Host Specificity of *Hypena opulenta*: A Potential Biological Control Agent of *Vincetoxicum* in North America

Author(s): Alex F. Hazlehurst, Aaron S. Weed, Lisa Tewksbury, and Richard A. Casagrande

Source: Environmental Entomology, 41(4):841-848. 2012.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/EN12093
URL: http://www.bioone.org/doi/full/10.1603/EN12093

Host Specificity of *Hypena opulenta*: A Potential Biological Control Agent of *Vincetoxicum* in North America

ALEX F. HAZLEHURST,[1,2] AARON S. WEED,[1,3] LISA TEWKSBURY,[1] AND RICHARD A. CASAGRANDE[1]

Environ. Entomol. 41(4): 841–848 (2012); DOI: http://dx.doi.org/10.1603/EN12093

ABSTRACT A biological control program has been initiated against the European swallow-worts *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopow) Barbar. (Family Apocynaceae) that have become invasive in North America. The leaf-feeding moth, *Hypena opulenta* Christoph (Lepidoptera: Erebidae), originating from eastern Europe, has been under measurement as a potential biological control agent of swallow-worts since 2006. In this study we measured the host range of *H. opulenta* by screening 82 potential host plant species for larval development under no-choice conditions. In addition, we also monitored female fecundity, longevity, and oviposition preference among suitable larval hosts. Successful larval development occurs only on *Vincetoxicum* spp. Partial larval development by one larva was observed on *Boehmeria cyclindrica* (L.) Sw. (Urticaceae) to the final instar, but this individual failed to pupate. Exploratory feeding occurred on *Gonolobus stephanotrichus* Griseb. (Apocynaceae) and *Urtica dioica* L. (Urticaceae), but all larvae failed to develop past the first and second instar, respectively. Additional testing with mature larvae on a subset of the plant species demonstrates that no species outside the genus *Vincetoxicum* are suitable for complete larval development of *H. opulenta*. The longevity and fecundity of females raised on each target weed are similar and gravid females do not display an oviposition preference among *Vincetoxicum* spp. *Hypena opulenta* does not present a risk to any native plant species or species of economic importance in North America. Petitions have been submitted for experimental open-field releases of *H. opulenta* in the United States and Canada.

KEY WORDS *Hypena*, dog-strangling vine, larval development, host range, fecundity

Populations of European swallow-worts (*Vincetoxicum* species) have become established in North America and their distribution has expanded dramatically during the past 40 yr, possibly because there are no effective antagonists to suppress population growth and prevent spread (Sheeley and Raynal 1996, Christensen 1998, Lawlor 2000, Milbrath 2010). *Vincetoxicum nigrum* (L.) Moench (black swallow-wort) and *V. rossicum* (Kleopow) Barbar. (pale swallow-wort or dog-strangling vine) were introduced from Europe into northeastern North America as ornamentals in the mid-19th century. Naturalized populations currently extend from northeastern to central United States and into the Canadian provinces of Ontario and Quebec. *Vincetoxicum nigrum* is widely distributed in the United States with populations recorded from 21 states and *V. rossicum* is established in seven states (USDA NRCS 2011). Both swallow-worts are considered invasive in Canada (Quebec and Ontario) and the United States and considered noxious weeds in Vermont and Ontario (DiTommaso et al. 2005, USDA NRCS 2011).

Vincetoxicum nigrum is native to Mediterranean regions of France, Italy, and Spain and typically grows in calcareous soils on forested slopes. *Vincetoxicum rossicum* is naturally distributed in southeast Ukraine and Russia and is restricted to forested ravines (Pobedimova 1952, Markgraf 1972). Both swallow-worts have a very limited geographical distribution in their native range with populations typically occurring as small, isolated patches consisting of fewer than 100 individuals (Weed et al. 2011). In Ukraine, *V. rossicum* is considered rare (Ostapko 1995). However, both species display a much greater tolerance to a diverse array of habitats and climates in North America.

