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Biology and larval feeding impact of *Hypena opulenta* (Christoph) (Lepidoptera: Noctuidae): A potential biological control agent for *Vincetoxicum nigrum* and *V. rossicum*

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ABSTRACT

A classical biological control program has been initiated against the invasive European swallow-worts *Vincetoxicum nigrum* and *Vincetoxicum rossicum* in North America. The noctuid moth *Hypena opulenta*, discovered feeding on *V. rossicum* in forests of southeastern Ukraine, is currently under evaluation as a potential biological control agent. In this study the life cycle of *H. opulenta* and factors affecting diapause induction were evaluated. Additionally, larval impact of *H. opulenta* on *Vincetoxicum* spp. performance was tested to determine whether future screening is worthwhile. Adults of *H. opulenta* begin oviposition 2 days after emergence and produce approximately 600 eggs. Larvae develop through five larval instars and overwinter as pupae. Pupal diapause is facultative, resulting in at least two generations per year. Diapause induction is affected by photoperiod and seasonal changes in plant quality as evidenced by increasing diapause induction when larvae are grown under a short day photoperiod and on senescing plants in the fall. Feeding by two larvae per plant caused reductions in aboveground biomass to *V. rossicum* resulting in decreased reproductive output (flower, seedpod, and seed production). Only flower production of *V. nigrum* was negatively affected by larval feeding. The results of this study indicate that *H. opulenta* is a promising agent against forested populations of *V. rossicum* and warrants completion of host specificity testing and examination of population dynamics of *H. opulenta*. This practice of conducting impact assessments of herbivores that are apparent specialist feeders prior to conducting the full battery of host specificity tests should improve agent selection by reducing the costs associated with screening ineffective agents.

1. Introduction

Biological control provides an effective method to suppress invasive plants, but low historical success of these programs (McFadyen, 1989) and growing concern for non-target effects (Louda et al., 2003; Pearson and Callaway, 2003, 2005) requires improvement of agent selection (Sheppard, 2003; van Klinken and Raghu, 2006). Weed biological control agents must have the capacity to reduce host populations either by attaining high densities of damaging stages or by feeding on sensitive plant stages that severely impact plant population dynamics (Sheppard, 2003). Because weed biological control can be achieved with a single agent (Myers, 1985; Denoth et al., 2002), it is important to identify and screen only the efficacious species out of a pool of available agents to reduce costs and possible non-target effects (McEvoy and Coombs, 1999; McClay and Balciunas, 2005; van Klinken and Raghu, 2006; Müller-Schärer and Schaffner, 2008).

Current methods used to measure agent efficacy prior to release have developed beyond previous scoring systems (Harris, 1973; Goeden, 1983) to empirical designs commonly used in ecological studies (e.g. Inouye, 1982; Meyer, 1993; Maron, 1998). These methods include herbivore exclusion with insecticides (Waloff and Richards, 1977; Balciunas and Burrows, 1993; Goolsby et al., 2004), studies in the native range (Sheppard et al., 1995; Briese, 2000; Briese et al., 2002; Häfliger et al., 2006), and impact studies (Briese, 1996; Häfliger et al., 2006; Gerber et al., 2007; Baars et al., 2007). These designs provide robust assessments of herbivore efficacy by measuring the effect of natural or manipulated herbivore densities on survival, growth, and reproduction of the targeted weed (McClay and Balciunas, 2005).

The European swallow-worts *Vincetoxicum nigrum* (L.) Moench (syn. = *Cynanchum louiseae*) and *Vincetoxicum rossicum* (Kleopow) Barbar (syn. = *Cynanchum rossicum*) are herbaceous perennials considered invasive in North America. *V. nigrum* (black swallow-wort) is native to France, Spain, Portugal, and Italy (Markgraf, 1972) and typically grows in calcareous soils on forested slopes. *V. rossicum* (pale swallow-wort or dog-strangling vine) is native to Eastern

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Europe and it is restricted to forested ravines and is considered rare in Ukraine (Ostapko, 1995). Swallow-worts arrived in northeastern North America in the mid-19th century and naturalized populations currently extend from northeastern North America to the central United States. Swallow-worts infest fencerows, grasslands, roadsides, pastures, stream banks, cliffs, and agricultural row crops and their populations are increasing in abundance in North America (DiTommaso et al., 2005). *V. rossicum* also grows within mixed forest types, where it inhibits regenerating understory species (DiTommaso et al., 2005). Infestations of *V. rossicum* reduce native arthropod biodiversity (Ernst and Cappuccino, 2005) and habitat quality for grassland birds (DiTommaso et al., 2005) and both species of swallow-worts are becoming increasingly problematic weeds in no-till agriculture (DiTommaso et al., 2005). Management of swallow-worts is challenging because they are resistant to mechanical control, require repeated herbicide applications for long-term control, thrive in a wide variety of soil types and light conditions, and produce a large number of wind-dispersed seeds (DiTommaso et al., 2005; McKague and Cappuccino, 2005; Averill et al., 2008). Their vining growth further complicates control by intertwining with native species (Lawlor and Raynal, 2002). In light of the current negative ecological effects of swallow-worts, and the lack of effective management alternatives, a biological control program was initiated (Tewksbury et al., 2002; Weed and Gassmann, 2006).

In 2006 the leaf-feeding moth *Hypena opulenta* (Christoph) (Lepidoptera: Noctuidae: Hypeninae) was collected from *V. rossicum* and the sympatric species *Vincetoxicum scandens* (Sommier et Levier) Pobed. in forested sites located in southeast Ukraine (survey details described in Weed and Gassmann, 2006). In the past, *H. opulenta* had been observed in Ukraine, Turkey, Iran, and Turkmenistan (Fibiger, personal communication), but its host plant was unknown and thus it was not initially considered a potential biological control agent (Tewksbury et al., 2002). Based upon observed defoliation of *Vincetoxicum* species by *H. opulenta* in the field (Weed and Gassmann, 2006), this species was investigated as a potential biological control agent.

The use of foliage feeders in weed biological control has been widespread but has resulted in varying levels of success (Crawley, 1989a; Julien and Griffiths, 1998). One reason may be that although leaf feeders generally have negative effects on plant performance (Crawley, 1989b; Wise and Sacchi, 1996; Hunt-Joshi et al., 2004; Ding et al., 2006), these insects may also have no effect (Verkaar, 1988; Obeso, 1993), or may actually enhance plant performance (Islam and Crawley, 1983). Therefore, an assessment of each leaf feeder's efficacy prior to release is critical to not only improve chances of success, but also to minimize unwanted compensatory effects that may further complicate management. Recently, the effect of artificial defoliation on *Vincetoxicum* performance has been evaluated at an open field site (McKague and Cappuccino, 2005) and in a greenhouse under two light conditions (Milbrath, 2008). Although artificial defoliation has potential drawbacks, it can provide important insight into the potential value of a defoliator as a biological control agent (Raghu and Dhileepan, 2005). Both studies showed that under high light conditions defoliation was not effective at reducing *Vincetoxicum* performance unless it was repeated, but Milbrath (2008) found that under low light the negative effects of defoliation were substantial. Therefore, a defoliator such as *H. opulenta* has the potential to be a valuable biological control agent for *Vincetoxicum* under certain conditions.

