetoxicum scandens, V. flavum* Ostapko, *V. donetizcum* Ostapko, and *V. ucrainicum* Ostapko were also surveyed at two sites in Ukraine. These additional species are grouped under the category 'Other'.

At each site we also recorded herbivore presence by visually examining or collecting stems, leaves, flowers, and seedpods from individual plants. Time constraints prevented us from conducting standardized quantitative sampling of insect populations. Populations of *V. nigrum* and *V. rossicum* were small enough (<100 plants) that all individuals could be thoroughly checked at each site. However, at some *V. hirundinaria* sites there were too many plants to effectively survey so 100 plants spaced at least 0.5 m apart were haphazardly selected for examination. Additional species of *Vincetoxicum* were observed at three sites in Ukraine and included in the survey. Commonly encountered herbivores were collected and brought to the laboratory at CABI Europe-Switzerland in Delémont, Switzerland (CABI) for identification or to establish cultures for conducting the herbivore performance tests described below. The majority of surveys were conducted from 7 June to 6 July 2006. An additional survey of *V. hirundinaria* was conducted in Switzerland on 6 and 7 September 2006 during the period of adult activity of *Chrysolina a. asclepiadis*.

Insect and plant rearing

We established cultures of the leaf feeders *A. asclepiadis*, *Hypena opulenta* (Christoph), and *C. a. asclepiadis*, the root feeder *E. asclepiadeus*, and the pre-dispersal seed feeder *E. connexa*. Field-collected herbivores completed development at CABI on vegetation that was harvested from potted *Vincetoxicum* plants grown outdoors since 2001. Once colonies were established, the leaf feeding herbivores were shipped to the quarantine facility at the University of Rhode Island, Kingston, Rhode Island, USA (URI) and *E. asclepiadeus* and *E. connexa* remained at CABI for study.

The *V. nigrum* used in experiments originated from seeds collected in Rhode Island and *V. rossicum* originated from New York and Connecticut, USA. *Vincetoxicum hirundinaria* was grown from seeds originating from Göttingen and Leipzig, Germany. Seeds were sown in potting mix in greenhouses at CABI during the winter of 2004/2005 and at URI in 2006. Seedlings at CABI were transplanted into 2 l pots into a peat-based growing mixture (1 : 4 : 30, vermiculite:sand:soil). Potted plants were moved outdoors, spaced 10 cm apart in a raised bed, and surrounded with coarse sawdust that was filled level with the pot edge. The raised beds were fully exposed to sun and irrigated regularly to ensure healthy plant growth. During the winter plants were protected with a layer of pine boughs. Seedlings at URI were transplanted into 2 or 3 l pots with Metromix 510 (Sun Gro Horticulture, Bellevue, WA) and all plants were transferred to raised beds where they were surrounded with coarse sawdust, maintained outdoors year around, and watered as needed. Excised leaves were used for the feeding experiments with leaf feeders (URI) and whole plants were used for studies with the root and seed feeder (CABI).

Larval performance

Leaf-feeding herbivores

Larval performance of the leaf-feeding moth *A. asclepiadis* was monitored with the F_1 generation of populations collected on *V. hirundinaria* in Kiev (N50°23.792′, E30°33.389′) and Donetsk, Ukraine (N48°08.955′, E37°50.594′). Near Donetsk over 10 species of *Vincetoxicum* are potential hosts for *A. asclepiadis* whereas in Kiev only *V. hirundinaria* is locally available (Weed and Gassmann 2007). Thus, both *A. asclepiadis* populations were tested to assess whether host plant availability may have affected local adaptation of this herbivore to host plants. Larval performance of the leaf-feeding moth *H. opulenta* was assessed using the F_1 generation of a population collected in southeastern Ukraine (N47°34.4970, E37°46.1680) attacking *V. rossicum* and *V. scandens* (Sommier et Levier) Pobed. Adults of both species emerge in late spring, females lay eggs typically on the undersides of leaves, and larvae take 4–6 weeks to develop (Förare 1995; Weed and Casagrande 2010). *Hypena opulenta* has facultative diapause and completes at least two generations per season (Weed and Casagrande 2010). Southern European populations of *A. asclepiadis* apparently are bivoltine, but univoltine in Central and Northern Europe (Förare 1995). Both species overwinter as pupae in the leaf litter.

Larvae of both moths used in the experiments originated from bulk egg collections obtained by placing multiple mating pairs into cages (40 × 40 × 40 cm) containing a potted plant of *Vincetoxicum* and a source of diluted honey. Cages were checked daily for eggs which were transferred to 50 mm Petri dishes lined with moistened filter paper. Larval development of *H. opulenta* and *A. asclepiadis* was monitored on *V. hirundinaria*, *V. nigrum*,

and *V. rossicum*. Neonate larvae were transferred individually from egg dishes directly onto excised *Vincetoxicum* leaves originating from the third to sixth plant nodes and held in 473 mL plastic jars lined with moistened filter paper and closed with a clear, ventilated lid. The development of 10–15 individual larvae of both insect species and each population of *A. asclepiadis* was monitored on each host plant. Jars were checked daily for cleaning and to replenish leaves. Both species were grown under 16 : 8 h (L : D) photoperiod. *Hypena opulenta* was raised under constant 20°C and 80% RH and *A. asclepiadis* was reared at ambient room conditions that averaged 23.0 ± 1.5°C (±SD) and 51.7% ± 9.0% RH. At the final instar, sterilized vermiculite or clean paper towel was added to the jars to serve as a pupation site. We recorded the number of days to reach the prepupal stage, pupal weight, and survival of each individual larva. Only pupae of *A. asclepiadis* were sexed.