Invasive swallow-worts flower from late spring to late August and flowers are insect- or self-pollinated. Each fertilized flower produces one or two elongate seedpods with ≈20 seeds. The polyembryonic seeds either germinate during late summer to fall or the next spring (DiTommaso et al. 2005). Swallow-worts display superior competition for resources among native plants and often form dense, nearly monospecific stands (Cappuccino 2004). In North America both species are hardy colonizers in a wide variety of primarily upland habitats including but not restricted to

[1] Department of Plant Sciences and Entomology, University of Rhode Island, Kingston, RI 02881.
[2] Corresponding author, e-mail: afhazlehurst@gmail.com.
[3] Department of Biological Sciences, Dartmouth College, Hanover, NH 03755.

pastures, old fields, hillsides, shores, flood plains, roadsides, and forest margins (DiTommaso et al. 2005). Both species can endure a broad range of moisture regimes and flourish in either full sun or partially shaded areas; however, *V. rossicum* also establishes within forest understories. Dense populations threaten sensitive plant communities of endemic flora (DiTommaso et al. 2005). Infestations of *V. rossicum* negatively affect native arthropod diversity in Canada and may decrease foraging habitat of native birds and small mammals (Ernst and Cappuccino 2005). Substantial efforts in the use of conventional control methods such as mowing, hand pulling, and applying herbicides have largely been unsuccessful in eliminating established infestations. Annual herbicide treatment and mowing have immediate effects on seedpod production, but control is temporary and not selective (Lawlor 2000, McKague and Cappuccino 2005, Averill et al. 2008).

A classical biological control program was initiated for swallow-worts in North America in 2001 (Tewksbury et al. 2002, Weed et al. 2011). During surveys conducted in 2006 the leaf-feeding moth *Hypena opulenta* (Christoph) was found attacking *V. rossicum* and *V. scandens* near Donetsk, Ukraine (Weed and Casagrande 2010) and was transported to a quarantine facility for further measurement as a biological control agent. Adult moths emerge in the spring, mate, and oviposit on the undersides of leaves or in grooves along the petioles. Larvae preferentially attack newly expanding leaves and complete development through five instars after which they pupate in the soil or in tied-up leaf material. Pupal diapause is facultative and at least two generations per year are possible. The potential for significant larval impact on *V. rossicum* is encouraging because only two larvae per plant are needed to reduce shoot biomass and reproduction (Weed and Casagrande 2010). However, the risk of attack by *H. opulenta* to North American plant species is unknown from the literature. In this study the host range of *H. opulenta* was assessed to determine whether any plant species outside of the genus *Vincetoxicum* are at risk of attack by this species in North America. In addition, we also measured the longevity, fecundity, and oviposition preference of adults reared on suitable larval hosts.

Materials and Methods

Insect Culture. All testing with *H. opulenta* was conducted using a population that was collected near Donetsk, Ukraine in 2006. The test population started from four pupae that were gathered from tied leaves of *V. rossicum* in a forested ravine (47° 34.497′ N, 37° 46.168′ E) along with 32 larvae collected on *V. rossicum* and *V. scandens* within a nearby forest (47° 48.681′ N, 38° 32.738′ E) (Weed et al. 2011). Field-collected *H. opulenta* initially were brought to CABI EU- CH (Delémont, Switzerland) and then shipped to the insect quarantine laboratory at the University of Rhode Island, Kingston, RI.

In rearing for the host range testing, pupae were overwintered for about 4 mo in plastic jars (473 ml) containing 10 pupae each within incubators set to 4°C. After 4 mo the temperature of the incubators was set to 20°C to initiate adult emergence. One mating pair of newly emerged adults was released into a cage (40 by 40 by 40 cm) containing a potted *Vincetoxicum* plant and a diluted honey solution provided on cotton dental wicks. Five to 10 cages were set up at a time and all cages were kept under ambient laboratory conditions (21–25°C with natural lighting from a large window supplemented with florescent lights on a photoperiod of 16:8 [L:D] h). Each day newly laid eggs were removed from the plant by first wetting them with a fine-tip brush and then gently transferring them to 50-mm petri dishes lined with moistened filter paper. All egg dishes were kept in an insulated box until hatch. Neonates were transferred with a fine-tip brush in batches of 10 to excised leaves of *Vincetoxicum* held in plastic 473-ml jars lined with moistened filter paper and closed with clear, ventilated lids. In every generation we raised ≈150–200 larvae divided equally among *V. nigrum* and *V. rossicum.* Jars were checked daily to remove frass and replenish leaves. Pupae were sexed and then transferred to clean jars partially filled with sterilized vermiculite and held at ambient conditions until overwintering. Adult emergence was monitored for 2–4 wk after pupation because *H. opulenta* undergoes facultative diapause. Larvae and adults used in the host specificity trials originated from bulk samples taken from this culture.

