

## Project Summary

This is a research project. Two species of European swallow-worts, *Vincetoxicum nigrum* (black) and *V. rossicum* (pale) have invaded the USA and are spreading throughout the Northeast and beyond. Swallow-worts contain a haemolytic glycoside which is toxic to grazing animals. In addition to pastures, swallow-worts invade gardens, lawns, shrubs, hedgerows, forests, ornamental plant nurseries, Christmas tree farms, and pine plantations. The twining climbing plants smother plants, serve as alternate hosts for *Cronartium* rusts of pines, and induce monarch butterflies to lay eggs on these plants where larvae cannot survive.

Swallow-worts are difficult to control mechanically or chemically and native herbivores have little impact. In response to stakeholder requests, we initiated a biological control program on swallow-worts and in 2006 we collected insect herbivores throughout Europe. We presently have five European insect herbivores of swallow-worts in quarantine where we have made good progress on host range testing and already identified two agents as particularly promising. We seek support to continue host range testing of promising natural enemies and to measure their impact on the target weeds toward the goal of bringing North American swallow-worts under biological control.

**The objective of this proposal is to evaluate the host range and potential impact of European insect herbivores under consideration for biological control of swallow-worts in North America.** We anticipate giving several research presentations, submitting five journal articles, and preparing a petition for biological control agent release during the funding period covered in this proposal.

This multi-disciplinary, international research project meets NEREAP-IPM goal 3: “..... reduce unreasonable adverse environmental effects from pests and pest management practices.” As serious weeds in ornamental nurseries and Christmas tree plantations, swallow-worts qualify under NEREAP-IPM priority one for 2006 and priority 3 (minor crop) for 2007.

## Problem

### Distribution

There are two swallow-wort species widely distributed in the USA. Black swallow-wort *Vincetoxicum nigrum* (synonym: *Cynanchum louiseae* L.) is found from Maine through Kansas and in California (Fig. 1) (Tewksbury et al. 2002). This European perennial herbaceous vine is commonly found in disturbed areas, pastures, roadsides, and forest margins (DiTommaso and Losey 2003). Pale swallow-wort *Vincetoxicum rossicum* (synonym: *Cynanchum rossicum* [Klepow] Borhidi) is also commonly known as dog-strangler vine. Pale swallow-wort, native to Ukraine and Russia, has a growth pattern and habitat preference similar to *V. nigrum*, and a discontinuous distribution that extends from the Great Lakes through New England and the Mid-Atlantic States (Fig. 1) (Tewksbury et al. 2002). Both swallow-wort species are considered highly invasive in North America where they out-compete and displace native plant species, including milkweeds (Haribal and Renwick 1998, DiTommaso and Losey 2003). The earliest record of *V. nigrum* in the USA is from Massachusetts in 1854 and *V. rossicum* was recorded in NY in 1897 (Sheeley and Raynal 1996).

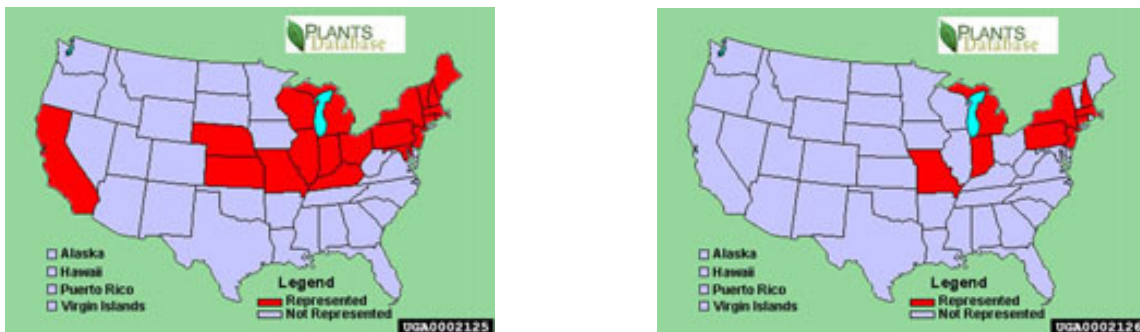


Figure 1. Distribution of Black swallow-wort (left) and Pale swallow-wort (right) in the USA (Plants Database).

### Ecological and Economic Impact

The introduction, spread, and resulting ecological damage caused by non-native invasive plants and animals not only threaten worldwide biodiversity and agricultural production, but also generate a variety of unforeseeable economic and political repercussions. Currently, the invasion of exotic species ranks second behind habitat fragmentation as the largest threat to biodiversity.

The European swallow-wort species, *Vincetoxicum nigrum* and *V. rossicum* are spreading throughout the northeastern USA, displacing native vegetation, altering native ecological processes, threatening endangered species, and becoming important pests in agriculture and natural areas. Both species have adverse impacts upon monarch butterflies, inducing oviposition upon plants where larvae cannot survive (Casagrande and Dacey 2007, Tewksbury et al. 2002). Additionally, they may be reducing monarch host plant

availability through competitive displacement (DiTommaso et al. 2005). They also serve as alternate hosts for *Cronartium* Fr. rusts attacking species of *Pinus* L. (DiTommaso et al. 2005).

The roots of swallow-worts contain the haemolytic glycoside vincetoxin which is toxic to humans and other mammals, resulting in reduced grazing and/or toxicity (including death) to grazing animals (DiTommaso et al. 2005). In addition to pastures, pale swallow-wort invades gardens, lawns, shrubs, hedgerows, fencerows, a variety of deciduous and mixed forest types and pine plantations (DiTommaso et al. 2005). Old field sites colonized by pale swallow-wort have fewer arthropods than comparable sites without this invasive species (Ernst and Cappuccino 2005). Dense stands of this species also discourage grasslands birds from nesting in summer (DiTommaso et al. 2005). Pale swallow-wort has recently been observed in no-till corn and soybean fields in several counties in central and western NY and may pose a management problem for these cropping systems. Some landowners in NY have abandoned horse pastures after control of pale swallowwort proved unsuccessful over a 5-10 year span (DiTommaso et al. 2005). The twining climbing plants adversely impact other plants (Fig. 2), smothering small trees in Ontario restoration sites and possibly reducing forest regeneration in New York (DiTommaso et al. 2005). Black swallow-wort is threatening the endangered Jessop's milkvetch, *Astragalus robbinsii* at Windsor, Vermont (DiTommaso et al. 2005).