Host plant effects on the larval performance of the leaf beetle *C. a. asclepiadis* collected on *V. hirundinaria* in southern Switzerland (N46°26.016′, E08°51.537′) were studied using larvae from the F$_2$ generation of our research colony. *Chrysolina a. asclepiadis* larvae hatch from overwintering eggs and feed on newly expanding shoots of *V. hirundinaria* in early spring. Pupation occurs in the soil and within a few weeks adults emerge for a brief period to feed. Adults are inactive in the soil during the summer and begin mating and oviposition with the onset of autumn (A.W. Aaron Weed, unpublished data).

In rearing for experiments, eggs were collected from 10 to 20 mating pairs of *C. a. asclepiadis* that were kept in clear, 1.3 l plastic cylinders. In each cylinder adults were provided cut shoots of *Vincetoxicum* fixed into moist florists' foam and 2-cm wide paper strips as an oviposition site. Eggs were transferred from cylinders with a fine-tip brush into 10 cm Petri dishes lined with moistened filter paper. Egg dishes were kept in incubators at 4°C for at least 12 weeks to break diapause. They were inspected weekly during this period to adjust moisture in dishes. After about 12 weeks, dishes were held at 10°C until eggs began hatching. Individual neonates were transferred onto shoots held within plastic jars (473 ml) fitted with water pics to monitor larval development. Fifteen jars were set up on each host plant species. Jars were checked daily for cleaning, to remove condensation, and to add new plant shoots. Larvae were raised at ambient room conditions that averaged 22.5 ± 0.66°C and 29.2% ± 8.7% RH under a 16 : 8 h (L : D) photope-

riod. We assessed larval performance using similar parameters as with *A. asclepiadis* and *H. opulenta* but also weighed and sexed teneral adults.

Root-feeding beetle

Host plant effects on larval performance of the root-feeding beetle *E. asclepiadeus* were studied using a population from Kiev, Ukraine (N50°23.572′, E30°33.315) collected on *V. hirundinaria*. Adults of *E. asclepiadeus* are active from June until September. Females oviposit at the shoot base and larvae feed externally on the roots. Most larvae require 2 years to complete development but some (<5%) take 1 or 3 years (A.W. Aaron Weed, unpublished data). *Eumolpus asclepiadeus* overwinter as larvae and pupation occurs during early spring.

Host plant effects on larval survival and development of *E. asclepiadeus* were studied on potted plants of *V. nigrum, V. rossicum,* and *V. hirundinaria*. We simultaneously examined the effects of larval infestation density on larval performance. Larvae used in this experiment originated from bulk egg collections obtained from 25 field-collected females. Eggs were acquired from adults as described for *C. a. asclepiadis* but kept at room temperature until hatch (10–12 days). In July of 2006, 5–10 plants of each *Vincetoxicum* sp. were infested with 10 larvae. In addition, 20 larvae were transferred to another set of 15–20 plants of each *Vincetoxicum* sp. After larval transfer, plants were kept under the same outdoor conditions as described above at CABI and randomly arranged within beds. Treatment effects on larval survival and development were assessed as follows. At 85 days, following larval transfer in 2006 the number of surviving larvae was counted from 5 to 10 randomly selected plants of each species and infestation density. At 335 days following larval transfer (11 June 2007) two plants of each species initially infested with 20 larvae were dissected to record the number, weight, and head capsule width of larvae. Survival was also evaluated by monitoring adult emergence in 2007 and 2008 from all remaining pots. The gender and weight of newly emerged adults was recorded in 2008. In sum, this design allowed us to evaluate short- and long-term treatment effects on *E. asclepiadis* survival and development.

Seed-feeding fly

Finally, host plant effects on adult acceptance, egg and larval developmental time, and pupal weight of the seed-feeding tephritid *E. connexa* were measured among *Vincetoxicum* spp. *Euphranta connexa* is widely

distributed throughout Europe and usually present wherever its host plant *V. hirundinaria* grows (Solbreck 2000). Adult emergence is tightly synchronized with seedpod production of its host. After copulating, females bore a hole into the wall of the seedpod and commonly deposit one egg (Solbreck 2000). Females apparently leave a marking pheromone, but multiple larvae commonly develop within one pod (Solbreck 2000; A. Weed, pers. obs.). The larvae consume the developing seeds, exit the seedpod at maturity, and burrow into the soil to pupate.

Adults of *E. connexa* used in the experiment were obtained from collections of mature larvae in pods of *V. hirundinaria* in Moutier, Switzerland (N47°17.073', E07°23.160') in 2007. Pupae were held in 250 ml cups containing vermiculite and cups were overwintered in an outdoor shelter at CABI. The adults were sexed at the time of emergence in spring of 2008 and individual mating pairs were held in plastic vials in an incubator at 10°C until the trials. Prior to trials, all potted plants were cleared of predators and individually covered with a mesh bag supported by a wire frame. Individual plants of *V. hirundinaria* (10 replicates), *V. nigrum* (23 replicates) and *V. rossicum* (20 replicates) were exposed to one mating pair of *E. connexa* for 24 h in a greenhouse. Seventeen days after releasing the adults, entire plant shoots with pods still attached were cut and fixed into moist florists' foam kept in clear, 1.3 l plastic cylinders to capture larvae that were exiting for pupation. Adult acceptance of each plant was measured either by locating oviposition scars (Solbreck 2000) from a subsample of pods taken immediately when adults were removed, by dissecting another subsample of pods 7 days after adults were removed, or by counting larval exit holes from the remaining pods. Each cylinder was checked daily to determine developmental times and pupal weight was measured within 3 days of pupation.