Preliminary host specificity trials have concluded that *H. opulenta* is a specialist feeder of *Vincetoxicum* spp. (Weed, 2010) and evidence from European surveys suggests that it is restricted to forested habitats (Weed and Gassmann, 2006). The biology of *H. opulenta* is generally similar to the multivoltine green cloverworm, *Hypena scabra* (F.) (Pedigo et al., 1973) and hop looper, *Hypena humuli* Harris, (Grasswitz and James, 2008). In this study, we report biological observations on *H. opulenta* and its impact on growth and reproduction of *V. nigrum* and *V. rossicum* under quarantine conditions.

2. Materials and methods

2.1. Collection site of H. opulenta and research culture

Exploratory surveys for potential biological control agents of *V. rossicum* were conducted during June 2006 near Donetsk, Ukraine (Weed and Gassmann, 2006). Four pupae of *H. opulenta* were discovered within tied leaves of *V. rossicum* from a forested ravine located in Starognatovkg village (N47°34.497′ E37°46.168′) south of Donetsk, Ukraine. Larvae were also collected from *V. rossicum* and *V. scandens* within a forest in Amvrosiivka (N47°48.681′ E38°32.738′) southeast of Donetsk, Ukraine. Four pupae and 32 larvae were transported back to the laboratory at CABI EU- Switzerland (Delémont, Switzerland). The research colony was then shipped to the USA where it has been maintained on *V. nigrum*, *V. rossicum*, and *Vincetoxicum hirundinaria* in the insect quarantine facility at the University of Rhode Island (URI). A few adults that emerged from field-collected material were frozen and then shipped to Dr. Michael Fibiger (Denmark) for species identification. Voucher specimens are maintained in the URI quarantine laboratory.

2.2. Plant culture

Plants used in the biological and diapause studies described below originated from rootstock collections in the USA of *V. nigrum* (Charlestown, Rhode Island) and *V. rossicum* (Cumberland, Rhode Island and Groton, Connecticut) in 2006. Rootstocks were transplanted into 2.8 L pots and grown in Metro-mix 510 (Sun Gro Horticulture, Bellevue, Washington). Seeds of *V. hirundinaria* from Germany were sown in the greenhouse during the winter of 2006. After the seedlings reached 4–6 leaf nodes, they were transplanted into pots and growing medium as described above and held in the greenhouse until they could be moved outdoors in spring. Plants were surrounded by coarse sawdust kept in raised beds located outdoors. All plants were watered as needed and fertilized every two weekly during the growing season with Peter's Professional All Purpose Plant Food, 20-20-20 (0.2 g N/L). The plants were maintained over the winter in the sawdust-filled raised beds with an insulating fabric covering which was removed in spring. Leaf material used in the experiments was cut from between the 3rd and 6th leaf nodes to standardize leaf size and age among replicates.

Plants used in the study of larval feeding on plant performance were started from seed. Seeds of *V. nigrum* were collected from mature seedpods in Charlestown, Rhode Island during the fall of 2005 and sown during February 2006. Seeds of *V. rossicum* were collected in Aurora, New York and Groton, Connecticut during the fall of 2006 and sown during April 2007. All seeds were stored in a refrigerator until sowing in the greenhouse. After producing 4–6 leaf nodes, seedlings of *V. rossicum* and *V. nigrum* were transplanted into 1.9 and 2.8 L pots, respectively, with Metro-mix 510. All plants were moved outdoors and held in the same raised beds and maintained as described above until used in the experiment.

2.3. Biology of H. opulenta

2.3.1. Life cycle and reproduction

The life cycle of *H. opulenta* was studied with the first generation adults that emerged from field-collected material. One newly

emerged female and two males were placed into cages (40 × 40 × 40 cm) containing a diluted honey solution provided on a cotton swab and a potted *V. rossicum* held at 20 °C, 80% RH, and at 16:8 (L:D) h photoperiod. The longevity and preoviposition period of five females was monitored and daily egg production was determined by removing the plants from the cages and intensively searching the leaves, petioles, and stems. Eggs were removed from the plant surface by first wetting them with a fine-tip brush and then gently transferring them to 50 mm Petri dishes lined with moistened filter paper. A subset of the egg dishes was checked daily to determine time to hatch and the proportion of eggs hatching. Larval development of 15 individuals was monitored by transferring neonates with a fine-tip brush to excised leaves of *V. rossicum* held in tight fitting Petri dishes lined with moistened filter paper. Larvae were checked daily to observe molting and to change leaves. Time to complete each instar and to reach pupation was recorded for all larvae. Pupae were moved to 250 ml cups with moist vermiculite, covered with ventilated lids, and checked daily to record adult emergence. All immature life stages were kept under the same conditions as ovipositing adults. Head capsule widths were measured for larvae (n = 21–25 per instar) raised on *V. rossicum*.

2.3.2. Environmental factors affecting diapause induction

Hypena opulenta is a multivoltine species that exhibits facultative diapause in the pupal stage. The factors controlling diapause induction are unknown, but may include larval host plant quality (Danks, 1987; Hunter and McNeil, 1997) and/or photoperiod (Danks, 1987; Miller et al., 2000; Kurban et al., 2005). The effect of these factors on pupal diapause induction was tested in two separate trials. In the first, the proportion of pupae entering diapause was determined when caterpillars were raised under summer conditions (long day photoperiod and over 20 °C) on *V. nigrum*, *V. hirundinaria*, and *V. rossicum*. All species support successful larval development, but larval performance is variable among species (Weed, 2010). A second trial evaluated the effects of temperature, photoperiod, and host species on pupal diapause induction just before leaf senescence in the fall. All caterpillars used in the trials were obtained from "bulk" egg collections originating from multiple females from our research colony. To account for the possibility that photoperiod in one generation affects diapause induction in the next (Danks, 1987), the females used in these tests were raised as larvae and held during oviposition at 20 °C under a long day photoperiod 16:8 (L:D) h.

In both trials caterpillars were raised in groups of 10 on excised leaves in 473 ml plastic jars fitted with moistened filter paper and closed with clear, ventilated lids. A jar of 10 caterpillars on one leaf species was considered one replicate. Jars were checked daily to remove frass and replenish leaves. Pupae were transferred to clean 473 ml jars partially filled with sterilized vermiculite and held under the same conditions as the larvae until the end of adult emergence. Pupae were visually inspected on a regular basis for fungal disease and checked for viability by stimulating the abdomen with a gentle squeeze from soft forceps. (This causes live pupae to wriggle.) All dead pupae were discarded. Jars were checked daily for adult emergence and the number of healthy pupae entering diapause was counted 2 weeks after adults stopped emerging.

In the first trial, starting on 30 May 2008, five replicates were set up on each of the three plant species (*V. hirundinaria*, *V. nigrum*, and *V. rossicum*) and held at ambient room temperature 25.2 ± 1.5 °C (mean ± SD) under a 16:8 (L:D) h photoperiod produced by fluorescent lights (GE Daylight F48T12DH0). In the second trial, beginning on 6 October 2008, three replicates per plant species were held at two temperature levels: a constant 18 °C in growth chambers and at ambient room temperature 22.5 ± 0.7 °C (mean ± SD). Insects at both temperature conditions were either exposed to long (16:8 [L:D] h) or short (12:12 [L:D] h) day light conditions. Larvae were raised in separate incubators and rooms to create photoperiod treatments. This combination of factor levels produced a 2 × 2 × 3 factorial design where 360 caterpillars were monitored in the trial.