Fig. 2 Black swallow-wort overgrowing trees, shrubs, and milkweed on Prudence Island, RI.

These plants are able to thrive under a wide range of environmental conditions. They are too widespread for eradication and control is difficult because we lack cost-effective, non-toxic methods to manage infestations or prevent spread into new areas. Swallow-worts are a particular challenge for managers of protected habitats since funding is typically limited for management and infestations need to be treated with repeated herbicide applications. Native biodiversity is becoming permanently degraded due to these invasive plants.

## Background

### Stakeholder Needs Assessment

Our swallow-wort biological control efforts were begun at the request of Don and Heather Minto of Watson Farm, (Jamestown RI) whose pastures became overrun with black swallow-wort following our successful program of Cypress Spurge biological control in their fields (Faubert and Casagrande 2002). As we have distributed Cypress Spurge natural enemies to other pastures (at owners request), we have noted increasing problems with black swallow-wort. Of the 53 pastures we have sampled throughout RI, 20 have black swallow-wort infestations including 6 of 12 horse pastures surveyed in the state.

This project meets goal 3 of the NE Regional IPM program: “..... reduce unreasonable adverse environmental effects from pests and the use of pest management practices.”

As serious weeds in ornamental nurseries and Christmas tree plantations, swallow-worts qualify under NEREAP-IPM priority one for 2006 and priority 3 (minor crop) for 2007.

Further, this research conforms to the General IPM Priorities for the Northeast (November, 2004) items 5 and 6 (of 16): weed management in nurseries.

And it particularly addresses the 2006 Livestock/Field Crop IPM Priorities for New York State: “..... and poisonous/noxious plants affecting cattle on pasture”

### Potential for Biological Control

The primary hypothesis explaining the success of non-native organisms in newly invaded habitats is the absence of co-evolved natural enemies. Surveys conducted in Rhode Island and New York indicate that no native insects or other organisms in the United States are causing significant damage to these plants. This is likely due to their toxic alkaloids which protect them from herbivory. Swallow-worts in North America have escaped their specialized herbivores in Europe and are thriving in enemy-free space. The species, *Vincetoxicum hirundinaria*, *V. nigrum*, and *V. rossicum* occur in Europe, but they are restricted to discrete (normally shady) habitats and are not pests in pastures, agricultural fields, or natural areas. The goal of our biological control program is to reacquaint these plants with the effective natural enemies that they left behind in Europe. **Since there are no closely-related native plants in North America, swallow-worts are good candidates for Classical Biological Control.**

### Research Progress to Date

This project got underway in 2000 and 2001 when Casagrande (URI), DiTommaso (Cornell) and Gassmann (CABI, Delémont Switzerland) prepared two proposals on biological control of swallow-worts for submission to NRI. Although neither project was funded, the literature work served as the basis for a publication on this topic (Tewksbury et al. 2002). Since then CABI colleagues have continued to study the literature and conduct limited surveys of swallow-worts and their natural enemies in Europe. This set the stage for the research of Aaron Weed, Ph.D. candidate working under Casagrande. Aaron spent four months during the 2006 field season working in Europe (at his own expense) with André Gassmann and other colleagues at CABI. By tagging along on other funded research projects, Aaron was able to travel extensively,

working with plant taxonomists and insect ecologists throughout Europe (see collaborators, below) and he has mapped out host plant and insect herbivores throughout much of Western Europe.

He collected and transported insects to the laboratory in Delémont for initial studies on biology and host range, including three *Vincetoxicum* species and a few putative host plants from North America. Initial rearing procedures were also developed in this lab and the insects were prepared for shipment to the URI. Plant and insect specimens were given to taxonomic specialists for species confirmation and deposited in recognized reference collections. Through this process, five species were determined to be promising enough for future investigation and they were delivered (under the proper authority) to the URI Biological Control Quarantine Laboratory where they are presently under study to determine if any have adequate host specificity and promise for release in North America. Results of the past 15 months of research are summarized in the paragraphs below and in Table 1.

#### Swallow-wort herbivores in URI Quarantine (11/07)

##### 1) *Eumolpus asclepiadeus* Pallas (Chrysomelidae)

This species is distributed throughout Central to Eastern Europe and most likely into Asia and is only reported developing on *Vincetoxicum hirundinaria*. It was collected in Kiev, Ukraine and at 4 locations in the Ticino region of Switzerland exclusively in open habitats. The life cycle can be completed in one year, but our observations have suggested that the majority of larvae take over one year to complete development. The factors controlling the length of the larval period will be studied further. Adults typically emerge starting mid-June and are present until early September in Switzerland. Females lay egg clutches at the shoot base just under the soil surface and cover the eggs in frass. Larvae emerge roughly 10 to 12 days later and immediately begin digging. The larvae feed externally on the roots and concentrate feeding on the primary roots. In 2006, a small percentage of larvae transferred to potted *V. hirundinaria*, *V. nigrum*, and *V. rossicum* successfully developed to adults in 2007. Dissections made in 2007 of a proportion of the pots setup during 2006 determined that larvae were still developing one year after transfer and the majority were 4<sup>th</sup> or 5<sup>th</sup> instars. Eggs were collected from the adults that emerged in the summer of 2007, thus confirming that this species can successfully reproduce on the target weeds. Resulting larvae were transferred to a number of North American species in 2007 to determine host range and assess potential plants at risk (Table 1). The results of this test are anticipated by December 2007, when pots will be dissected to assess feeding behavior and larval survival.