Test Plant List. A test plant list of 58 species comprised of 37 native North American species and 21 introduced taxa of economic importance has been approved by the Technical Advisory Group (TAG) to USDA-APHIS for host specificity testing of *Vincetoxicum* biological control agents (Milbrath and Biazzo, unpublished). Our test plant list contained 53 species from this list and when possible we found suitable representative substitutes for the six species that were not tested. Of these, we could not procure *A. welshii* N. & P Holmgren from the subtribe Asclepiadinae but we did test 15 other species in this subtribe. We did not test the European species *Cynanchum acutum* L. but we did test four other species in the subtribe Cynanchinae (two species native to North America). We acquired *Marsdenia floribunda* (Brongn.) to test as a replacement for *M. edulis* Wats from the tribe Marsdenieae. We could not obtain *Cycladenia humilis* Benth. variety *humilis* or *Amsonia kearneyana* Woods because of scarcity. Both species only exist in remote, desert areas of California and Arizona. We could not locate *Mitreola petiolata* (J.F. Gmel.) Torr. & Gray (Family Loganiaceae) but we did test *Spigelia marilandica* L. as a representative of this family.

As we learned more about the host ranges of other species in the genus *Hypena* (McCabe and Vargas 1998, Kravchenko et al. 2006, Grasswitz and James 2008), we added eight plant species within the families Urticaceae and Cannabaceae. An additional 15 plant species were added to increase the number of different representatives in several TAG plant categories

(http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/tag.pdf) and increase the power of our testing. Seven additional plant species (in Asteraceae and Convulvulaceae) that were procured to address specific requirements of host range testing on other potential biocontrol control agents of *Vincetoxicum* also were screened with *H. opulenta.* The final test plant list consisted of 82 species from 10 families (Table 1). We tested 51 species within the family Apocynaceae, of which, 31 are species native to North America.

Test plants were grown primarily from seed but some field-collected rootstock and vegetative cuttings were used to initiate cultures, depending on the species. Seeds were sown in the greenhouse and transplanted after reaching four to six leaf nodes. Rootstocks were transplanted immediately and cuttings were held in mist tents and transplanted when rooted. All plants were transplanted into 1.9- or 2.8-liter pots containing Metro-mix 510 (Sun Gro Horticulture, Bellevue, WA) and kept in greenhouses or in outdoor raised beds located at the University of Rhode Island in Kingston before testing. Plants were watered as necessary and treated with liquid fertilizer (Peter's Professional All Purpose Plant Food, 20N-20P-20K, Madison, WI) every 2 wk during the growing season. Hardy plants were maintained outdoors over the winter in sawdust-filled raised beds covered with an insulating fabric that was removed in spring. Plants unable to survive winter temperatures were kept in the greenhouse until spring.

No-Choice Larval Development. The host range of *H. opulenta* was measured by monitoring larval feeding and development on leaves of each test plant. Newly emerged larvae were transferred from egg dishes directly to excised leaves of each test plant held in plastic 473-ml jars lined with moistened filter paper and closed with clear, ventilated lids. Feeding and development of 10 larvae was monitored individually in separate jars on each test plant species. Larvae were exposed to foliage clipped from the top three leaf nodes of test plants because *H. opulenta* larvae attack newly expanded leaves in the field (Weed et al. 2011). After larval transfer, jars were checked daily to assess survival, developmental stage, and replenish leaves when necessary. We added a piece of moistened paper towel into jars once larvae reached the L5 stage to serve as a pupation substrate. Trials of nontarget plant species were conducted over a period of 2 yr spanning six generations of *H. opulenta.* Each time a group of nontarget plant species was tested, the survival of 10 larvae was monitored on *V. nigrum* or *V. rossicum* to serve as controls. The proportion of larvae pupating was determined for each test plant and among all replicates of control leaves (*Vincetoxicum*) during the experiment. A test plant was considered outside of the fundamental host range of *H. opulenta* when none of the larvae survived to pupation.

We conducted additional tests to determine if *H. opulenta* larvae would move to other plants in later instars if *Vincetoxicum* were defoliated. In these tests, larvae were reared on *V. nigrum* for two instars and then 10 larvae were transferred to a different nontarget test plant. We chose eight plant species to screen for these tests, which included two species of the subtribe Asclepiadinae (*A. speciosa* Torr., *A. syriaca* L., and *A. verticillata* L.); three species in the subtribe Cynanchinae [*C. ascyrifolium* Matsumura and *C. laeve* (Michx.) Pers.]; two species in the subtribe Gonolobinae [*G. stephanotrichus* Griseb. and *M. gonocarpos* (Walt.) Shinners]; and one species in the family Urticaceae (*Urtica dioica* L.). Each test plant was paired to controls where similar larvae were reared on *Vincetoxicum* leaves. We monitored survival daily and noted any feeding.