2.4. Effect of larval feeding on performance of V. nigrum and V. rossicum

Fifty potted *V. rossicum* and 44 *V. nigrum* plants of similar size that had been grown from seed were randomly assigned to the larval density treatments of zero, two, four, and eight larvae per plant. The zero larval density (control) received double the number of plants so that half could be sacrificed prior to the experiment to establish pre-infestation measures of above- and belowground biomass. Prior to infestation, the number of shoots, maximum shoot height, length and width of the largest leaf, and the number of flowers and seedpods were measured from each plant. An ANOVA confirmed that there were no significant pre-treatment differences among these plant growth parameters. *V. rossicum* were, on average, 66.2 ± 2.3 cm tall, with 1.5 ± 0.5 shoots and leaves 8.9 ± 0.1 cm long and 4.4 ± 0.1 cm wide, with 11.2 ± 1.4 flowers and 5.3 ± 0.9 seedpods (mean ± SE). *V. nigrum* were 56.5 ± 3.7 cm tall, with 6.4 ± 0.3 shoots, with leaves 7.3 ± 0.1 cm long and 4.0 ± 0.1 cm wide, with 8.6 ± 2.9 flowers and 7.4 ± 1.3 seedpods (mean ± SE).

One day prior to larval transfer, plants were moved from outdoors into quarantine and randomly arranged by treatment on plastic trays spaced 5 cm apart and held under a 16:8 (L:D) h photoperiod under fluorescent lights. Second instars of *H. opulenta* were transferred to leaves of *V. rossicum* on 5 July 2008 and to *V. nigrum* on 2 August 2008. (Space constraints precluded simultaneous evaluation.) Larvae were confined to individual plants with a white mesh sleeve supported by four 27-cm stakes placed in each corner of the pot. The bottom of the mesh sleeve was secured to the pot with a rubber band and the top of the sleeve was tied. The experiment with *V. rossicum* was conducted at ambient room temperatures of 27.5 ± 0.04 °C (mean ± SD) and room temperatures during the *V. nigrum* experiment averaged 25.7 ± 0.04 °C. Plants were watered twice per week during the experiment. Over the course of the experiment four *V. nigrum* plants were discarded due to infection by an unidentified pathogen. Remaining plants were closely examined at harvest for this pathogen but none was observed.

All plants were harvested after larval development was complete. The total experiment from larval transfer to plant harvest took 26 d for *V. rossicum* and 32 d for *V. nigrum*. Prior to plant dissection, pupae were counted and weighed from each plant. At harvest, the number and height of all shoots, number of axillary branches, and number of flowers and seedpods was counted from each plant. Two observers estimated the percentage of defoliation per plant by closely examining remaining leaf tissue. The aboveground plant parts were clipped and the seedpods were separated from the vegetative growth and weighed separately. The roots were cleaned of all soil and weighed. All plants were dried at 50 °C for 2 weeks to a constant dry weight and reweighed. Finally, after whole dry seedpods were weighed, the number of seeds per plant was counted.

2.5. Statistical analysis

In the first trial of the experiment which examined the effects on pupal diapause induction, an ANOVA was used to determine whether plant species influenced the proportion of pupae entering diapause. In the second trial, a three-way ANOVA was performed on the proportion of pupae entering diapause with plant species, temperature, and photoperiod as the fixed effects. An arcsine

square root transformation was performed on the proportion data to meet ANOVA assumptions.

Plant variables at final harvest in the larval feeding study were analyzed separately for each plant species. Percent defoliation and *H. opulenta* survivorship were compared among larval density treatments using a Kruskal–Wallis test followed by Dunn's multiple comparison of ranks test (Dunn, 1964). Pupal weight among larval densities was compared using one-way ANOVA. The effects of larval density were tested on the growth variables: above- and belowground biomass, number of axillary branches, and stem growth (difference between the maximum shoot height after the experiment [T_1] and maximum initial shoot height [T_0]). In these analyses, larval density was set as the main factor and in some cases a covariate was added. For instance, shoot height was a good predictor of dry aboveground biomass (linear regression: $F_{1,9} = 7.20$; $r^2 = 0.47$; $P = 0.0278$) for the individuals of *V. rossicum* that were allocated to the control treatment (T_0) harvested prior to larval infestation. Consequently, initial shoot height of experimental plants measured just prior to larval infestation was set as a covariate to adjust for plant size of *V. rossicum*. For a similar set of *V. nigrum* plants, seedpod number was a good predictor of dry aboveground biomass (linear regression: $F_{1,7} = 5.80$; $r^2 = 0.45$; $P = 0.0469$). Thus, initial seedpod number, counted prior to larval infestation, was set as the covariate to control for differences in plant size of *V. nigrum*. The effects of larval density on reproduction were analyzed similarly to the growth parameters. First, flower counts were not normally distributed, so these data were analyzed using a generalized linear model with a Poisson error structure. The number of flowers recorded prior to larval infestation was added as a covariate. Next, the effects of larval density were assessed on seedpod number, dry seedpod weight and the number of seeds. The initial seedpod number was set as a covariate in these analyses to adjust for prior seedpod load. The number of seedpods from *V. rossicum* plants harvested prior to larval infestation was a good predictor of dry seed weight (linear regression: $F_{1,8} = 31.68$; $r^2 = 0.80$; $P = 0.0005$). For *V. nigrum*, seedpod number was a good predictor of the number of seeds (linear regression: $F_{1,7} = 178.41$; $r^2 = 0.96$; $P < 0.0001$) and dry seed weight (linear regression: $F_{1,7} = 213.07$; $r^2 = 0.96$; $P < 0.0001$). Data were transformed as necessary to meet assumptions of the analyses (normality and homoscedasticity), but back-transformed values are presented in tables and figures. All analyses were performed using JMP Version 7.0.1 (SAS Institute Inc., Cary, NC, 1989–2007).

3. Results

3.1. Biology of H. opulenta

Males and females are similar in appearance with dull, light brown forewings with a central, dark brown band and light orange hindwings. Both sexes are similar in size, averaging 1.1 ± 0.1 cm in length with a wingspan of 2.9 ± 0.1 cm ($n = 10$, mean ± SD). Females lived for an average of 17.2 ± 4.0 d and laid 409.8 ± 156.9 eggs each after a 1.8 ± 0.8 d preoviposition period. Small, pale yellow eggs were deposited primarily on the undersides of the leaves near the midrib or in the depressions on the upper side of the petiole near the stem. Egg hatch at 20 °C required 3.6 ± 0.1 d with 80% of 335 eggs hatching. White neonates immediately move to a leaf undersurface and initiate feeding. They drop on a silk thread when disturbed. As they develop, larvae turn a darker green, acquire black spots, and their head capsule turns yellow. One larva died during the larval rearing experiment and 10 adults emerged. Larvae develop through five instars, requiring 18.9 ± 2.9 d at 20 °C to reach pupation and total development from egg hatch to adult requires 36.8 ± 4.2 d at 20 °C (Table 1). Larvae skeletonize leaves during the first three instars and consume all leaf tissues but avoid major leaf veins during the L4 and L5 stages. Fifth instars prepare pupation sites either by tying leaves together or by constructing a silk chamber in soil at the base of plants. Pupae are dark brown, 1.2 ± 0.01 cm long, and weigh 91.2 ± 7.1 mg ($n = 14$, mean ± SD).