##### 2) *Chrysolina aurichalcea (asclepiadis) ssp. bohémica* (Gebler) (Chrysomelidae)

There are many similar species within this species complex that extends from Central Europe throughout Asia to Japan. One author suggests that *C. aurichalcea* is from the eastern Palearctic (*aurichalcea*) whereas *asclepiadis* is restricted to the western Palearctic. The host range of this species complex is also not well understood. Our colony originated from adult collections made during early September from 5 locations in the Ticino region of Switzerland on *V. hirundinaria*. After one year of testing with this species, we have determined that the larvae are leaf feeders and appear to feed gregariously as 1<sup>st</sup> instars. Larvae were offered a number of test plants (see Table 1) and

at 18°C, the larvae that survived took an average of 27 d to complete development on *Vincetoxicum* spp. through 4 instars. The larvae also developed successfully on *Artemisia* spp. and *Asclepias tuberosa*, but their survival was much lower than those individuals raised on *Vincetoxicum* spp. There was no larval development or feeding on the other plant species tested. Mature larvae excavate a cavity below ground and enter the prepupal stage for roughly 1 week and then pupate for another 1 to 2 weeks. Adults emerge and begin feeding immediately. Females begin laying eggs after 6d when fed the target weeds but those females raised as larvae and fed plants outside the genus *Vincetoxicum* never matured their ovaries. It appears that *Artemisia* and *A. tuberosa* may be at risk of feeding by the adults. Future assays will be designed to determine whether the adults and larvae accept either *Artemisia* or *Asclepias* when in the presence of *Vincetoxicum*. However, because this is the only insect so far that we have observed feeding outside of the genus *Vincetoxicum*, we are prioritizing studies with the other insect candidates.

### 3) *Euphranta connexa* (Fabricius) (Tephritidae)

This species has a distribution similar to *E. asclepiadeus*, but extends into Scandinavia as well. Larvae infest seedpods of *V. hirundinaria* at high densities. During 2006, Aaron found this species in Donetsk and Kiev, Ukraine and at seven locations in Switzerland. Typically, populations were located in open habitats with dense populations of *V. hirundinaria* similar to *E. asclepiadeus*. However, one population was located infesting the seedpods at a forested site in Moutier, Switzerland. The adults were active in mid-June in Ukraine and from late June to early July in Switzerland. Aaron collected adults of this species, but mainly concentrated on collecting infested seedpods. Typically, 3 or 4 larvae infested the seedpods, but as many as 7 were found in one pod. The larvae completely destroy the seed embryo and after reaching the 3<sup>rd</sup> (?) instar they dig out of the seedpod and pupate within the soil to overwinter.

During 2007, we were unable to work with this species in quarantine due to high pupal mortality or because pupae require an additional year to develop. However, colleagues in Europe collected hundreds of additional pupae, which will be shipped to us next year for testing. Although host range testing was initiated in 2007, our EU colleagues were able to determine that *E. connexa* will infest pods of *V. rossicum* under common garden conditions. We also suspect that this species will accept *V. nigrum* as well. We will continue to evaluate the potential of this species regardless of laboratory rearing difficulties because it infests such a high percentage of seedpods in the field. This work may have to be supplemented with field-collected individuals every year.

### 4) *Abrostola asclepiadis* (Denis & Schiffermüller) (Noctuidae)

This species also has a distribution similar to *E. asclepiadeus*. In southern Europe there are reports of two generations per year, but one is more typical. In 2006, larvae or eggs were collected in Kiev and Donetsk, Ukraine, Pottenstein, Germany, and Moutier, Switzerland and empty eggshells and larval damage were observed at one location in Ticino, Switzerland. Our surveys in 2006 determined that this species was the most common leaf-feeding insect on *V. hirundinaria*, but populations were never very high. Damage to the plant is also small and concentrated to the lower leaves.

In 2007, hundreds of larvae were offered a variety of North American test plants and three *Vincetoxicum* spp. (Table 1). Larval development and feeding were only observed on the three *Vincetoxicum* spp. while the other test plants were never accepted. At 23°C, all larvae took on average 17 d to complete development and ate roughly 98cm<sup>2</sup> to reach pupation although this varied slightly among hosts. Pupal weight was higher for larvae reared on *V. nigrum* compared to all other host plants suggesting that females may be more fecund when reared on this host. We will begin evaluating how each host plant influences the fecundity of the resulting females next season. Due to the specificity of larvae, the ability of this species to persist in open and forested habitats, and its ability to develop on both target weeds we will begin evaluating the realized impact of this species on the growth and reproduction of the target weeds.

#### 5) *Hypena opulenta* (Christoph) (Noctuidae)

This species was collected in the Donetsk region of Ukraine at two locations and is reported from Ukraine, Turkey, Iran and Turkmenistan. After the original field collection this species has been reared in the laboratory and during this time we have been able to conduct extensive feeding and oviposition testing. In 2007, larvae were offered excised leaves of the test plants and only successfully developed to pupae on *Vincetoxicum* spp. (Table 1). No larvae ever fed on the test plants other than *Vincetoxicum*. The larvae took approximately 2 weeks at 25°C to develop and another 2 weeks for adults to emerge. Larvae developed faster and attained greater pupal weights when raised on *V. nigrum* and *V. rossicum* compared to larvae provided *V. hirundinaria*. Oviposition occurs roughly 2d after emergence and is concentrated on the undersides of the leaves. In the laboratory, females have survived up to 35d and can lay up to 600 eggs, but on average lay 365 eggs per female. Female fecundity is similar between females raised on both target weeds. This species has great potential as a biocontrol agent due to multiple generations per year, specificity to *Vincetoxicum*, and ability to inhabit forest edge and interior sites.

Table 1. Results of no-choice larval development testing on the target weeds and a subset of the TAG plants for each insect herbivore. Successful larval development is indicated by '+' and unsuccessful development by '-'. Plants that have not been tested, but will be in 2008 are designated by 'not tested' and those plants specified as 'ongoing' will be completed by December 2007.