Performance of adult herbivores

Leaf-feeding moths
Host plant effects on the potential fecundity of *A. asclepiadis* were assessed by comparing female pupal mass among host plants and between insect populations based upon the assumption that pupal mass correlates with fecundity (Awmack and Leather 2002). Newly emerged females of *H. opulenta* originating from larvae reared as in the performance experiment were kept in 50 ml plastic vials at 12°C for 6–9 days. After this period, the abdomens of females reared on different hosts (10 on *V. hirundi-*

naria, 15 on *V. nigrum*, and 14 on *V. rossicum*) were removed with a scalpel and transferred into 80% ethanol. The entire reproductive system was carefully removed using forceps and the immature and mature eggs were counted under a dissecting microscope.

Leaf beetles
The longevity and fecundity of newly emerged individual *C. a. asclepiadis* and *E. asclepiadeus* females was monitored on each *Vincetoxicum* sp. Reproduction was monitored using the same approach described in the general rearing section above. The longevity, number of days to first oviposition, daily egg production (*E. asclepiadeus* only), and total egg production was monitored for five to six females held individually on each *Vincetoxicum* sp. Eggs were collected every 4 days for a period of 44 days after first oviposition for *C. a. asclepiadis* and every 4 days over the life span of *E. asclepiadeus*.

Statistical analysis

Larval survival of insect herbivores was compared among plant species by logistic regression and performance measures (developmental time, insect weight, and egg count) were compared among plant species using ANOVA. Plant species acceptance by *E. connexa* was compared among hosts using logistic regression. Differences in survival of the root feeder *E. asclepiadeus* were compared among plant species and larval densities using two-way ANOVA. We compared *E. asclepiadeus* larval weights among host plants with ANCOVA, using head capsule width as the covariate. Factors affecting adult emergence of *E. asclepiadeus* were analyzed as a repeated measures experiment using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Plant species, larval density, year, and interactions were set as the main effects, adult counts per plant as the repeated variable, and an unstructured covariance pattern was requested. A two-way ANOVA was used to compare adult weight of *E. asclepiadeus* by host plant species and gender. Lastly, measures of adult *C. a. asclepiadis* and *E. asclepiadeus* performance (longevity, days to oviposition, and egg production) were compared among plant species using one-way ANOVA. In all analyses, data were transformed to meet assumptions of statistical methods as needed. Tukey's HSD was used for comparisons among means. All analyses except the repeated measures test were performed using JMP Version 7.0.1 (SAS Institute Inc., Cary, NC, 1989–2007).

Table 2 Herbivores observed attacking *Vincetoxicum* during field surveys

Order	Family	Species	Insect Stage[1]	Plant part attacked	V. hirundinaria	V. nigrum	V. rossicum	Other
Heteroptera	Aphididae	Undetermined	N	Leaves	+	−	−	−
	Cicadellidae	Undetermined	N & A	Leaves/Stems	+	−	+	+
	Membracidae	Undetermined	N & A	Leaves/Stems	+	−	−	−
	Pseudococcidae	Undetermined	N	Flowers	−	−	+	−
	Lygaeidae	*Lygaeus equestris* L.	N & A	Seedpods	+	−	−	−
	Lygaeidae	*Tropidothorax leucopterus* Goeze	N & A	Seedpods	+	−	−	−
	Pentatomidae	*Graphosoma italicum* (Müller)	N & A	Flowers/Seedpods	+	−	−	−
Coleoptera	Chrysomelidae	*Chrysolina a. asclepiadis* (Gebler)	A	Leaves	+	−	−	−
	Chrysomelidae	*Eumolpus asclepiadeus* Pallas	A	Leaves/Roots	+	−	−	−
	Curculionidae	Undetermined	A	Leaves	+	−	−	−
Diptera	Tephritidae	*Euphranta connexa* (Fab.)	L	Seeds	+	−	−	−
Lepidoptera	Noctuidae	*Abrostola asclepiadis* (Denis & Schiffermüller)	L	Leaves	+	−	−	−
		Abrostola sp.	L	Leaves	−	−	−	+
		Hypena opulenta (Christoph)	L	Leaves	−	−	+	+
	Arctiidae	Undetermined	L	Leaves	+	−	−	−
	Unidentified	Undetermined	L	Leaves	+	−	−	−
				Total	14	0	3	3

[1]Insect stages: N: nymph, L: larva, and A: adult.

Results

Distribution of herbivores in Europe

Fifteen herbivores were found attacking *Vincetoxicum*. Most feeding was on leaves, but stem, flower, and seedpod herbivory was also observed (table 2). Herbivore richness was highest on *V. hirundinaria* and no herbivores were found attacking *V. nigrum* (table 2). Three newly documented herbivores were observed attacking *V. rossicum* including the leaf-feeding moth *H. opulenta*, an unidentified mealybug, and a leafhopper (table 2). *Hypena opulenta* and *Abrostola* sp. were observed attacking *V. scandens* (table 2). We were unable to confirm whether the species of *Abrostola* was *A. asclepiadis* or *A. clarrisa* (Staudinger) because specimens were damaged.