Table 1
Developmental period of *Hypena opulenta* life stages at 20 °C and larval head capsule widths.

Stage	Developmental period		Head capsule width	
	n	(d ± SE)	*n*	(mm ± SD)
Egg	335	3.6 ± 0.1		
Larval				
L1	15	2.0 ± 0.0	21	0.32 ± 0.03
L2	15	2.9 ± 0.3	23	0.49 ± 0.04
L3	14	2.3 ± 0.5	25	0.78 ± 0.04
L4	14	3.6 ± 0.6	25	1.24 ± 0.06
L5	14	6.1 ± 1.3	24	1.71 ± 0.06
Prepupal	14	1.9 ± 1.1		
Total	14	18.9 ± 2.9		
Pupal	10	14.3 ± 1.4		
Total	10	36.8 ± 4.2		

3.2. Environmental factors affecting diapause induction

In trial one, 24% of pupae entered diapause. This did not vary among host plants ($F_{2,17} = 0.75$; $P = 0.4910$) (Fig. 1a). In trial two (Fig. 1b), rearing temperature had no effect on diapause but larvae reared under a short day photoperiod experienced 100% pupal diapause as opposed to 68% under long day conditions – a significant difference ($F_{1,54} = 63.2$; $P < 0.0001$). Pupal diapause induction was lower (24%) for larvae reared under summer conditions than those reared under a similar photoperiod during the fall (68%) (Fig. 1).

3.3. Effect of larval feeding on performance of V. nigrum and V. rossicum

Increasing larval density of *H. opulenta* caused increasing levels of defoliation to *V. rossicum* ($H = 35.2$; df = 3; $P < 0.0001$) and *V. nigrum* ($H = 22.4$; df = 3; $P < 0.0001$) (Fig. 2). Median survival of *H. opulenta* on *V. rossicum* ranged from 63% to 100% with no significant relationship to larval density ($H = 2.60$; df = 2; $P = 0.2722$), although larval density significantly affected pupal weight ($F_{1,93} = 21.04$; $P < 0.0001$) on *V. rossicum* (Fig. 3). Median survival of *H. opulenta* on *V. nigrum* ranged from 50% to 75% and was not related to larval density ($H = 4.06$; df = 2; $P = 0.312$) which in turn had no effect on pupal weight ($F_{1,47} = 1.49$; $P = 0.2344$) (Fig. 3).

No plants died from larval feeding. All larval densities of *H. opulenta* caused a significant reduction in aboveground biomass of *V. rossicum* ($F_{3,35} = 16.0$; $P < 0.0001$) (Fig. 4a) but larval density did not affect belowground biomass of this plant ($F_{3,36} = 0.94$; $P = 0.4318$) (Fig. 4b). Feeding of *H. opulenta* had no effect on aboveground or belowground biomass of *V. nigrum* ($F_{3,27} = 0.91$; $P = 0.4488$ and $F_{3,27} = 0.79$; $P = 0.1404$, respectively) (Fig. 4a and b). Stem growth was not affected by larval feeding on *V. rossicum* ($F_{3,36} = 1.30$; $P = 0.2892$) or on *V. nigrum* ($F_{3,27} = 1.98$; $P = 0.1404$) (Fig. 4c). Finally, the number of new axillary branches increased with larval density on *V. rossicum* ($F_{3,36} = 4.61$; $P = 0.0080$) but not *V. nigrum* ($F_{3,27} = 1.45$; $P = 0.2513$) (Fig. 4d).

All larval densities reduced flower production of *V. rossicum* ($\chi^2 = 69.2$; df = 3; $P < 0.0001$) while four or eight larvae per plant were required to reduce flower production of *V. nigrum* ($\chi^2 = 148.6$; df = 3; $P < 0.0001$) (Fig. 5a). The number of seedpods per plant was unaffected by larval feeding for *V. nigrum* ($F_{3,26} = 0.76$; $P = 0.0511$) but significantly decreased on infested

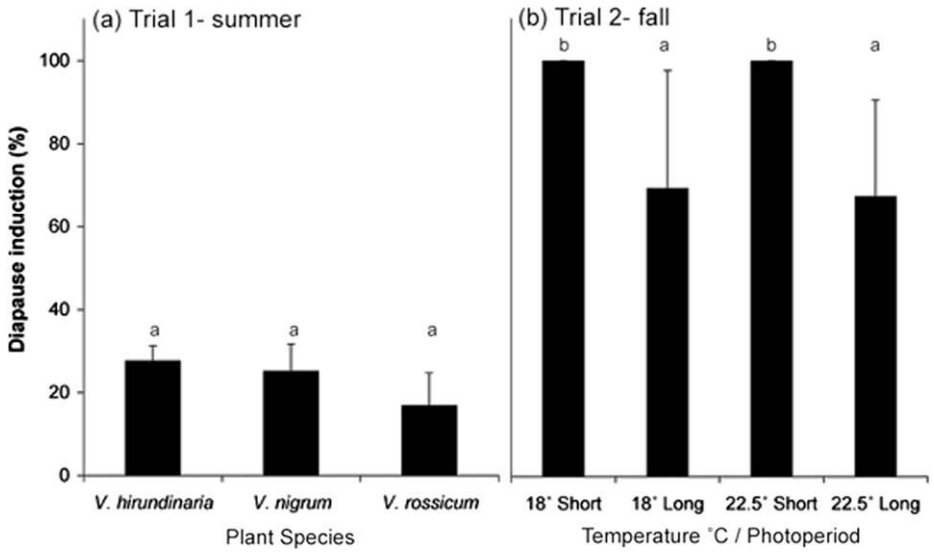

Fig. 1. Percent diapause (±SE) of *H. opulenta* pupae. (a) Trial 1 – effect of host plant during summer conditions (20 °C, 16:8 [L:D] h). (b) Trial 2 – effect of photoperiod and temperature during the fall (long: 16:8 [L:D] h, short: 12:12 [L:D] h). Different letters indicate significant differences among means within a trial.