Adult Longevity and Fecundity. Female longevity and fecundity were monitored from females emerging from nondiapausing pupae on the target weeds *V. nigrum* and *V. rossicum.* Although larvae completed development on *Vincetoxicum hirundinaria* (Medic.), adult performance was not monitored on reared females because this species is not established in North America (DiTommaso et al. 2005). One newly emerged female and two males were placed into cages (40 by 40 by 40 cm) containing the appropriate potted plant species and a diluted honey solution provided on a cotton dental wick. Cages were held at ambient room conditions of 16–28°C, 15–50% RH, and a photoperiod of 16:8 (L:D) h. Every day plants were removed from the cages to examine the leaves, petioles, and stems for eggs until the female died. We originally monitored the reproduction of 15 and 10 females raised on *V. nigrum* and *V. rossicum*, respectively. However, two females raised on *V. nigrum* and one from *V. rossicum* failed to lay >10 eggs so their data were discarded. Longevity (days alive) and total egg production were compared between host plants by using *t*-tests.

Multiple-Choice Oviposition. Oviposition preference of *H. opulenta* was measured among three *Vincetoxicum* sp. in a multiple-choice design. One potted plant of each *Vincetoxicum* sp. was placed within 15 cages (40 cm^3) and the three pots were set in a triangular arrangement in each cage. The location of each plant species within the cage was randomized among the 15 cages. One female and two males (2–3-d-old) were released into each cage and held at ambient room conditions of 16–28°C, 15–50% RH, and a photoperiod of 16:8 (L:D) h. After 72 h, eggs were counted on each plant. If after 72 h <10 eggs were laid on all plant surfaces, the female was monitored every 24 h until at least 10 eggs were laid. Females that died during the test were replaced and the test was rerun until 15 females were tested. Female preference was measured by comparing the number of eggs laid among plant species by using a linear mixed model. Egg counts were square root transformed before the analysis to meet statistical assumptions and cage was set as a random effect in the model to account for correlated errors within cages (SAS Institute 2009).

Results

No-Choice Larval Development. *Hypena opulenta* only completed successful development on *Vincetoxi-*

Table 1. Results of no-choice larval development testing for *H. opulenta* on test plants

Plant species	Origin[a]	No. larvae tested	Survival to pupation (% mean ± SE)
Family Apocynacaeae			
Subfamily Asclepiadoideae			
Tribe Asclepiadeae			
Target weeds (Subtribe Tylophorinae)			
Vincetoxicum nigrum (L.) Moench	I	190	78.1 ± 4.4
Vincetoxicum rossicum (Kleopow) Barb	I	120	75.0 ± 15.0
Vincetoxicum hirundinaria (Medic.)	I	40	78.9 ± 12.8
Subtribe Asclepiadinae			
Asclepias asperula (Dcne.) Woods.	N	10	0
Asclepias curassavica L.	N	10	0
Asclepias fascicularis Dcne.	N	10	0
Asclepias fruticosa L.	I	10	0
Asclepias incarnata L.	N	10	0
Asclepias hirtella (Pennell) Woodson	N	10	0
Asclepias meadii Torr. Ex Gray	N	10	0
Asclepias purpurascens L.	N	10	0
Asclepias speciosa Torr.	N	10	0
Asclepias sullivanti Engelm. Ex Gray	N	10	0
Asclepias syriaca L.	N	10	0
Asclepias tuberosa L.	N	10	0
Asclepias verticillata L.	N	10	0
Asclepias viridiflora Raf.	N	10	0
Asclepias viridis Walt.	N	10	0
Subtribe Cynanchinae			
Cynanchum ascyrifolium Matsumura	F	10	0
Cynanchum laeve (Michx.) Pers.	N	10	0
Cynanchum marnierianum Rauh	F	10	0
Cynanchum racemosum (Jacq.) Jacq.	N	10	0
Subtribe Gonolobinae			
Matelea carolinensis (Jacq.) Woods.	N	10	0
Matelea decipiens (Alexander) Woods.	N	10	0
Matelea gonocarpos (Walt.) Shinners	N	10	0
Matelea oblique (Jacq.) Woods.	N	10	0
Gonolobus stephanotrichus Griseb.	N	20	0[b]
Subtribe Metastelmatinae			
Funastrum angustifolium (Pers.) Liede & Meve	N	10	0
Funastrum cynanchoides (Dcne.) Schlechter	N	10	0
Metastelma aff. pringlei A. Gray	N	10	0
Metastelma barbigerum Scheele	N	10	0
Subtribe Oxypetalinae			
Araujia sericifera Brot.	I	10	0
Tribe Ceropegieae			
Ceropegia woodii Schltr.	I	10	0
Stapelia gigantea N.E. Br.	I	10	0
Tribe Marsdenieae			
Hoya carnosa (L. f.) R. Br.	I	10	0
Marsdenia floribunda (Brongn.)	N	10	0
Subfamily Periplocoideae			
Periploca graeca L.	I	10	0
Subfamily Apocynoideae			
Tribe Wrightieae			
Nerium oleander L.	I	10	0
Tribe Malouetieae			
Pachypodium lamerei Drake	I	10	0
Tribe Apocyneae			
Apocynum androsaemifolium L.	N	10	0
Apocynum cannabinum L.	N	10	0
Trachelospermum difforme (Walt.) Gray	N	10	0
Trachelospermum jasminoides (Lindl.) Lem.	I	10	0
Trachelospermum mandianum	I	10	0
Subfamily Rauvolfioideae			
Tribe Vinceae			
Amsonia illustris Woodson	N	10	0
Amsonia tabernaemontana Walter	N	10	0
Catharanthus roseus (L.) G. Don.	I	10	0
Vinca minor L.	I	10	0
Tribe Plumerieae			
Allamanda cathartica L.	I	10	0
Plumeria rubra L.	I	10	0