The most notable herbivores collected from *V. hirundinaria* were *A. asclepiadis*, *C. a. asclepiadis*, *E. asclepiadeus*, and *E. connexa*. We were unable to locate the two *Contarinia* gall midges, which were most likely overlooked or missed due to timing. More recent observations in 2008 confirmed that the fruit gall midge *Co. asclepiadis* is at one of our sites in Switzerland. In southern Switzerland we also observed the lygaeid *T. leucopterus*, which attacks vegetative and reproductive parts of *V. hirundinaria*.

However, not enough specimens were collected during surveys to establish a research colony.

The highest herbivore richness on *V. hirundinaria* occurred in Switzerland, although richness in Ukraine was roughly comparable-lacking only *C. a. asclepiadis* (table 2). Specialized herbivores were present at all sites of *V. hirundinaria* surveyed in Switzerland and Ukraine while only one specialized herbivore, the leaf-feeding moth *A. asclepiadis*, was located at one site (of four) in Germany. Multiple herbivores were present at 67% of the *V. hirundinaria* sites and *E. connexa* was the most frequently encountered herbivore (78% of *V. hirundinaria* sites). *Abrostola asclepiadis* and *E. asclepiadeus* occurred at fewer than 40% of *V. hirundinaria* sites but were found in every country where *V. hirundinaria* was found. *Chrysolina a. asclepiadis* was only found in southern Switzerland (28% of *V. hirundinaria* sites) and *E. connexa* was not observed in Germany.

Vincetoxicum nigrum and *V. rossicum* were less abundant and habitat-restricted whereas *V. hirundinaria* was found in all habitat types and populations were much larger (figs 1a and 2a). The leaf beetles *E. asclepiadeus* and *C. a. asclepiadis* were only found in open sites and *H. opulenta* was located exclusively in forested sites (fig. 1b). *Euphranta connexa* and *A. asclepiadis* were found on *V. hirundinaria* in all habitat
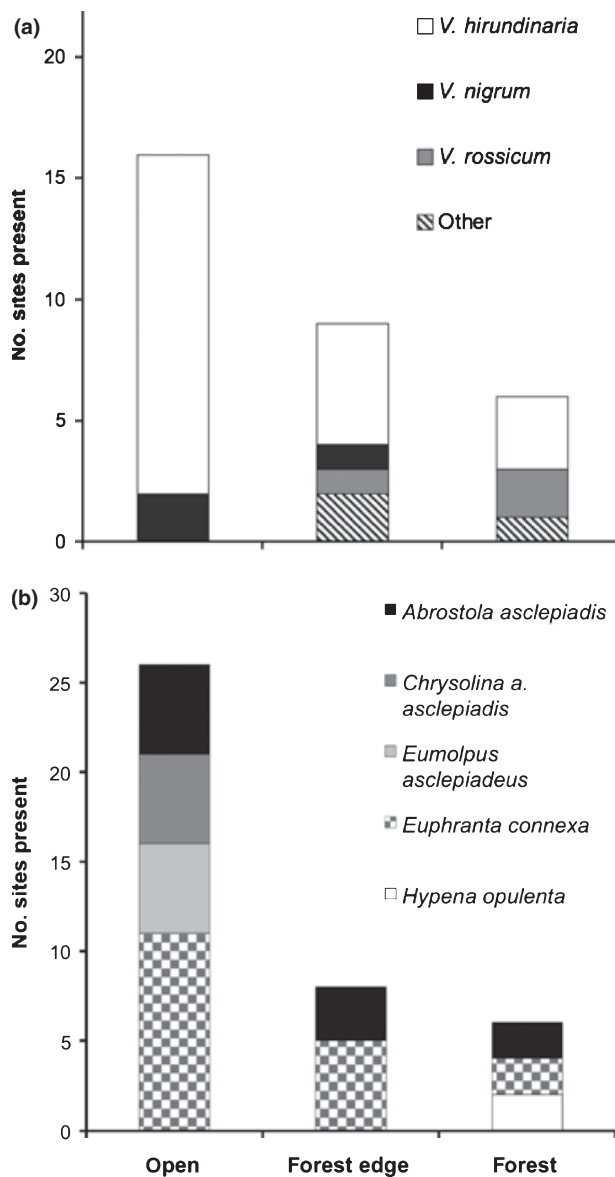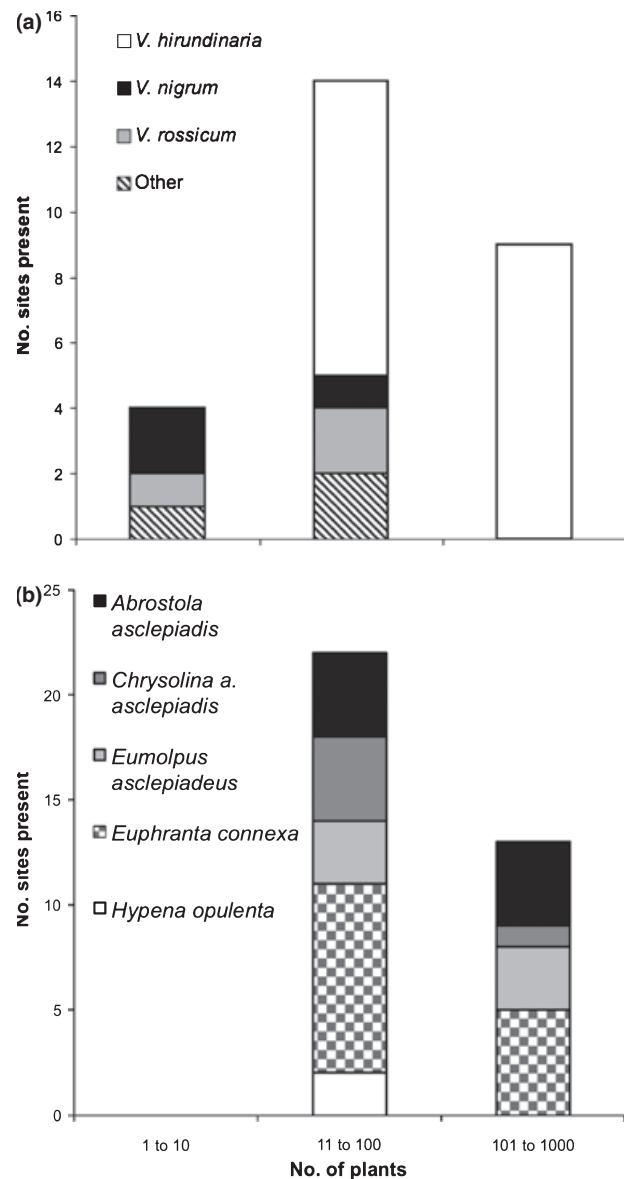
Fig. 1 Frequency of (a) *Vincetoxicum* spp. and (b) common herbivores in each habitat type.