Plant Species	Potential insect biological control agent				
	<i>Abrostola asclepiadis</i>	<i>Chrysolina aurichalcea</i>	<i>Eumolpus asclepiadeus</i>	<i>Euphranta connexa</i>	<i>Hypena opulenta</i>
<b>Target weeds (Subtribe Tylophorinae)</b>					
<i>Vincetoxicum nigrum</i> (L.) Moench	+	+	+	not tested	+
<i>Vincetoxicum rossicum</i> (Kleopow) Barb	+	+	+	+	+
<b>Family Asclepiadaceae</b>					
<b>Subtribe Asclepiadinae</b>					
<i>Asclepias curassavica</i> L.	-	-	not tested	not tested	-
<i>Asclepias fascicularis</i> Dcne.	-	-	-	not tested	-

Plant Species	Potential insect biological control agent				
	<i>Abrostola asclepiadis</i>	<i>Chrysolina aurichalcea</i>	<i>Eumolpus asclepiadeus</i>	<i>Euphranta connexa</i>	<i>Hypena opulenta</i>
<i>Asclepias fruticosa</i> L.	-	-	-	not tested	-
<i>Asclepias incarnata</i> L.	-	-	-	not tested	-
* <i>Asclepias speciosa</i> Torr.	-	-	-	not tested	-
<i>Asclepias syriaca</i> L.	-	-	-	not tested	-
* <i>Asclepias tuberosa</i> L.	-	+	-	not tested	-
* <i>Asclepias viridiflora</i> Raf.	-	-	-	not tested	-
<b>Subtribe Cynanchinae</b>					
<i>Cynanchum laeve</i> (Michx.) Pers.	-	-	ongoing	not tested	-
<b>Subtribe Gonolobinae</b>					
<i>Gonolobus suberosa</i> (L.)	-	-	ongoing	not tested	-
<b>Subtribe Metastelmatinae</b>					
<i>Funastrum angustifolium</i> (Pers.) Liede & Meve	-	-	not tested	not tested	-
<b>Subtribe Oxypetalinae</b>					
<i>Araujia sericifera</i> Brot.	-	-	ongoing	not tested	-
<b>Tribe Ceropegieae</b>					
<i>Ceropegia woodii</i> Schltr.	-	-	ongoing	not tested	-
<i>Stapelia gigantea</i> N.E. Br.	-	not tested	not tested	not tested	-
<b>Tribe Marsdenieae</b>					
<i>Hoya carmosa</i> (L. f.) R. Br.	-	-	ongoing	not tested	-
<b>Subfamily Periplocoideae</b>					
<i>Periploca graeca</i> L.	-	-	ongoing	not tested	-
<b>Subfamily Apocynoideae</b>					
<b>Tribe Wrightieae</b>					
<i>Nerium oleander</i> L.	-	-	ongoing	not tested	-
<b>Tribe Malouetieae</b>					
<i>Pachypodium lamerei</i> Drake	-	not tested	not tested	not tested	-
<b>Tribe Apocyneae</b>					
<i>Apocynum cannabinum</i> L.	-	-	ongoing	not tested	-
<i>Trachelospermum</i> sp.	-	-	ongoing	not tested	-
<b>Subfamily Rauvolfioideae</b>					
<b>Tribe Vinceae</b>					
<i>Amsonia tabernaemontana</i> Walter	-	-	ongoing	not tested	-
<i>Vinca minor</i> L.	-	-	-	not tested	-
<b>Tribe Plumerieae</b>					
<i>Allamanda cathartica</i> L.	-	-	ongoing	not tested	-
<b>Tribe Carisseae</b>					
<i>Carissa macrocarpa</i> (Eckl.) A. DC.	-	not tested	not tested	not tested	not tested
<i>Carissa grandiflora</i>	-	-	ongoing	not tested	-

Plant Species	Potential insect biological control agent				
	<i>Abrostola asclepiadis</i>	<i>Chrysolina aurichalcea</i>	<i>Eumolpus asclepiadeus</i>	<i>Euphranta connexa</i>	<i>Hypena opulenta</i>
<b>OUTGROUPS</b>					
<b><u>Family Loganiaceae</u></b>					
<i>Spigelia marilandica</i> (L.) L.	-	-	ongoing	not tested	-
<b><u>Family Gelsemiaceae</u></b>					
<i>Gelsemium sempervirens</i> (L.) St. Hil.	-	-	ongoing	not tested	-
<b><u>Asteraceae</u></b>					
<i>Artemisia absinthium</i> L.	-	not tested	not tested	not tested	-
<i>Artemisia ludoviciana</i> Nutt.	-	+	not tested	not tested	-
<b><u>Convulvulaceae</u></b>					
<i>Calystegia (Convulvulus) sepium</i> R. Br.	not tested	not tested	not tested	not tested	-

In addition the research described above, we have also entered into three collaborative research arrangements to assure an expeditious and quality evaluation of these potential biological control agents. In addition to the collaborative research with André Gassmann of CABI in Delémont, Switzerland, we are working with Lindsey Milbrath, a USDA/ARS scientist stationed at Cornell University and Naomi Cappuccino at Carlton University in Ontario – all of whom will be participating in host specificity testing of potential swallow-wort biological control agents (see Collaborative Arrangements). This frees Aaron Weed, who has already completed much host range testing, to focus more attention on herbivore impact studies that will determine which of these (apparently host specific) natural enemies would be the most effective candidates for release.

## Justification

### Multi-state Benefit

Swallow-wort species are distributed throughout the northeast (and Canada) and a successful biological control program against these weeds will benefit the entire region.

### Importance

From an environmental stewardship perspective, expanding swallow-wort populations represent a threat to populations of monarch butterflies and several threatened and endangered plant species. In agriculture, they represent one of the key weed species in commercial nursery production – the number one crop in Rhode Island, valued at 109 million dollars in RI and approximately a billion dollars in New England. Invasive weed problems in nurseries are particularly troublesome since if left uncontrolled, they get transplanted into landscapes throughout the region. Swallow-worts are serious pasture pests and pests of Christmas tree plantations throughout the northeast. Surveys of Rhode Island pastures (cited above) indicate that 38% have black swallow-wort infestations. Additional problems with swallow-worts are discussed under Ecological and Economic Impact (above).