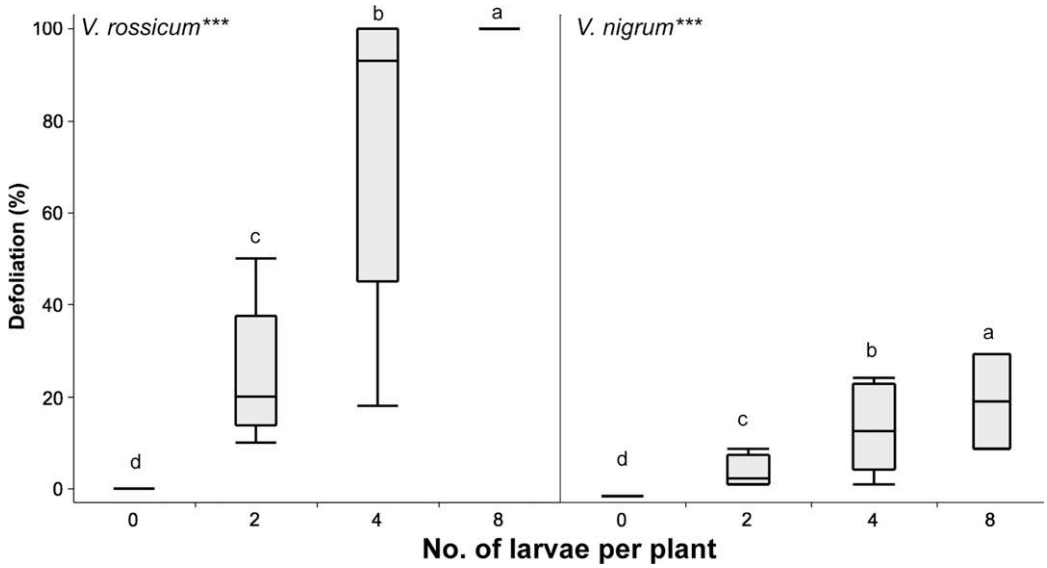

Fig. 2. Defoliation from three larval densities of *H. opulenta* on *V. rossicum* and *V. nigrum*. The boxes represent the 25th and 75th percentiles about the median and the bars extend to the 10% and 90% values. Different letters indicate significant differences at $\alpha < 0.001$ within species (Dunn's multiple comparison of ranks test). Asterisks indicate significance at $\alpha < 0.001$.

V. rossicum plants ($F_{3,36} = 11.03$; $P = 0.0002$) (Fig. 5b). Seedpod mass of control *V. rossicum* increased by 20% compared to plants harvested prior to larval infestation. Mass of *V. rossicum* seedpods was lower at all larval densities compared to controls ($F_{3,35} = 10.01$; $P < 0.0001$) (Fig. 5c), resulting in a reduction in seed number ($F_{3,36} = 4.93$; $P = 0.0057$) (Fig. 5d). However, only the highest larval density caused a significant reduction in seed number compared to the control (Fig. 5d). Larval feeding on *V. nigrum* did not cause reductions in seedpod mass ($F_{3,26} = 0.86$; $P = 0.4738$) (Fig. 5c) or seed number ($F_{3,26} = 0.77$; $P = 0.5172$) (Fig. 5d).

4. Discussion

Hypena opulenta undergoes facultative diapause in the pupal stage. In contrast, *H. scabra* overwinters in the adult and pupal stages (Pedigo et al., 1973) and *H. humuli* diapause in the adult stage (Grasswitz and James, 2008). It is unlikely that *H. opulenta* overwinter as adults because they have never survived more than 14 d when held at 7 °C (unpublished data). Diapause induction of *H. opulenta* is regulated by photoperiod and in our experiment, short day conditions induced diapause in all individuals during the fall trial. This is similar to other species such as the cotton bollworm, *Helicoverpa armigera* (Hübner) (Kurban et al., 2005), and the field bindweed moth, *Tyta luctuosa* (Dennis and Schiffermüller) (Miller et al., 2000), where long day photoperiods inhibit diapause induction. As shown for other insects (Danks, 1987; Hunter and McNeil, 1997), diapause induction of *H. opulenta* did not vary among host plant species. However, the trials in summer compared to fall provide preliminary evidence that seasonal changes in host plant quality may affect diapause induction of *H. opulenta*. Although temperature differed by about 3 °C between trials, this discrepancy did not affect diapause induction when tested in the fall trial. Hence it is likely that increased diapause from summer

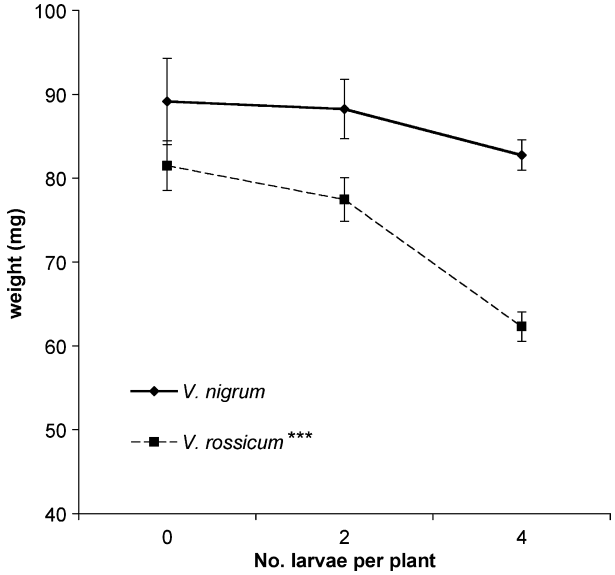

Fig. 3. Effect of larval density on pupal weight of *H. opulenta* grown on *V. rossicum* (dashed line) and *V. nigrum* (solid line). Error bars represent ±SE. Asterisks indicate significance at $\alpha < 0.001$.

to fall under long day conditions was due to decreases in leaf quality as reported in other species (Danks, 1987). Therefore, it appears that photoperiod and seasonal changes in host plant quality of *Vincetoxicum* both affect diapause induction in *H. opulenta*.

A single generation of larval feeding by *H. opulenta* did not kill plants. Increasing densities of *H. opulenta* larvae inflicted increasing levels of defoliation, but defoliation differed between species. Eight larvae (and occasionally four) per plant caused nearly 100% defoliation of *V. rossicum*, but this level of damage never occurred on *V. nigrum*, where eight larvae per plant removed about 30% of total leaf area. Lower defoliation on *V. nigrum* may have occurred because of size differences between species. The *V. nigrum* plants used in this experiment were approximately 1 year older than *V. rossicum* and their above- and belowground biomass was, respectively, 2.7 and 5.6 times greater than the biomass of *V. rossicum*. Lower survival rates of *H. opulenta* on *V. nigrum* (57% on *V. nigrum* compared to 76% on *V. rossicum* over all densities), may have also reduced defoliation.

Many defoliating insects reduce aboveground biomass (Ding et al., 2006; Schooler et al., 2006) and a common response to this herbivory is increased production of secondary shoots (Häfliger et al., 2006; Stanley et al., 2007), and decreased reproductive effort (Wise and Sacchi, 1996; Hunt-Joshi et al., 2004). Larval densities of