Fig. 2 Frequency of sites with each (a) *Vincetoxicum* sp. and (b) herbivore within plant abundance classes.

types (fig. 1b). No specialized herbivores were recorded at very small *Vincetoxicum* populations (fig. 2b).

Larval performance

Leaf-feeding herbivores

Survival of the leaf feeders *A. asclepiadis* (χ^2 = 0.65; d.f. = 2; P = 0.7243), *H. opulenta* (χ^2 = 1.25; d.f. = 2; P = 0.5355), and *C. a. asclepiadis* (χ^2 = 1.24; d.f. = 2; P = 0.5392) was similar among hosts (fig. 3a). Larvae of *A. asclepiadis* completed development roughly

2 days faster on *V. nigrum* and *V. rossicum* compared to *V. hirundinaria* ($F_{2,38}$ = 6.14; P = 0.0049) (fig. 3b). Survival of *A. asclepiadis* larvae was similar between the Kiev and Donetsk populations (χ^2 = 0.02; d.f. = 1; P = 0.8752) and developmental time did not vary between the two populations ($F_{1,38}$ = 1.56; P = 0.2232). Similarly, *H. opulenta* took on average four days longer to complete development on *V. hirundinaria* compared to *V. nigrum* and *V. rossicum* ($F_{2,30}$ = 28.40; P < 0.0001) (fig. 3b). Larvae of *C. a. asclepiadis* completed development 2 and 3 days faster when raised on *V. nigrum*
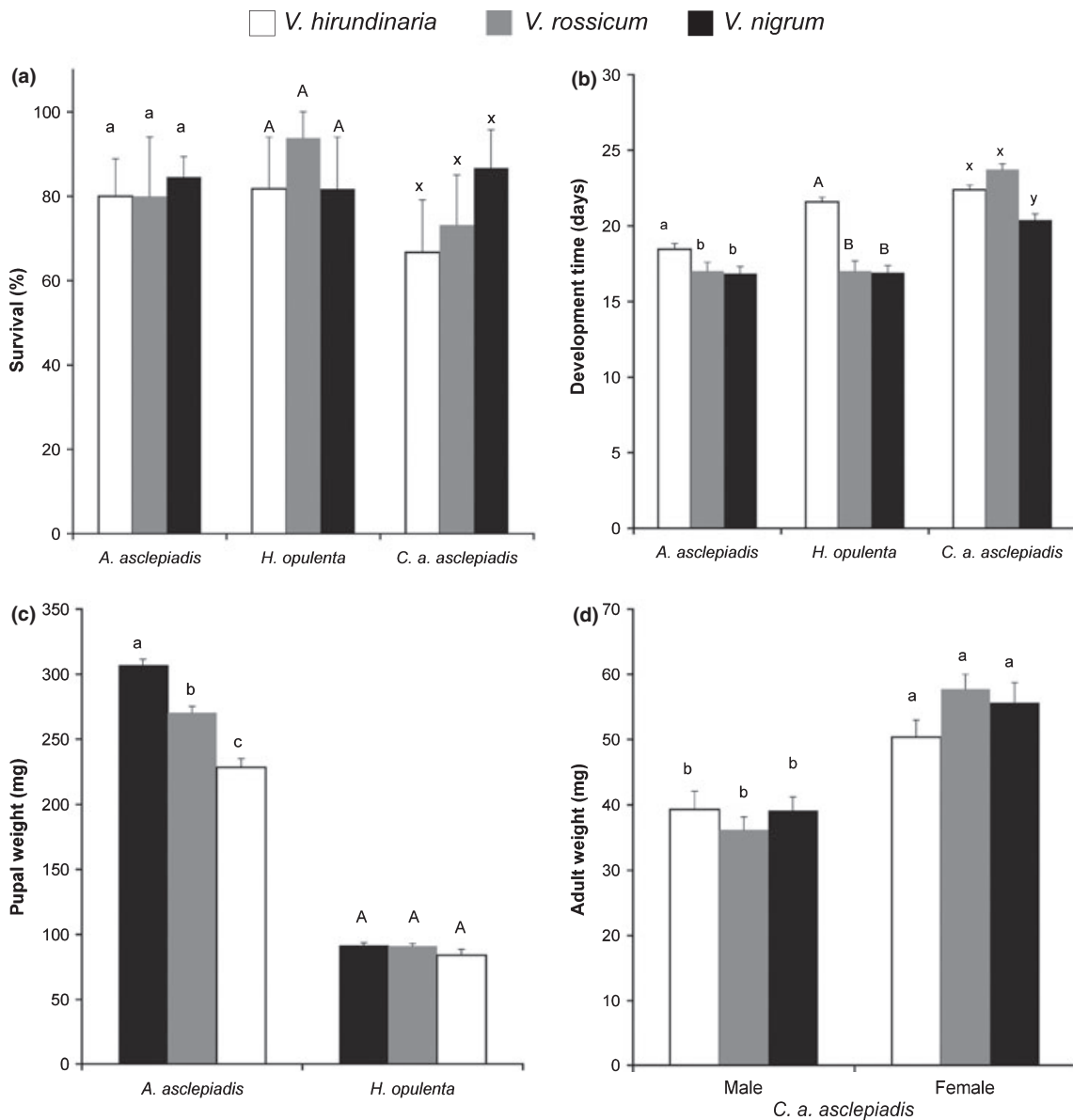
Fig. 3 Host plant effects on (a) survival and (b) developmental time of the leaf feeders *A. asclepiadis*, *H. opulenta*, and *C. a. asclepiadis*, (c) pupal weight of *A. asclepiadis* and *H. opulenta*, and (d) teneral adult weight of *C. a. asclepiadis*. Letters over bars (mean ± SE) denote the results of one-way ANOVA and Tukey's HSD test for host plant effects on individual herbivore parameters.