### Management alternatives

Herbicides provide some control of mature plants, but they are expensive and impractical for use over large areas where selective management of invasive species is desired. Christensen (1997) found that three applications of glyphosate were needed per year to reduce pale swallow-wort cover by over 90%. Lawlor and Raynal (2002) also found that repeated applications of glyphosate or triclopyr were needed to suppress this plant. A large seed bank may also greatly affect the cost and effectiveness of herbicide use against swallow-worts (DiTommaso et al. 2005). Mowing, cultivation, grazing, and trampling do not reduce cover of pale swallow-wort (Christensen 1998, DiTommaso et al. 2005).

### Need for intervention

Although swallow-worts have been in the northeast for over a century, the distribution of these plants and the problems that they cause are rapidly expanding with no evidence that they are approaching their maximum geographic or ecological distribution (DiTommaso et al. 2005). Indeed, “spread of these two [*Cynanchum*] species is expected to increase exponentially as more colonies establish, coalesce and become seed sources” (DiTommaso et al. 2005). Bjeruke (2006) has noted a similar exponential increase in pale swallow-wort in Norway in the past two decades where the plant, introduced in 1865, has gone from being a “rare botanical curiosity” to “an invasive threat to indigenous vegetation”. Again, quoting DiTommaso et al. (2005) on the North American situation: “In addition to the loss of floral and faunal biodiversity in native and semi-native communities, various perennial cropping systems are at risk from the increased costs associated with control. Agroecosystems, such as pastures, perennial forage crops, tree nurseries, sugar maple woodlots, vineyards and orchards, are likely to be vulnerable.” DiTommaso et al. (2005) conclude their 20-page monograph with the statement: “Given the difficulties of control, perhaps the most effective single means of slowing spread and reducing competitive abilities of the *Cynanchum* species will be through the development of a biological control program with multiple agents.”

Additional justification, including stakeholder requests and relevance to NE IPM goals is addressed under Background – Stakeholder Needs Assessment (above).

## **Objectives**

The general objective of this project is to evaluate the potential of European insect herbivores to provide biological control of swallow-worts in eastern North America.

Our specific objectives for this proposal include:

- 1) Completing host range testing of agents now in quarantine.
- 2) Evaluating potential impact of these agents upon swallow-worts.

## **Anticipated Outcomes and Impacts**

Contemporary weed biological control projects typically require 5-7 years and funding on the order of a million dollars to accomplish from onset to field release. This project has short-cut much of the foreign research and accompanying expense and we have already initiated impact and host range testing on five natural enemies of swallow-worts. This rapid progress was possible because we completed the literature work and outlined the

basic project seven years ago (Tewksbury et al. 2002) so when Aaron Weed explored Europe in 2006, he knew exactly where to go, who to work with, and what to look for. By working cooperatively with European, USDA, and Canadian scientists, we hope to keep this program on a fast track - completing host range testing and identifying optimal control agents to a point of requesting release permits in as little as two years.

There are many anticipated (and some realized) outputs, outcomes, and impacts from this research program. In the past 8 months we have given invited presentations on this work in Albany NY, Harrisburg PA, and at the International Congress of Weed Biocontrol in France. The published results of this research will provide the foundation for a program of swallow-wort biological control in North America. Right now there is only one scientific article in the literature on biological control of swallow-worts (Tewksbury et al. 2002). The five journal articles anticipated to come from Aaron's PhD dissertation will greatly expand our scientific knowledge and literature base in this area. The new knowledge that we disseminate through presentations and publications will impact other researchers who are studying this and related problems. In addition to the scientific contributions from the proposed research, we anticipate the practical outcome of moving one or more biological control agent to a point where we can prepare a petition for release. Thus we anticipate giving several additional presentations, submitting five journal articles, and preparing a petition for biological control agent release during the funding period covered in this proposal.

However, additional beneficial outcomes will likely follow over the next few years. Based upon what we have already learned about these insects, it is likely that we would be granted permission to release and that release would result in establishment. Thus it is quite possible that three years from now we could have one or more new biological control agents of swallow-worts established in North America and studies on agent distribution and impact could begin.

In the long run, we anticipate that this project will result in favorable impacts such as reduced herbicides, dollars saved, improved environmental conditions, etc. However the interim impacts of our research on the scientific community are also of considerable consequence. For instance, based upon the presentation of our very favorable results at an international meeting in France in April, 2007, Ag. Canada decided to support complementary work which is now underway by Naomi Cappuccino at Carlton University in Ontario and at CABI in Switzerland (see Collaborative Arrangements and Robert Bouchier letter of support in project appendices). This research will save us time and money in completing evaluation studies on potential biological control agents.

### **Approach and Procedures**

Potential biological control agents are subject to stringent evaluations of host specificity before release permits can be granted by the USDA – APHIS (Animal and Plant Health Inspection Service). This agency has overall responsibility for regulating weed biological control programs and relies upon the advice of an independent Technical Advisory Group

for Biological Control Agents of Weeds (TAG). TAG reviews release requests, providing advice to researchers and to APHIS regarding permits for importation, testing, and field release. The decision making process is described at ([www.aphis.usda.gov/ppq/permits/tag/tag.pdf](http://www.aphis.usda.gov/ppq/permits/tag/tag.pdf)). Key to this process is the development of a list of plants to be evaluated for host specificity – a TAG list. As described in Collaborative Arrangements (below) we have a tentative TAG list for swallow-wort biological control agents. All of these plants will need to be tested. However, at this point we have elected to culture only a subset of the TAG plant list at URI. This was done to expedite the process of selecting agents with the most promise. By testing plant species of varying degrees of relatedness to the target weeds while also including some outgroups, we will have a good indication of whether insects will ultimately pass host specificity tests. Our test plant list (Table 1) includes 30 species representing the major divisions of the Asclepiadaceae (milkweed family) and from a few closely related families (outgroups).