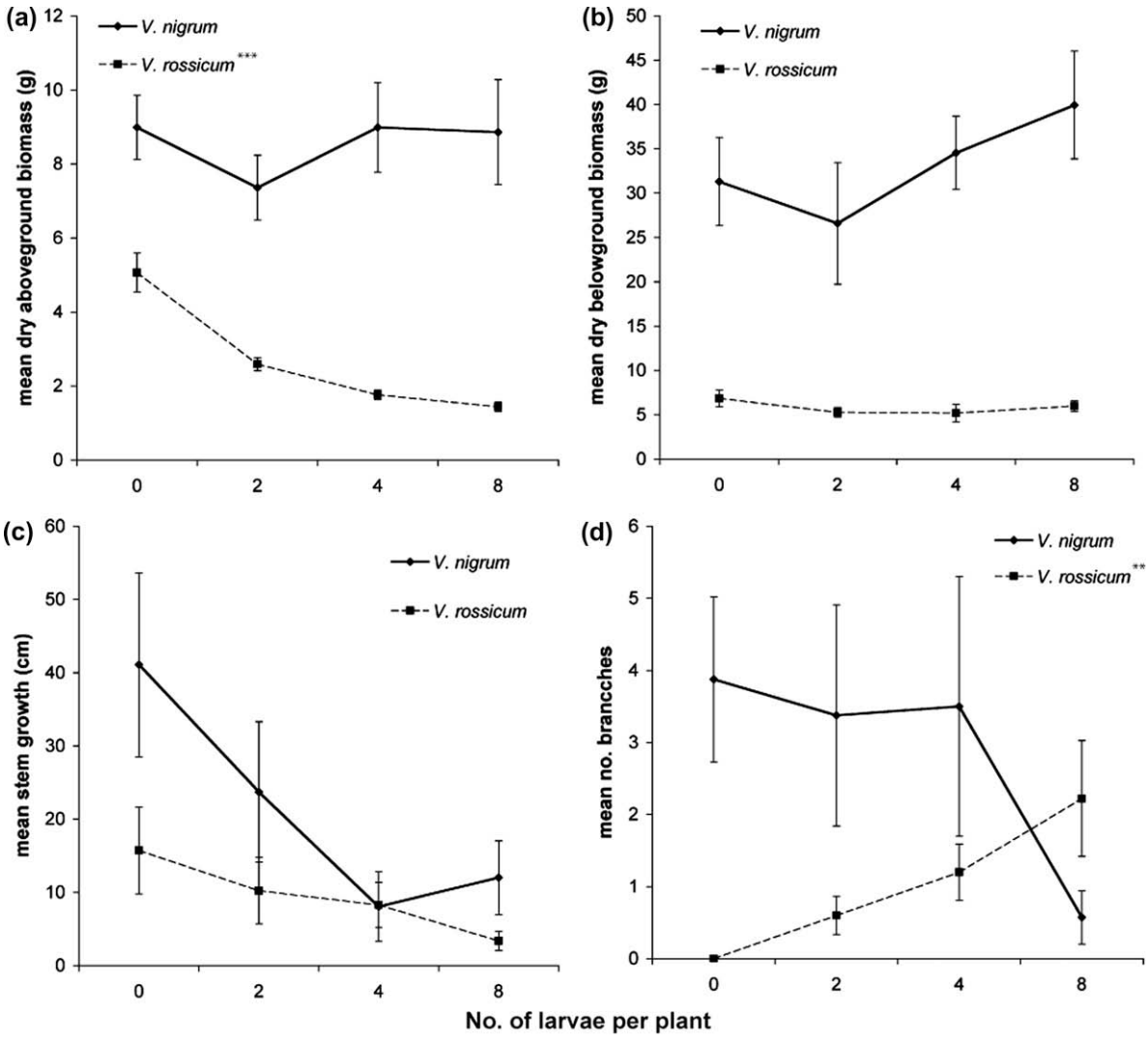

Fig. 4. Effect of *H. opulenta* larval density on (a) aboveground biomass, (b) belowground biomass, (c) stem growth, and (d) axillary branch production of *V. rossicum* (dashed line) and *V. nigrum* (solid line). Error bars represent ±SE. Aboveground biomass values are adjusted means from the ANCOVA (see Section 2). Asterisks indicate significance at $\alpha < 0.01$ (**) and $\alpha < 0.001$ (***) levels.

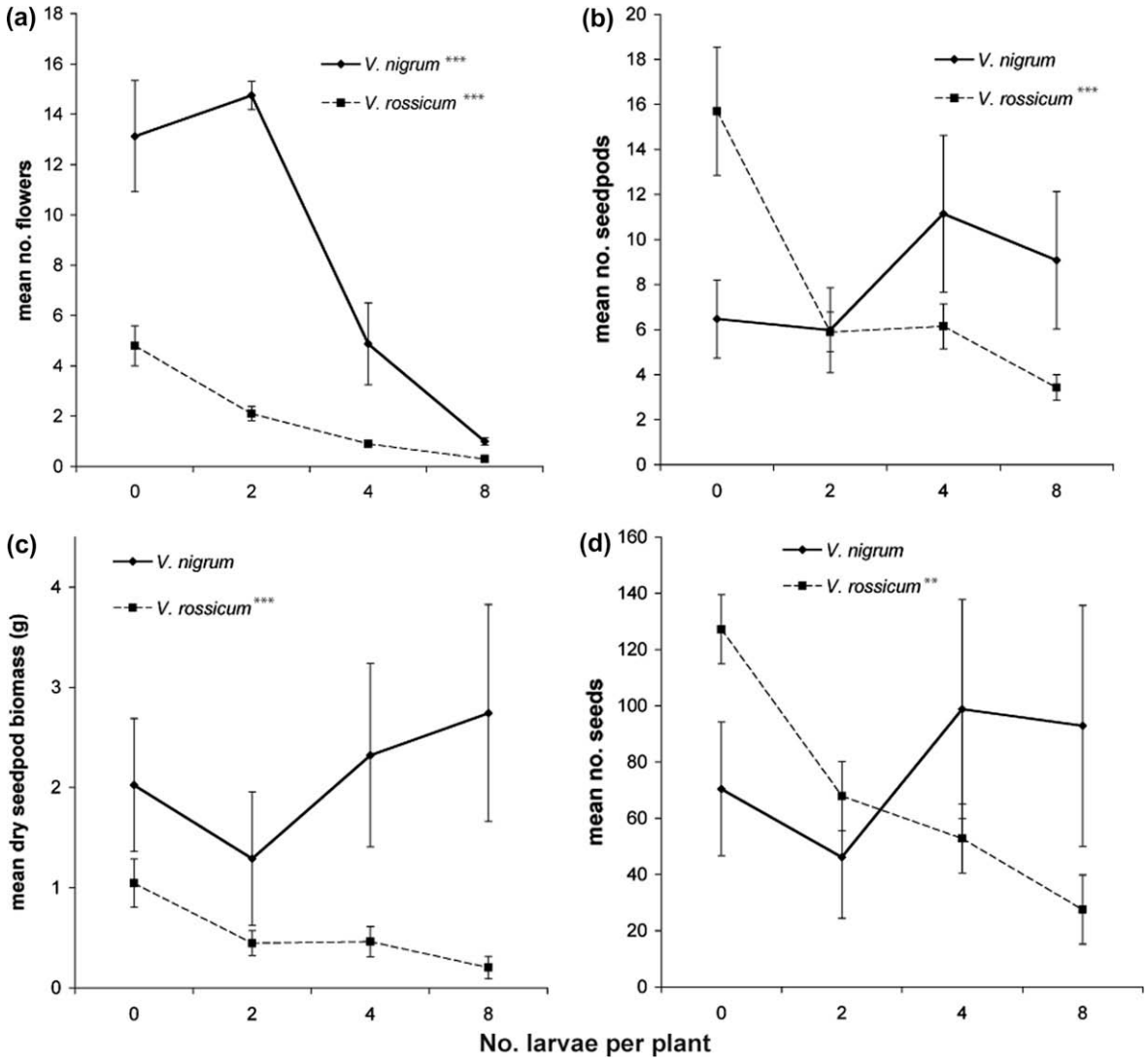

Fig. 5. The effect of *H. opulenta* larval density on the number of (a) flowers and (b) seedpods, (d) dry seedpod mass, and (c) seed production of *V. rossicum* (dashed line) and *V. nigrum* (solid line). Values displayed for flower and seedpod counts, seedpod mass, and number of seeds (*V. nigrum* only) are adjusted means from the ANCOVA (see Section 2). Error bars represent ±SE. Asterisks indicate significance at $\alpha < 0.01$ (**) and $\alpha < 0.001$ (***) levels.