compared to *V. rossicum* and *V. hirundinaria*, respectively ($F_{2, 32} = 10.22$; $P = 0.0004$) (fig. 3b). Finally, larvae of *A. asclepiadis* attained heavier pupal weights on *V. nigrum* than *V. hirundinaria* and attained intermediate weights on *V. rossicum* ($F_{2,38} = 56.5$; $P < 0.0001$) (fig. 3c). Pupal weight of *H. opulenta* ($F_{2,30} = 2.18$; $P = 0.1308$) (fig. 5c) and adult weight of *C. a. asclepiadis* ($F_{2, 22} = 0.23$; $P = 0.7954$) (fig. 3d) did not vary among host plants. Female *C. a. asclepiadis* were heavier than males ($F_{1, 22} = 18.93$; $P = 0.0003$) (fig. 3d).

Root-feeding beetle

After 85 d of development 80% of *E. asclepiadeus* root-feeding larvae were recovered from *V. nigrum*, 72% from *V. rossicum*, and 70% from *V. hirundinaria* ($F_{2, 15} = 0.29$; $P = 0.7564$). Infestation density had no effect on larval survival at this date ($F_{1,15} = 0.12$; $P = 0.7305$). At 335 days of development [June 2007] larvae raised on *V. nigrum* roots were heavier (123.4 ± 1.5 mg, n = 46) (mean ± SE) than those raised on *V. rossicum* (83.7 ± 4.3 mg, n = 43) and *V. hirundinaria* (58.2 ± 9.4 mg, n = 39) ($F_{2, 50} = 18.18$;
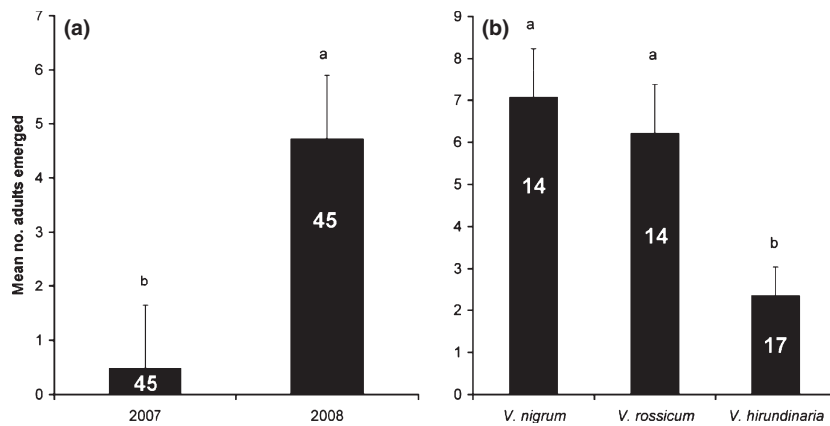
Fig. 4 Mean number (+SE) of *E. asclepiadeus* adults emerging (a) per year (b) and among *Vincetoxicum* spp. over both years and infestation densities. Letters above bars signify the results of Tukey's HSD test. Numbers inside bars denote the number of plants monitored for adult emergence.
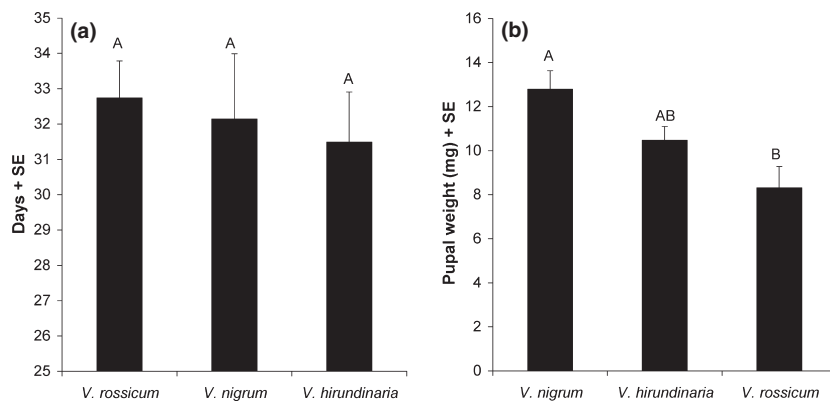
Fig. 5 Host plant effects on the (a) total developmental time (b) and pupal weight of *E. connexa* among *Vincetoxicum* spp. Letters denote the results of one-way ANOVA and Tukey's HSD test.