**Objective 1) Completing host range testing of agents now in quarantine.**

Plants for host range testing will be started in the URI greenhouse range and then transplanted into containers held outside the greenhouses or in the case of field-collected material - transplanted directly into containers and held outdoors. Plant quality is critically important in host acceptance studies (Blossey et al. 1994a, b). Growing plants outdoors avoids the complex of common greenhouse pests (aphids, thrips, spider mites, etc.) and also allows plants to better develop their normal growth and chemical characteristics (Blossey et al. 1994a, b). Growing them in containers gives us flexibility to move entire plants into quarantine when needed for experiments. Under this objective testing will be completed for all plants listed under all insects in Table 1 in 2008.

Host range tests follow Wapshere's protocol (Wapshere 1974) of first screening for larval survival on plant parts. Plant parts will be collected from potted plants and insects will be confined with them in Petri dishes in the quarantine laboratory. These tests are known to have a high probability of false positives (wrongly identifying a plant as a host), but plant species not attacked under these test conditions are considered safe from attack. For each plant trial, appropriate plant parts (roots, leaves, stems, seed pods – depending upon the insect) will be placed in a minimum of 10 Petri dishes and one newly hatched larva will be introduced into each dish. Equivalent numbers of controls (*Vincetoxicum* spp.) will be used for each test. We will monitor larval survival on a daily basis and will record developmental time and instar weights (if possible). Insects that successfully develop on plant parts as larvae will then be evaluated with oviposition tests and by studying the completion of entire generations on whole plants in cages in quarantine. For these tests 10 gravid females will be introduced into each of three cages, again using *Vincetoxicum* spp. as a control. These will be no-choice tests using naive adults (not previously exposed to hosts) in separate cages. As with the Petri dish study, this approach is conservative – possibly leading to false positives, but unlikely to give false negatives. Depending upon behavior results in the whole plant studies, these tests will be supplemented with caged or uncaged outdoor common garden experiments in Switzerland. This series of experiments is designed to determine potential host range and specificity of the organisms in the laboratory.

**Objective 2) Evaluating the potential impact of these agents upon swallow-worts.**

So far we have studied the ability of each insect agent to develop on the target weeds and we are prioritizing those that minimize potential risks to nontarget species. The next step is determining if they are effective at suppressing the weed, not simply using it as a developmental host. These determinations require impact studies, where the herbivore is exposed to the target weeds at varying densities under controlled conditions to determine how herbivore damage affects biomass production and reproductive output of the target weeds. Based upon our results to date, we believe the best candidates for impact evaluation are the root feeder, *E. asclepiadeus* and the leaf feeder *A. asclepiadis*. This study will include direct effects of these two insect species upon the target plants both singly and in combination.

Direct effects of *E. asclepiadis*.

We will study the effects of root herbivory by *E. asclepiadeus* on the fitness and growth of *V. rossicum* and *V. nigrum* in outdoor studies at CABI in Delémont Switzerland during the 2008 field season. Five insect densities will be established around June 1 by transferring 10, 20, 30, 40, and 0 (control) neonate larvae (5 replicates of each) to 1-year-old plants growing in 8 liter nursery pots. With five densities, five replicates, and two species, there will be 50 potted plants in this test. All plants will be held outside and watered periodically throughout the five-month duration of the trial. Ten uninoculated plants of each species will be sacrificed at the beginning of the trial to establish a baseline. All plants (including controls) will be covered with a gauze cage to exclude other insect species (as in Fig. 3). Plant measurements in late October at the end of the trial (and of the pre-treatment controls) will include the number of leaves and stems, flowers, perennating buds, and seedpods and the wet weights of the plant parts. The plants will then be dried in an oven at 80°C for 48h and weighed immediately to determine dry weights of the same plant parts. The number of seeds per pod will also be recorded. The number of larvae (by instar) will be determined for each pot by visual examination of the roots and soil when the plants are destructively sampled. The impact of root herbivory on plant growth will be determined by a series of regression analyses of growth of entire plants and plant parts against insect density per pot for each plant species.



Fig. 3 Herbivore impact study underway at CABI. (This study involves *Phragmites australis*, but is similar to the setup envisioned for swallow-wort experiments.)

Direct effects of *Abrostola asclepiadis*.

The second study will evaluate the impact of leaf herbivory by larvae of *Abrostola asclepiadis* at CABI by placing second instars on potted plants of *V. rossicum* and *V. nigrum*. Five densities of larvae will be released (0, 2, 4, 6, 8, larvae per plant) with 5 replicates of each treatment. As in the first experiment, there will be 50 potted plants used in this trial. The 4 liter pots will be covered in gauze cages, held with the plants described above, and larvae will be allowed to feed until pupation, which should take around five weeks for *A. asclepiadis* (Förare 1995). The plants will be sampled 5 months after larvae are released. The total leaf area consumed will be estimated and the same plant parameters will be measured and the impact on plant growth and fitness will be analyzed by the same procedures described above.

Direct effects of both herbivores combined.

The third aspect of this study will combine both herbivores. Seven combination treatments will be created and each replicated 5 times as indicated below:

- 0 *Eumolpus* and 0 *Abrostola*
- 20 *Eumolpus* and 2 *Abrostola*
- 20 *Eumolpus* and 4 *Abrostola*
- 20 *Eumolpus* and 6 *Abrostola*
- 40 *Eumolpus* and 2 *Abrostola*
- 40 *Eumolpus* and 4 *Abrostola*
- 40 *Eumolpus* and 6 *Abrostola*

This trial will be set up, managed, harvested, and evaluated the same as in the individual treatments. This design is intended to not only determine how increasing larval density of two herbivores affects biomass production by the plant, but also to determine how the interaction of *Abrostola* and *Eumolpus* densities affects biomass production. It should

determine whether the impact of *Abrostola* is similar between two *Eumolpus* densities and *vice versa*.