Continued on following page

Table 1. Continued

Plant species	Origin[a]	No. larvae tested	Survival to pupation (% mean ± SE)
Tribe Carisseae			
Carissa macrocarpa (Eckl.) A.DC.	I	10	0
Family Gelsemiaceae			
Gelsemium sempervirens (L.) St. Hil.	N	10	0
Family Gentianaceae			
Bartonia paniculata (L.) B.S.P.	N	10	0
Centaurium erythraea Rafn.	I	10	0
Gentiana andrewsii Griseb.	N	10	0
Gentianella quinquefolia (L.) Small	N	10	0
Family Loganiaceae			
Spigelia marilandica (L.) L.	N	10	0
Family Rubiaceae			
Cephalanthus occidentalis L.	N	10	0
Coffea arabica L.	I	10	0
Galium boreale L.	N	10	0
Gardenia jasminoides J. Ellis.	I	10	0
Hedyotis purpurascens	F	10	0
Houstonia caerulea L.	N	10	0
Houstonia longifolia Gaertn.	N	10	0
Mitchella repens L.	N	10	0
Rubia tinctoria L.	I	10	0
Family Scrophulariaceae			
Buddleja davidii Franch.	I	10	0
Polypremum procumbens L.	N	10	0
Family Asteraceae			
Artemisia absinthium L.	I	10	0
Artemisia caudata (Michx.) H.M. Hall & Clem.	N	10	0
Artemisia ludoviciana Nutt.	N	10	0
Artemisia stelleriana Besser	I	10	0
Artemisia vulgaris L.	I	10	0
Tanacetum vulgare L.	I	10	0
Family Cannabaceae			
Humulus lupulus L. var. "Newport"	I	10	0
Humulus lupulus L. var. "Golden Nugget"	I	10	0
Family Convulvulaceae			
Calystegia (Convulvulus) sepium R. Br.	I	10	0
Family Urticaceae			
Urtica dioica L.	I	20	0[c]
Boehmeria cylindrica (L.) Sw.	N	10	0[d]
Laportea canadensis L.	N	10	0
Parietaria floridana Nutt.	N	20	0
Pilea microphylla (L.) Liebm.	N	10	0
Pipturus albidus	N	10	0
Total species tested		82	

[a] Plant origin: introduced (I), native (N) to North America, or (F) foreign not in North America (Milbrath and Biazzo 2007 or USDA NRCS 2011 Plants Database).
[b] One larva fed but died in the first instar.
[c] One larva fed but died in the second instar.
[d] One larva fed and survived to the final instar (L5) but died before pupation.

cum spp. with survival averaging >75% (Table 1). One larva developed to the second instar on *U. dioica.* One larva survived to the final instar on *Boehmeria cylindrica* but died before pupation. An additional 10 larvae were screened for development on these species, but none fed or developed past the first instar in these follow-up trials.

In supplemental transfers with third instars, some larvae fed on *A. syriaca* (60%), *Cynanchum racemosum* (Jacq.) Jacq. (50%), and *C. laeve* (Michx.) Pers. (30%), but all larvae died in <6 d without further development. No feeding was observed on *A. verticillata* L., *C. ascyrifolium* Matsumura, *Matelea gonocarpos* (Walt.) Shinners, *G. stephanotrichus,* and *U. dioica.* All larvae transferred to *Vincetoxicum* (control group) completed development to pupation in these trials.