$P < 0.0001$). Teneral adult weight did not differ between sexes ($F_{1, 177} = 3.08$; $P = 0.0821$) but adults emerging from *V. nigrum* were significantly heavier (86.5 ± 1.5 mg, $n = 49$) (mean $\pm$ SE) than those raised on *V. rossicum* (73.3 ± 1.9 mg, $n = 68$) and *V. hirundinaria* (75.8 ± 1.5 mg, $n = 67$) ($F_{2, 177} = 14.51$; $P < 0.0001$). The number of *E. asclepiadeus* adults emerging from plants was similar between infestation densities ($F_{1, 42} = 0.27$; $P = 0.6031$) and the plant $\times$ year interaction was marginally significant ($F_{2, 42} = 3.18$; $P = 0.0516$). In 2007, one year after larval transfer, less than one *E. asclepiadeus* adult emerged per plant, whereas, 2 years after transfer, in 2008, nearly five adults emerged per plant ($F_{2, 42} = 42.81$; $P < 0.0001$) (fig. 4a). Nearly three and four times more adults emerged over both years from *V. rossicum* and *V. nigrum* than *V. hirundinaria*, respectively ($F_{2, 42} = 6.02$; $P = 0.0051$) (fig. 4b).

Seed-feeding fly
Euphranta connexa successfully oviposited in seedpods of each *Vincetoxicum* sp. and the number of plants accepted did not differ among plant species ($60.1\% \pm 8.7\%$ [mean $\pm$ SD], $n = 53$) ($\chi^2 = 0.77$; d.f. $= 2$; $P = 0.6800$). Developmental time from oviposition to pupation was similar among hosts ($F_{2,47} = 0.0980$; $P = 0.9068$) (fig. 5a), but larvae attained heavier pupal weights on *V. nigrum* compared to *V. rossicum* ($F_{2,47} = 7.48$; $P = 0.0015$) (fig. 5b).

Performance of adult herbivores

As already reported, larvae of *A. asclepiadis* attained heavier pupal weights on *V. nigrum* than *V. rossicum* than *V. hirundinaria* (fig. 3c). Thus, it was assumed that adult fecundity would follow a similar pattern among hosts (Awmack and Leather 2002). Ovaries of *H. opulenta* females raised on *V. nigrum* contained significantly more eggs (400.3 ± 33.5, $n = 15$) (mean $\pm$ SE) than females raised on *V. hirundinaria* (270.0 ± 39.6, $n = 7$) ($F_{2, 36} = 4.28$; $P = 0.0215$); *V. rossicum* produced intermediate results (310.6 ± 35.5 eggs, $n = 9$). Reproduction by leaf beetles was generally similar among hosts but with a couple of notable exceptions (table 3). First, *C. a. asclepiadis*

Table 3 Host plant effects on adult beetle fecundity of *C. a. asclepiadis* and *E. asclepiadeus*. Letters indicate results of one-way anova and Tukey's HSD tests for each response variable among plant species

Herbivore	Plant			
	V. hirundinaria	*V. nigrum*	*V. rossicum*[1]	*All*[1]
Chrysolina a. asclepiadis				
Preoviposition (d ± SE)	26.8 ± 1.7a	28.1 ± 1.7a	23.8 ± 1.2a	26.3 ± 1.0
Egg production				
Mean ± SE	119.8 ± 19.1b	186.1 ± 20.3a	196.8 ± 22.0a	158.6 ± 16.1
Range	61–171	101–248	166–262	61–262
Eumolpus asclepiadeus				
Longevity (d ± SE)	95.7 ± 5.5a	79.5 ± 8.1a	70.4 ± 4.8a	82.5 ± 4.3
Preoviposition (d ± SE)	6.3 ± 1.6a	5.7 ± 0.8a	5.4 ± 1.7a	5.8 ± 0.3
Eggs per day ± SE	10.2 ± 0.6a	10.9 ± 1.0a	10.6 ± 1.1a	10.6 ± 0.5
Egg production				
Mean ± SE	972.0 ± 73.4a	865.8 ± 107.0a	750.0 ± 96.9a	869.2 ± 54.8
Range	810–1,276	525–1,229	454–990	454–1,276

[1] Excluding data from one female that died during each experiment.

produced fewer eggs when reared on *V. hirundinaria* compared to *V. nigrum* or *V. rossicum* over the 44 days experimental period ($F_{2, 14} = 3.90$; $P = 0.0474$) (table 3). Second, *E. asclepiadeus* survived longer on *V. hirundinaria* compared to the other hosts but the relationship was only marginally significant ($F_{2, 15} = 4.63$; $P = 0.0502$) (table 3).