### Research Timetable

Objective 1: Research conducted during the 2008 season.

All plant and insect cultures needed to complete Table 1 are already available in our greenhouses in our quarantine laboratory. Experiments will be run during the growing season when plants can be grown outdoors.

Objective 2

This research will be conducted during the 2008 field season in Europe.

**Dec. 07** start 300 seeds of *V. nigrum*, 300 *V. rossicum* at CABI. (Done by CABI colleagues.)

**Feb. 08** Transplant seedlings into 1 liter pots. (Done by CABI colleagues.)

**May/June 08** Aaron goes to CABI for 3 weeks.

Transplant plants into 8 liter pots and inoculate with insects as described above

**June-Oct. 08** Maintain plants as described. (Done by CABI colleagues.)

**Oct./Nov. 08** Aaron goes to CABI for 2 weeks to harvest and evaluate as described.

**Nov. 08 – May 09** Aaron compiles and analyzes results, prepares manuscripts.

URI results are combined with Canadian, USDA, and CABI results to see if a package can be prepared for a release petition.

Collaborative and multi-institutional and multi-disciplinary aspects of this work are not included in the timetable, but are documented under Collaborative Arrangements, Budget Justification, and at the end of the Background section.

### Evaluation of Project Impacts

As described above, we do anticipate positive outcomes, but our impacts will primarily be limited to the scientific community over the next two seasons. In reporting on this research, we'll document outcomes such as progress toward completing TAG requirements and enumerate publications, presentations, and plants and insects given to cooperating scientists. Under the best of circumstances, we may be in a position of making field releases of biological control agents against swallow-worts in 3 years. Following release, we typically measure the additional outcomes of natural enemy establishment and distribution of natural enemies for at least one season before measuring the field impacts of the program. For instance in our current program on lily leaf beetle biological control, a documented outcome is that *Tetrastichus setifer* is now established in four New England states and it has spread several miles from release sites. An impact is that lily leaf beetle populations have declined to non-pest levels at and near release sites and gardeners have stopped using insecticides against this pest. We can also measure pest density and pesticide use against lily leaf beetle and compare it to control areas and quantify pest and pesticide reduction.

If successful, we anticipate distributing swallow-wort natural enemies to cooperators throughout the northeast (much like our lily leaf beetle project) and thus facilitate the spread of these agents in this region. With a successful biological control program, there would be no need for other control measures against these weeds.

### **Key Personnel**

The research described in this proposal will be conducted by URI students, staff, and graduate students with assistance from researchers and assistants at CABI. Aaron Weed, PhD candidate working under Richard Casagrande has performed the research described above under "Research Progress to Date". This proposal will allow him to continue this research at URI under quarantine conditions and outdoors at CABI in Europe. Lisa Tewksbury, lab director and quarantine officer for the URI Biological Control Laboratory, assists in maintaining plant collections and insect colonies, hires and directs undergraduate students working on this project, and assures compliance with quarantine regulations. André Gassmann of CABI in Delemont Switzerland supervised Aaron's European research in 2006 and will again supervise research conducted at CABI in 2008. Richard Casagrande has overall supervisory responsibilities for the project. Many other scientists (local, regional, and international) provide assistance in this research as described in Collaborative Arrangements.

It is important to note that the overall effort in classical biological control of swallow-worts, initiated by URI, has grown well beyond the URI program. We are key cooperators with the USDA/ARS effort as well as the Canadian research program and the CABI program in Europe as described in Collaborative Arrangements and supporting letters in appendices. The research described in this proposal is a key discrete project fitting into a much larger interdisciplinary program.

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## RELEVANCE STATEMENT

Program Directors: Richard A. Casagrande, Professor of Entomology, Univ. of R.I.  
 André Gassman, CABI Europe, Delémont Switzerland

Title: Biological control of invasive swallow-worts in the northeast

Project Type: Research

### Project Summary

Two species of European swallow-worts, *Vincetoxicum nigrum* (black) and *V. rossicum* (pale) have invaded the USA and spread throughout the Northeast, raising the attention of ecologists and habitat managers. Swallow-worts contain a haemolytic glycoside which is toxic to mammals, resulting in reduced grazing and/or toxicity (including death) to grazing animals. In addition to pastures, pale swallow-wort invades gardens, lawns, shrubs, hedgerows, fencerows, and a variety of deciduous and mixed forest types as well as ornamental plant nurseries, Christmas tree farms, and pine plantations. The twining climbing plants smother small trees and also serve as alternate hosts for *Cronartium* rusts attacking *Pinus* species. Both species have adverse impacts upon monarch butterflies, inducing oviposition upon plants where larvae cannot survive. These problems will increase as swallow-worts continue to spread throughout the USA.

Swallow-worts are difficult to control mechanically or chemically. Native insect herbivores associated with these plants cause little damage, which is the likely reason for the successful invasion and spread of these plants. In response to stakeholder requests, we have initiated a biological control program on swallow-worts. We studied the European literature, corresponded with European specialists, and in 2006 we investigated and collected insect herbivores on swallow-worts throughout Europe. We presently have five European species of insect natural enemies of swallow-worts in our Rhode Island quarantine laboratory where we are determining their potential for release as biological control agents in North America. In two seasons of research, we have made good progress on host range testing and we have identified two agents which appear particularly promising. We seek support to continue host range testing of promising natural enemies and to measure their impact on the target weeds toward the goal of bringing North American swallow-worts under biological control.