Adult Longevity and Fecundity. Female longevity was similar when larvae had been raised on *V. nigrum* and *V. rossicum* ($t = 0.59$; df = 22; $P = 0.281$) (Table 2). Longevity extended to 36 d and about half of the females were still alive after 15 d (Fig. 1a). The preoviposition period was similar between hosts ($t = 0.89$; df = 20; $P = 0.193$) and oviposition typically began within 3 d after emergence but in some cases took as many as 10 d (Table 2). Daily egg production was similar for females raised on each host ($t = 0.10$; df = 22; $P = 0.459$) (Table 2). Newly-emerged females reached peak oviposition within a few days and continued ovipositing until death, although daily egg production declined over time (Fig. 1b). By 10 d, females had laid 74% of their eggs, and by 20 d, 98% of all eggs were laid.

Table 2. Longevity and fecundity of *H. opulenta* raised on *Vincetoxicum*

Plant species	n^a	Longevity (days)		Preoviposition (days)		No. eggs per female per day	
		Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Vincetoxicum nigrum	13	16.7 ± 2.4	4–36	4.1 ± 0.7	2–10	17.6 ± 2.7	5.6–36.0
Vincetoxicum rossicum	9	14.9 ± 1.5	7–23	3.1 ± 0.6	2–8	17.1 ± 3.3	5.4–32.9

No variables differed significantly between plant species (*t*-test: $P > 0.05$).
[a] Number of females tested.

Multiple-Choice Oviposition. Two of 15 females failed to produce eggs within the 72-h period. Females did not display an oviposition preference among the *Vincetoxicum* spp., laying an average of 18.8 ± 4.1 eggs per host plant (mean ± SE) ($F = 0.72$; df = 2, 24; $P = 0.496$).

Discussion

The range of plants suitable for larval development by *H. opulenta* was measured in quarantine to determine whether this moth is adequately host specific to be considered as a potential biological control agent of *Vincetoxicum* in North America. *Hypena opulenta* only completed larval development on species in the target weed genus *Vincetoxicum.* Minimal feeding occurred on *U. dioica* (Urticaceae) and *G. stephanotrichus* (Apocynaceae), but larvae never developed past the first and second instar on these species, respectively. We were able to raise one larva to the final instar on *B. cylindrica* (Urticaceae), but this individual died before pupation. We do not consider *B. cylindrica* to be at risk of attack by *H. opulenta* for the following reasons: 1) we have never observed oviposition on *B. cyclindrica* under no-choice conditions in the laboratory, but females readily accept *Vincetoxicum* under these conditions (A. W., unpublished data); and 2) supplemental screening with L1 (30 larvae) and L5 (10 larvae) corroborated the results of our standardized larval development tests—some individuals initially accept *B. cylindrica* for feeding, but the majority die as L1 and never successfully pupate (no larvae developed past L2 in these tests).

Hypena is primarily a tropical genus with relatively few temperate species (Arnett 2000, Kravchenko et al. 2006) and is placed with the subfamily Hypeninae, which was recently moved from the family Noctuidae and assigned to the new family Erebidae (Fibiger and Lafontaine 2005, Lafontaine and Fibiger 2006). Members of the Hypeninae are considered to be monopha-

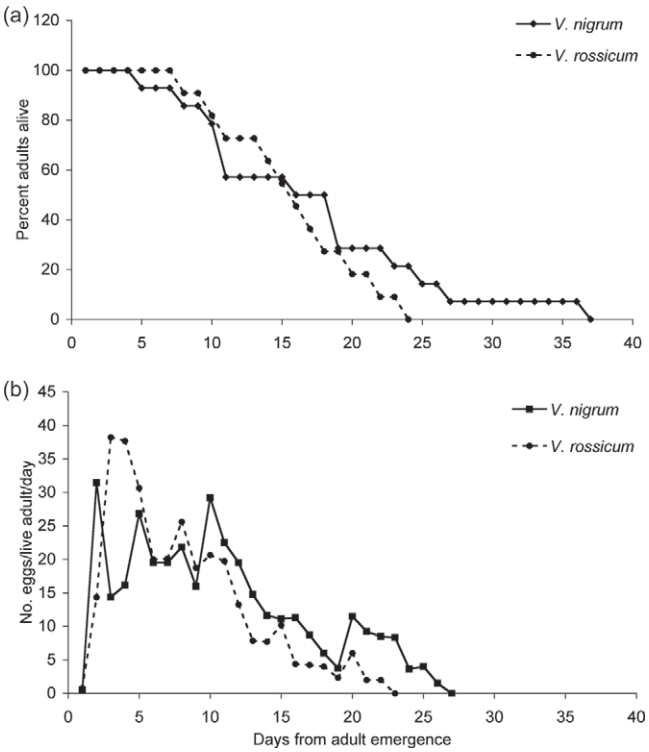

Fig. 1. Daily (a) proportion of surviving adults and (b) mean egg production of *H. opulenta* raised on *V. nigrum* and *V. rossicum.* Mean error bars in (b) are excluded to improve clarity.