Discussion

One goal of this study was to identify potential biological control agents for *V. nigrum* and *V. rossicum*. We were unable to find herbivores attacking *V. nigrum* in France. However, we found the leaf-feeder *H. opulenta* attacking *V. rossicum* and *V. scandens* in Ukraine, which, to our knowledge, is the first time this has been reported.

The second goal of this study was to study the performance of *H. opulenta* and known *V. hirundinaria* herbivores among *Vincetoxicum* spp. to determine their suitability as biological control agents against *V. nigrum* and *V. rossicum* in North America. Our data indicate that the leaf feeders *A. asclepiadis*, *C. a. asclepiadis*, and *H. opulenta*, root feeder *E. asclepiadeus*, and pre-dispersal seed feeder *E. connexa* are potential biological control agents of *V. rossicum* and *V. nigrum*. Despite the fact that only *H. opulenta* was collected on *V. rossicum*, insects generally performed at least as well, if not better on the target weeds than on their field host *V. hirundinaria* by measures such as development rate, pupal and adult weights, and fecundity. A good example of this was observed with the root feeder *E. asclepiadeus*. Larvae of this insect not

only grew faster, but we were able to rear nearly three to four times the number of adults in two years from *V. nigrum* and *V. rossicum* compared to *V. hirundinaria*.

Although *V. nigrum* and *V. rossicum* are suitable hosts for all herbivores in the laboratory, it is unknown whether the herbivores will oviposit and demonstrate preferences on these hosts in the field. Recent tests indicate that *H. opulenta* does not show an oviposition preference among the *Vincetoxicum* spp. in the laboratory (Weed 2010) and feeding damage and oviposition by *E. asclepiadeus* is also similar among these hosts in outdoor cages in Switzerland (A.W. Aaron Weed, unpublished data). Tests examining the oviposition behaviour of the pre-dispersal seed feeder *E. connexa* and leaf feeder *A. asclepiadis* are underway. Thus, for the herbivores tested, it appears that both invasive *Vincetoxicum* species are suitable hosts for oviposition, but further experiments are needed to determine whether other factors such as habitat type may affect host colonization in North America.

Despite the limitations of our time-constrained surveys, these results are useful for generating testable hypotheses on factors affecting herbivore distribution in Europe. It is well recognized that biological control agent colonization and impact can vary with habitat type (Larson and Grace 2004; Jonsen et al. 2007; Myers et al. 2009; Sims-Chilton et al. 2009) so an understanding of factors affecting herbivore colonization prior to release may improve the success. This is particularly important since swallow-worts invade multiple habitats in North America (Di-Tommaso et al. 2005).

In our survey herbivore distribution was evaluated in terms of habitat type defined by the extent of sun exposure. The leaf beetles *E. asclepiadeus* and *C. a. asclepiadis* were primarily associated with open, sun-exposed sites while *A. asclepiadis* and *E. connexa* were found in all habitat types. *Hypena opulenta* was the only species that was found exclusively within forested sites. This insect may have an affinity for these habitats because it was never found attacking *V. hirundinaria*, which was locally available (within 100 m) but was located outside the forest. It is still unclear whether sun exposure is the only factor affecting herbivore distribution, but our habitat categories demonstrate that herbivore distribution is not random. Work completed in Scandinavia indicates that *A. asclepiadis* may avoid plants in open habitats that are prone to wilting (Förare and Engqvist 1996). For *E. asclepiadeus,* local soil conditions may also influence its distribution by affecting the development of its root-feeding larvae.

Identifying successful agents prior to release still remains a challenge (Schroeder and Goeden 1986; McClay and Balciunas 2005; van Klinken and Raghu 2006) and *a priori* predictions of agent efficacy have seldom been explicitly tested by quantifying effectiveness in the field after release (Morin et al. 2009). A few authors have focused on evaluating the success of agents released in the past to gauge which taxonomic groups make the best biological control agents (Crawley 1989; Gassmann 1996; Syrett et al. 2000; Blossey and Hunt-Joshi 2003). These reviews showed that beetles, in particular Chrysomelidae and Curculionidae, are the most effective weed biological control agents in the temperate world. Other approaches have focused on scoring agents based on life history traits (Harris 1973; Goeden 1983) and quantifying agent impact in the native range (Sheppard et al. 1995; Gerber et al. 2007) or under varying resource conditions (Häfliger et al. 2006; Stanley et al. 2007). In this study, we utilized information, albeit limited, on herbivore distribution in the native range to assist in making predictions of herbivore colonization in the introduced range. This approach was effective in the leafy spurge (*Euphorbia esula* L.) biological control program (Gassmann and Schroeder 1995; Nowierski et al. 2002; Kalischuk et al. 2004; Larson and Grace 2004). However, further sampling is needed to identify additional explanatory variables of *Vincetoxicum* herbivore presence.

Our first priority for evaluating potential biological control agents for *Vincetoxicum* spp. in North America is given to the root-feeding beetle *E. asclepiadeus* because it attacks above and below ground parts of the target weeds. Based on our survey data we expect this insect to primarily colonize open habitats. Our second priority is given to leaf-feeding moth *H. opulenta* because it produces multiple generations per year, larval feeding suppresses *Vincetoxicum* growth and reproduction (Weed and Casagrande 2010), and it appears to utilize different habitats than *E. asclepiadeus*. We have initiated host range testing to further evaluate the potential of these herbivores for biological control of *Vincetoxicum*.

Acknowledgements

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