between two and eight per plant caused significant reductions in aboveground biomass and increased the production of axillary branches of *V. rossicum*. However, new axillary branching of *V. rossicum* was unable to fully compensate for aboveground biomass loss due to herbivory. Larval feeding did not affect any measure of *V. nigrum* growth which was most likely due to low levels of damage inflicted on this species. Stem growth of both species was not affected by larval feeding, indicating that larvae did not damage the apical buds. Larval feeding significantly reduced flowering, seedpod production, seedpod mass, and the number of seeds of *V. rossicum* but not *V. nigrum*. Caterpillar-free *V. rossicum* produced 11 seedpods per plant during this experiment, whereas seedpod production on infested plants only increased slightly (by one or two pods), resulting in lower seed output at harvest. This demonstrates that even at low densities, *H. opulenta* has a strong effect on *V. rossicum* reproduction.

Hypena opulenta feeding consistently reduced performance of *V. rossicum* but not *V. nigrum*. This is in contrast to another study which found similar effects on performance of both species caused by 100% artificial defoliation (Milbrath, 2008). The discrepancy between our results and those of Milbrath (2008) may be explained by the inability of *H. opulenta* to inflict comparable levels of damage to both *Vincetoxicum* species in our experiment. Both studies agree that defoliation has the capacity to reduce aboveground biomass, change plant architecture, and delay reproduction but this study highlights that 100% defoliation is not necessary to reduce plant performance. It is also probable that the negative effects of *H. opulenta* larval damage will be greater in forested habitats with low light availability (Milbrath, 2008).

We expect that, if released in North America, *H. opulenta* would be restricted to forested habitats as it is in Europe and hence, it would be more effective against *V. rossicum* which is often found in forests. This insect would only occasionally encounter *V. nigrum* which infrequently grows along forest margins (DiTommaso et al., 2005). Based upon the results of our diapause induction experiments, *H. opulenta* will produce multiple, overlapping generations typical of other *Hypena* spp. (Pedigo et al., 1973; Grasswitz and James, 2008). These generations may continually suppress the smothering growth of *V. rossicum* in forested sites and ultimately allow native species to regenerate. If compensation to herbivory by *V. rossicum* under field conditions is similar to laboratory results, continual defoliation is likely to lead to reductions in root mass (Meyer, 1993; Schat and Blossey, 2005), possibly by changes in resource allocation patterns away from roots to shoot growth

(Rogers and Siemann, 2003; Norghauer et al., 2008). The results of this study warrant completion of host specificity testing with this promising agent. Furthermore, this study lends support to the suggestion of McClay and Balciunas (2005) that when there is *a priori* evidence that the herbivore is host specific, as is the case for *H. opulenta*, greater emphasis should be placed on the completion of agent impact before extensive host specificity testing ensues.

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References

Averill, K.M., DiTommaso, A., Morris, S.H., 2008. Response of pale swallow-wort (*Vincetoxicum rossicum*) to triclopyr application and clipping. Invasive Plant Science and Management 1, 196–206.

Baars, J.-R., Hill, M.P., Heystek, F., Neser, S., Urban, A.J., 2007. Biology, oviposition preference and impact in quarantine of the petiole-galling weevil, *Coelocephalapion camarae* Kissinger, a promising candidate agent for biological control of *Lantana camara*. Biological Control 40, 187–195.

Balciunas, J.K., Burrows, D.W., 1993. The rapid suppression of the growth of *Melaleuca quinquenervia* saplings in Australia by insects. Journal of Aquatic Plant Management 31, 265–270.

Briese, D.T., 1996. Potential impact of the stem-boring weevil *Lixus cardui* on the growth and reproductive capacity of *Onopordum* thistles. Biocontrol Science and Technology 6, 251–262.

Briese, D.T., 2000. Impact of the *Onopordum capitulum* weevil *Larinus latus* on seed production by its host-plant. Journal of Applied Ecology 37, 238–246.

Briese, D.T., Thomann, T., Vitou, J., 2002. Impact of the rosette crown weevil *Trichosirocalus briesei* on the growth and reproduction of *Onopordum* thistles. Journal of Applied Ecology 39, 688–698.

Crawley, M.J., 1989a. The successes and failures of weed biocontrol using insects. Biocontrol News and Information 10, 213–223.

Crawley, M.J., 1989b. Insect herbivores and plant population dynamics. Annual Review of Entomology 34, 531–562.

Danks, H.V., 1987. Insect Dormancy: An Ecological Perspective. Biological Survey of Canada, Ottawa.

Denoth, M., Frid, L., Myers, J.H., 2002. Multiple agents in biological control: improving the odds? Biological Control 24, 20–30.

Ding, J., Blossey, B., Du, Y., Zheng, F., 2006. Impact of *Galerucella birmanica* (Coleoptera: Chrysomelidae) on growth and seed production of *Trapa natans*. Biological Control 37, 338–345.

DiTommaso, A., Lawlor, F.M., Darbyshire, S.J., 2005. The biology of invasive alien plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [=*Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [=*Vincetoxicum nigrum* (L.) Moench]. Canadian Journal of Plant Science 85, 243–263.

Dunn, O.J., 1964. Multiple comparisons using rank sums. Technometrics 6, 241–252.

Ernst, C.M., Cappuccino, N., 2005. The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. Biological Invasions 7, 417–425.

Gerber, E., Hinz, H.L., Blossey, B., 2007. Impact of the belowground herbivore and potential biological control agent, *Ceutorhynchus scrobicollis*, on *Alliaria petiolata* performance. Biological Control 42, 355–364.

Goeden, R.D., 1983. Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. Protection Ecology 5, 287–301.

Goolsby, J.A., Zonneveld, R., Bourne, A., 2004. Prerelease assessment of impact on biomass production of an invasive weed, *Lygodium microphyllum* (Lygodiaceae: Pteridophyta), by a potential biological control agent, *Floracarus perrepae* (Acariformes: Eriophyidae). Environmental Entomology 33, 997–1002.

Grasswitz, T.R., James, D.G., 2008. Biology, phenology and control of *Hypena humuli* (Lepidoptera: Noctuidae), an emerging pest of hops (*Humulus lupulus*) (Cannabaceae) in the USA. International Journal of Pest Management 54, 333–338.

Häfliger, P., Schwarzländer, M., Blossey, B., 2006. Impact of *Archanara geminipuncta* (Lepidoptera: Noctuidae) on aboveground biomass production of *Phragmites australis*. Biological Control 38, 413–421.

Harris, P., 1973. The selection of effective agents for the biological control of weeds. Canadian Entomologist 105, 1495–1503.

Hunt-Joshi, T.R., Blossey, B., Root, R.B., 2004. Root and leaf herbivory on *Lythrum salicaria*: implications for plant performance and communities. Ecological Applications 14, 1574–1589.

Hunter, M.D., McNeil, J.N., 1997. Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. Ecology 78, 977–986.