gous or oligophagous and closely associated with Urticaceae (McCabe and Vargas 1998, Wagner et al. 2011). However, many species attack a variety of trees (McCabe and Vargas 1998, Wagner et al. 2011) and the green cloverworm, *H. scabra* (F.), is an important pest of a variety of crops (Pedigo et al. 1973). *Hypena laceratalis* Walker feeds on *Lantana camara* L. (Verbenaceae) and has been released as a biological control agent against this weed in Hawaii, Indonesia, and South Africa (Day and Neser 2000). Specialization on Apocynaceae species (milkweeds) is apparently uncommon for this genus and subfamily or remains poorly studied. We are aware of only two Asian species that attack milkweeds in addition to *H. opulenta* (Sridhar and Rani 2003, Kravchenko et al. 2006). Like *H. opulenta*, both species are reported attacking members of the Palearctic subtribe Tylophorinae (Sridhar and Rani 2003, Kravchenko et al. 2006), which includes *Vincetoxicum* (target weeds) (Liede 1996). To our knowledge, Nearctic *Hypena* do not attack Apocynaceae.

We did not measure larval performance among acceptable hosts, but an earlier study has demonstrated that larval performance is similar between the target weeds *V. nigrum* and *V. rossicum* (Weed et al. 2011). In our tests, longevity and fecundity of females raised on *V. nigrum* and *V. rossicum* were similar. This finding also is in accordance with an earlier study observing similar ovariole development of unmated, 7-d-old females that were raised on *V. nigrum* and *V. rossicum* (Weed et al. 2011). In the laboratory, *H. opulenta* females are relatively long-lived and peak oviposition occurs soon after adult emergence with a steady decline in daily egg production over time, similar to *H. humuli* on hops (Grasswitz and James 2008). We also observed that gravid females do not discriminate between the target weeds in cages. Collectively, our results indicate that both target weeds should be attacked in the field unless other factors such as habitat preferences prevent *H. opulenta* from contacting either target weed in North America. This moth has only been collected from forested sites so it is possible that *H. opulenta* will only attack forested populations of *Vincetoxicum* (Weed et al. 2011).

Hypena opulenta has a number of traits that make it an attractive biological control agent for swallow-worts in addition to the demonstrated host specificity. Impact studies with *H. opulenta* indicate that it only takes one generation to significantly reduce vegetative and reproductive growth of *V. rossicum* (Weed and Casagrande 2010). This species has the potential to produce multiple, overlapping generations under field conditions (Weed and Casagrande 2010) and recent herbivory simulations conducted in the field suggest *H. opulenta* may have its greatest impact in the shade on *V. rossicum* (Doubleday and Cappuccino 2011). Given the severity of current swallow-wort infestations and lack of effective control measures, we have petitioned for the open-field release of *H. opulenta* as a biological control agent for *V. rossicum* and *V. nigrum* in the United States and in Canada.

Acknowledgments

André Gassmann and Robert Bourchier provided helpful comments for finalizing and submitting the release petition. We also thank Nicholas Larghi, Kerry Barton, Elwood Roberts, Elena Olsen, Jennifer Mulcahy, and Theresa Murphy for their assistance in data collection. We thank John Row, John D. Byrd Jr., Mike Bordelon, Hope Leeson, Nancy J. Bisset, and Lindsey Milbrath for providing plant material and two anonymous reviewers for improving the manuscript. This work was funded by the USDA Northeast integrated pest management (IPM) program and the University of Rhode Island College of Environment and Life Sciences Agricultural Experiment Station (Contribution 5297).

References Cited

Arnett, R. A. 2000. American insects: a handbook of the insects of America north of Mexico. CRC, Boca Raton, FL.

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

Cappuccino, N. 2004. Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). Oikos 106: 3–8.

Christensen, T. 1998. Swallow-worts, the ecology and control of *Vincetoxicum* spp. Wildflower 14: 21–25.

Day, M. D., and S. Neser. 2000. Factors influencing the biological control of *Lantana camara* in Australia and South Africa, pp. 897–908. *In* N. R. Spencer (ed.), Proceedings of the X International Symposium on Biological Control of Weeds, 4–14 July 1999, Montana State University, Bozeman, MT. CABI Publishing, Wallingford, UK.