### Background

Distribution. There are two swallow-wort species widely distributed in the USA. Black swallowwort *Vincetoxicum nigrum* is found from Maine through Kansas and in California (Fig. 1). This European perennial herbaceous vine is commonly found in disturbed areas, pastures, roadsides, and forest understories. Pale swallow-wort *Vincetoxicum rossicum*, also commonly known as dog-strangler vine, is native to the Ukraine and Russia. It has a growth pattern and habitat preference similar to *V. nigrum*, and a discontinuous distribution that extends from the Great Lakes through New England and the Mid-Atlantic States (Fig. 1). Both swallow-wort species are highly invasive in North America where they out-compete and displace native plant species, including milkweeds. The earliest record of *V. nigrum* in the USA is from Massachusetts in 1854 and *V. rossicum* was recorded in NY in 1897.

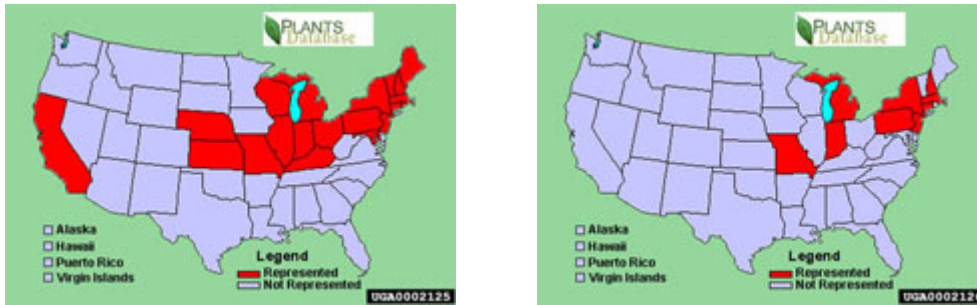


Figure 1. Distribution of Black swallow-wort (left) and Pale swallow-wort (right) in the USA (Plants Database).

**Potential for Biological Control.** Surveys conducted in Rhode Island and New York indicate that no native insects or other organisms in the United States are causing significant damage to these plants. This is likely due to their toxic chemicals which protect them from herbivory. Swallow-worts in North America have escaped their specialized herbivores in Europe and are thriving in enemy-free space. The goal of our biological control program is to reacquaint these plants with the effective natural enemies that they left behind in Europe. Since there are no closely-related native plants in North America, swallow-worts are good candidates for Classical Biological Control

In 2006 Aaron Weed, URI Ph.D. candidate, studying under Dr. Casagrande, spent the summer in Europe surveying for swallow-wort species and their natural enemies. Working with Dr. André Gassman and colleagues at CABI in Delémont, Switzerland, Aaron traveled extensively throughout Europe. He found *V. hirundinaria* widespread throughout Europe. *Vincetoxicum nigrum* is restricted to Northern Italy, France, and Spain; and *V. rossicum* is limited to southwestern Russia and Ukraine. Unlike North America, swallow-worts in Europe are restricted to discrete (normally shady) habitats and they were not found to be invasive in pastures, agricultural fields, or natural areas. Aaron identified several insect species that feed upon swallow-wort species and established colonies of five promising species. After conducting basic studies on biology and host range, he returned these species (with USDA permits) to the URI Insect Quarantine Laboratory where he has conducted research on biology and host range over the past 15 months. He has identified a root feeding beetle *Eumolpus asclepiadeus* and the leaf-feeding larvae of the moth *Abrostola asclepiadis* as particularly promising and he is focusing on these species while completing preliminary host range testing of other species.

## Justification

### Stakeholder Priorities

Our swallow-wort biological control efforts were begun at the request of Don and Heather Minto of Watson Farm, (Jamestown RI) whose pastures became overrun with black swallow-wort following our successful program of Cypress Spurge biological control. As we have distributed Cypress Spurge natural enemies to other pasture managers in the state, we have noted increasing problems with black swallow-wort. Of the 53 pastures we have sampled throughout RI, 20 have black swallow-wort infestations.

This project meets goal 3 of the NE Regional IPM program: “..... reduce unreasonable adverse environmental effects from pests and the use of pest management practices.”

As serious weeds in ornamental nurseries and Christmas tree plantations, swallow-worts qualify under NEREAP-IPM priority one for 2006 and priority 3 (minor crop) for 2007.

Further, this research conforms to the General IPM Priorities for the Northeast (November, 2004) items 5 and 6 (of 16): weed management in nurseries.

And it particularly addresses the 2006 Livestock/Field Crop IPM Priorities for New York State: “..... and poisonous/noxious plants affecting cattle on pasture”

### Non-pesticidal Tactics

Classical biological control represents the premier alternative to pesticides for managing widespread pests of agriculture and the environment. Through permanent establishment and spread of effective biological control agents, we hope to end use of herbicides against swallow-worts in pastures, nurseries, and Christmas tree plantations, and control the weeds in natural areas where widespread use of herbicides is not an alternative.

### Multi-state Involvement/Benefit

Swallow-wort species are distributed throughout the northeast and a successful biological control program against these weeds will benefit the entire region. URI researchers work in close collaboration with Lindsey Milbrath (USDA/ARS) and Antonio DiTommaso (weed science) at Cornell, Naomi Cappuccino of Carlton University in Ontario, and André Gassman of CABI Switzerland. We consult on this research with 10 additional specialists in three states and five countries

### Importance

From an environmental stewardship perspective, expanding swallow-wort populations represent a threat to populations of monarch butterflies and several threatened and endangered plant species. In agriculture, they represent one of the key weed species in commercial nursery production – the number one crop in Rhode Island, valued at 109 million in RI and approximately a billion dollars in New England. Invasive weed problems in nurseries are particularly troublesome, since if left uncontrolled, they get transplanted into landscapes throughout the region. Swallow-worts are serious pasture pests and pests of Christmas tree plantations throughout the northeast.

### Implementation

Research to date indicates that four of the five agents we hold may have adequate host specificity for release in North America and two look particularly promising. With our current research plan, we could be seeking release permits in two years. If released, we expect biological control agents to spread throughout the entire region.

## **Objective**

**The objective of this proposal is to evaluate the host range and potential impact of European insect herbivores under consideration for biological control of swallow-worts in North America.** We anticipate giving several research presentations, submitting five journal articles, and preparing a petition for biological control agent release during the funding period covered in this proposal.