Inouye, D.W., 1982. The consequences of herbivory: a mixed blessing for *Jurinea mollis* (Asteraceae). Oikos 39, 269–272.

Islam, Z., Crawley, M.J., 1983. Compensation and regrowth in ragwort (*Senecio jacobaea*) attacked by cinnabar moth (*Tyria jacobaeae*). Journal of Ecology 71, 829–843.

Julien, M.H., Griffiths, M.W., 1998. Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds. CABI Publishing, Wallingford, UK.

Kurban, A., Yoshida, H., Izumi, Y., Sonoda, S., Tsumuki, H., 2005. Pupal diapause of *Helicoverpa armigera*: sensitive stage for photoperiodic induction. Applied Entomology and Zoology 40, 457–460.

Lawlor, F.M., Raynal, D.J., 2002. Response of swallow-wort to herbicides. Weed Science 50, 179–185.

Louda, S.M., Pemberton, R.W., Johnson, M.T., Follett, P.A., 2003. Nontarget effects – the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology 48, 365–396.

Markgraf, F., 1972. *Vincetoxicum* N.M. Wolf. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, vol. 3. Cambridge University Press, Cambridge, pp. 71–73.

Maron, J.L., 1998. Insect herbivory above- and belowground: individual and joint effects on plant fitness. Ecology 79, 1281–1293.

McClay, A.S., Balciunas, J.K., 2005. The role of pre-release efficacy assessment in selecting classical biological control agents for weeds-applying the Anna Karenina principle. Biological Control 35, 197–207.

McEvoy, P.B., Coombs, E.M., 1999. Biological control of plant invaders: regional patterns, field experiments, and structured population models. Ecological Applications 9, 387–401.

McFadyen, R.E.C., 1989. Biological control of weeds. Annual Review of Entomology 43, 369–393.

McKague, C.I., Cappuccino, N., 2005. Response of pale swallow-wort, *Vincetoxicum rossicum*, following aboveground tissue loss: implications for the timing of mechanical control. Canadian Field Naturalist 119, 525–531.

Meyer, G.A., 1993. A comparison of the impacts of leaf-and sap-feeding insects on growth and allocation of goldenrod. Ecology 74, 1101–1116.

Milbrath, L.R., 2008. Growth and reproduction of invasive *Vincetoxicum rossicum* and *V. nigrum* under artificial defoliation and different light environments. Canadian Journal of Botany 86, 1279–1290.

Miller, N., Nechols, J.R., Horak, M.J., Loughin, T.M., 2000. Photoperiodic regulation of seasonal diapause induction in the field bindweed moth, *Tyta luctuosa* (Lepidoptera: Noctuidae). Biological Control 19, 139–148.

Müller-Schärer, H., Schaffner, U., 2008. Classical biological control: exploiting enemy escape to manage plant invasions. Biological Invasions 10, 859–874.

Myers, J.H., 1985. How many insect species are necessary for successful biocontrol of weeds? In: Delfosse, E.S. (Ed.), Proceedings of the Sixth International Symposium on Biological Control of Weeds, 19–25 August 1984, Vancouver, Canada. Agriculture Canada, Ottawa, Ontario, Canada, pp. 77–82.

Norghauer, J.M., Malcolm, J.R., Zimmerman, B.L., 2008. Canopy cover mediates interactions between a specialist caterpillar and seedlings of a neotropical tree. Journal of Ecology 96, 103–113.

Obeso, J.R., 1993. Does defoliation affect reproductive output in herbaceous perennials and woody plants in different ways? Functional Ecology 7, 150–155.

Ostapko, V.M., 1995. Genus *Vincetoxicum* N.M. Wolf in the South-East of Ukraine. Ukrainian Botanical Journal 52, 388–394.

Pearson, D.E., Callaway, R.M., 2003. Indirect effects of host-specific biological control agents. Trends in Ecology and Evolution 18, 456–461.

Pearson, D.E., Callaway, R.M., 2005. Indirect nontarget effects of host-specific biological control agents: implications for biological control. Biological Control 35, 288–298.

Pedigo, L.P., Stone, J.D., Lentz, G.L., 1973. Biological synopsis of the green cloverworm in Central Iowa. Journal of Economic Entomology 66, 665–673.

Raghu, S., Dhileepan, K., 2005. The value of simulating herbivory in selecting effective weed biological control agents. Biological Control 34, 265–273.

Rogers, W.E., Siemann, E., 2003. Effects of simulated herbivory and resources on Chinese tallow tree (*Sapium sebiferum*, Euphorbiaceae) invasion of native coastal prairie. American Journal of Botany 90, 243–249.

Schat, M., Blossey, B., 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). Environmental Entomology 34, 906–914.

Schooler, S., Baron, Z., Julien, M., 2006. Effect of simulated and actual herbivory on alligator weed, Alternanthera philoxeroides, growth and reproduction. Biological Control 36, 74–79.

Sheppard, A.W., 2003. Prioritising agents based on predicted efficacy: beyond the lottery approach. In: Spafford Jacob, H., Briese, D.T. (Eds.), Improving the Selection, Testing and Evaluation of Weed Biological Control Agents. CRC for Australian Weed Management, Tech. Ser. no. 7, pp. 11–22.

Sheppard, A.W., Aeschlimann, J.P., Sagliocco, J.L., Vitou, J., 1995. Below-ground herbivory in *Carduus nutans* (Asteraceae) and the potential for biological control. Biocontrol Science and Technology 5, 261–270.

Stanley, J.N., Julien, M.H., Center, T.D., 2007. Performance and impact of the biological control agent *Xubida infusella* (Lepidoptera: Pyralidae) on the target

weed *Eichhornia crassipes* (waterhyacinth) and on a non-target plant, *Pontederia cordata* (pickerelweed) in two nutrient regimes. Biological Control 40, 298–305.

Tewksbury, L., Casagrande, R., Gassmann, A., 2002. Swallow-worts. In: Van Driesche, R., Lyon, S., Blossey, B., Hoddle, M., Reardon, R. (Eds.), Biological Control of Invasive Plants in the Eastern United States. USDA Forest Service Publication FHTET-2002-04, pp. 209–216.

van Klinken, R.D., Raghu, S., 2006. A scientific approach to agent selection. Australian Journal of Entomology 45, 253–258.

Verkaar, H.J., 1988. Are defoliators beneficial for their host plants in terrestrial ecosystems: a review? Acta Botanica Neerlandica 37, 137–152.

Waloff, N., Richards, O.W., 1977. The effect of insect fauna on growth mortality and natality of broom, *Sarothamnus scoparius*. Journal of Applied Ecology 14, 787–798.

Weed, A.S., 2010. Biology and ecology of European natural enemies of swallow-worts (VIncetoxicum) and the potential for biological control. PhD dissertation, Dept. Plant Sciences, University of Rhode Island, Kingston, RI, p.176.

Weed, A.S., Gassmann, A., 2006. Evaluating the potential for biological control of swallow-worts, *Vincetoxicum nigrum* and *V. rossicum*. Unpublished Annual Report 2006, CABI EU – Switzerland, 20 pp.

Wise, M.J., Sacchi, C.F., 1996. Impact of two specialist insect herbivores on reproduction of horse nettle, *Solanum carolinense*. Oecologia 108, 328–337.