DiTommaso, A., F. M. Lawlor, and S. J. Darbyshire. 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]. Can. J. Plant Sci. 85: 243–263.

Doubleday, L.A.D., and N. Cappuccino. 2011. Simulated herbivory reduces seed production in *Vincetoxicum rossicum.* Can. J. Bot. 89: 235–242.

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

Fibiger, M., and J. D. Lafontaine. 2005. A review of the higher classification of the Noctuoidea (Lepidoptera) with special reference to the Holarctic fauna. Esperiana 11: 7–92.

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

Kravchenko, V., O. Orlova, M. Fibiger, J. Mooser, C. Li, and G. C. Muller. 2006. The Acronictinae, Bryophilinae, Hypenodinae and Hypeninae of Israel (Lepidoptera: Noctuidae). Sociedad Hispano-Luso-Americana de Lepidopterología, Rev. de Lepidopterología 34: 255–264.

Lafontaine, J. D., and M. Fibiger. 2006. Revised higher classification of the Noctuoidea (Lepidoptera). Can. Entomol. 138: 610–635.

Lawlor, F. M. 2000. Herbicidal treatment of the invasive plant *Cynanchum rossicum* and experimental post control restoration of infested sites. M.S. thesis, State University of New York, Syracuse.

Liede, S. 1996. *Cynanchum - Rhodostegiella - Vincetoxicum - Tylophora* (Asclepiadaceae): new considerations on an old problem. Taxon 45: 193–211.

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

McCabe, T. L., and V. Vargas. 1998. The larva of *Hypena manalis* (Lepidoptera: Noctuidae: Hypeninae). J. N.Y. Entomol. Soc. 106: 109–113.

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

Milbrath, L. R. 2010. Phytophagous arthropods of invasive swallow-wort vines (*Vincetoxicum* spp.) in New York. Environ. Entomol. 39: 68–78.

Ostapko, V. 1995. Genus *Vincetoxicum* N. M. Wolf in the south-east of Ukraine. Ukr. Bot. J. 52: 388–394.

Pedigo, L. P., J. D. Pedigo, and G. L. Lentz. 1973. Biological synopsis of the green cloverworm in central Iowa. J. Econ. Entomol. 66: 665–673.

Pobedimova, E. 1952. Family CXXXIII Asclepiadaceae Lindl., pp. 487–527. *In* B. Shishkin and E. Bobrov (eds.), Flora of the U.S.S.R., vol. 18. Metachlamydeae. Translation by N. Landau, 1967, Israel Program for Scientific Translations Ltd., Jerusalem, for The Smithsonian Institution and The National Science Foundation, Washington, DC.

SAS Institute. 2009. JMP version 8. SAS Institute, Cary, NC.

Sheeley, S. E., and D. J. Raynal. 1996. The distribution and status of species of *Vincetoxicum* in eastern North America. Bull. Torrey Bot. Club 123: 148–156.

Sridhar, V., and B. Rani. 2003. New record of *Canthecona parva* (Distant) (Heteroptera: Pentatomidae) as a predator of *Hypena quadralis* (Walker), a pest of Antamul, *Tylophora indica* (Burm. f) Merr. J. Biol. Control 17: 179–180.

Tewksbury, L., R. A. Casagrande, and A. Gassmann. 2002. Swallow-worts, pp. 209–216. *In* R. Van Driesche, S. Lyon, B. Blossey, M. Hoddle, and R. Reardon (eds.), Biological control of invasive plants in the eastern United States. U.S. Dep. Agric. Forest Service Publication FHTET-2002–04, Morgantown, WV.

[USDA NRCS] U.S. Department of Agriculture Natural Resources Conservation Service. 2011. The PLANTS Database. National Plant Data Center, Baton Rouge, LA. (http://plants.usda.gov).

Wagner, D. L., D. F. Schweitzer, J. B. Sullivan, and R. C. Reardon. 2011. Owlet caterpillars of eastern North America (Lepidoptera: Noctuidae). Princeton University Press, Princeton, NJ.

Weed, A. S., and R. A. Casagrande. 2010. Biology and larval feeding impact of *Hypena opulenta* (Christoph) (Lepidoptera: Noctuidae): a potential biological control agent for *Vincetoxicum nigrum* and *V. rossicum*. Biol. Control 53: 214–222.

Weed, A. S., A. Gassmann, A. M. Leroux, and R. A. Casagrande. 2011. Performance of potential European biological control agents of *Vincetoxicum* spp. with notes on their distribution. J. Appl. Entomol. 135: 700–713.

Received 28 March 2012; accepted 14 June 2012.