

Implementing Swallow-wort Biocontrol R.A. Casagrande, University of Rhode Island

PROJECT SUMMARY

This is a research project. Two species of European swallow-wort, *Vincetoxicum nigrum* and *Vincetoxicum rossicum*, have become invasive in North America, where there are no effective natural enemies able to suppress populations and deter further spread. Swallow-worts are toxic to mammals, causing reduced grazing in pastures, toxicity, and death. They invade gardens, lawns, shrubs, fencerows, and a variety of forest types as well as ornamental plant nurseries, Christmas tree farms, and pine plantations. The twining climbing plants smother small trees and adversely impact monarch butterflies, inducing oviposition upon plants where larvae cannot survive. These problems will increase as swallow-worts continue to spread throughout the USA. Conventional control methods are largely unsuccessful in managing established infestations, and biological control appears to be the most promising alternative. At the request of local stakeholders, we initiated a program of classical biological control against swallow-worts a decade ago. With Northeast Regional IPM funding in 2008 and 2009, we determined that the European leaf-feeding moth, *Hypena opulenta* has potential for successful biological control of swallow-worts. Host range testing shows that these larvae are monophagous on swallow-worts, not posing a risk to any native North American plant. This insect causes extensive defoliation of *V. nigrum* and *V. rossicum* under laboratory conditions in quarantine and it is expected that it would adversely impact plants under field conditions with repeated defoliation and in the presence of competing plant species. In November, 2011 we petitioned the USDA and Canadian authorities for the open-field release of *H. opulenta* as a biological control agent for *V. rossicum* and *V. nigrum* in the United States and Canada in 2012.

This research proposal requests funding to release and monitor this agent in plots carefully selected and pre-monitored to determine its potential for controlling both swallow-wort species in a range of habitats. Grant funding will cover agent release and three years of monitoring in the Northeast. We will also provide this agent to Canadian colleagues who will rear, release, and monitor *H. opulenta*. By conducting essentially paired experiments in Canada, we will have effectively bracketed the range of climates where swallow-worts grow in North America. This Canadian research, conducted at essentially no cost to this program, will greatly strengthen the value of research conducted in southern New England, allowing us to quickly determine whether to widely distribute this agent or to consider other agents presently under study. Through the permanent establishment and spread of effective biological control agents, we hope to end the use of herbicides against swallow-worts in agricultural enterprises and further control the weeds in natural areas where widespread use of herbicides is not an alternative.

This proposal meets 13 of the 14 goals of the current NE Regional IPM program and addresses several priorities established for IPM research in the Northeast. Evaluation includes monitoring establishment, spread, and impact of this biological control agent as well as establishing baseline data on swallow-wort control costs for RI land managers, against which we can measure impact of this biological control project in the future.

Nature of the problem

Populations of European swallow-wort (*Vincetoxicum* species) have become established in northeastern North America, where there are no effective arthropod herbivores to suppress populations and deter further spread into surrounding environments (Sheeley, 1992; Christensen, 1998; Lawlor, 2000; Milbrath, 2010). The two weed species, *Vincetoxicum nigrum* and *V. rossicum*, are now widely distributed along the Atlantic coast of the United States and in Ontario and Quebec in Canada. *Vincetoxicum nigrum* is native to Mediterranean regions of France, Italy, and Spain; *V. rossicum* is naturally distributed in southeast Ukraine and Russia. The earliest record of *V. nigrum* in the USA is from Massachusetts in 1854, and *V. rossicum* was first documented in New York in 1897 (Sheeley and Raynal, 1996). In Canada, the earliest record for *V. rossicum* in Ontario was 1889 (DiTommaso et al. 2005). *Vincetoxicum nigrum* has been confirmed to be in Ontario since the early 1950's (DiTommaso et al. 2005). Despite the long history of *Vincetoxicum* spp. presence in North America, **they have only become a significant problem in recent decades due to range expansion and unhindered population growth (Lawlor, 2000) and thus are considered emerging invasive pests.**

***Vincetoxicum rossicum* is currently distributed in seven states ranging from the Atlantic Coast west to Missouri as well as Ontario, Quebec, in Canada (USDA PLANTS database, 2011). *Vincetoxicum nigrum* has a greater distribution and has become established in 21 states from Maine through Kansas and in California as well as in Ontario and Quebec (DiTommaso et al. 2005, USDA PLANTS database, 2011).** Swallow-worts display superior competition for resources among native plants and often form dense monocultures in a variety of habitats (Cappuccino, 2004). 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. Swallow-worts pose a major threat to native species diversity and ecosystem functioning along with disruption of farmlands and pastures as substantial agricultural pests (DiTommaso et al., 2005).

Economic Impact. Swallow-worts negatively affect farming practices, livestock, and ornamental landscapes. Pastures create ideal conditions for swallow-wort establishment and growth because grazing reduces competition from other plants (Fig. 1).



Figure 1. Pale swallow-wort overgrowing a former pasture, Jefferson Co., New York (Fran Lawlor, The Nature Conservancy)

Swallow-wort contains the haemolytic glycoside vincetoxin, which is toxic to humans and most other mammals (DiTommaso et al., 2005). Cattle have demonstrated minimal consumption of swallow-wort; horses, goats, and sheep will graze around it, leaving those pastures open for successful colonization by swallow-wort (DiTommaso et al., 2005). Farmers, conservationists and gardeners often devote costly and extensive efforts towards manual removal and mowing of swallow-wort but underground rhizomes continuously send up new buds which create additional shoots (Lawlor and Raynal, 2002; Douglass et al. 2009). To eliminate populations, the entire rhizome must be removed, requiring substantial labor.

In addition to disrupting agricultural crops such as no-till corn, swallow-worts have been reported as a major pest in Christmas tree farms in central New York (DiTommaso et al., 2005). The twining vines of swallow-worts have been documented pulling down small trees and smothering vegetation (Fig. 2) planted at restoration sites (Christensen, 1998) and pine plantations in Ontario (DiTommaso et al., 2005).



Figure 2. Black Swallow-wort overgrowing forest trees. (Leslie J. Mehrhoff, UConn.)

Herbicides can be effective against swallow-wort in the short-term; however, repeated application over several years is necessary and may never completely eliminate plants (Lawlor and Raynal, 2002; McKague and Cappuccino, 2005; Weston et al., 2005; Averill et al., 2008; Douglass et al., 2009). Herbicide use is expensive, potentially harmful to applicators and the environment, and it may result in resistance among target weeds (Lawlor, 2000). Herbicides are often used against large infestations of swallow-wort, but since swallow-wort is often intertwined with other plants, foliar application is a major risk to non-target plants or crops in the surrounding area (Lawlor and Raynal, 2002; DiTommaso et al., 2005). **There are obviously large costs associated with swallow-wort management and reductions in land values associated with infestation by these weeds (Fig. 1 is a good example), but to date there are no economic data available on these costs – an issue we will begin to address through this proposal (see Approach and Procedures and Evaluation).**

Effects on Native Plant and Animal Populations. In North America, *Vincetoxicum* species affect ecosystems by reducing local biodiversity of native plants, vertebrates, and arthropods (DiTommaso et al., 2005). Studies in, Ontario have shown significantly lower arthropod diversity and abundance in old-fields where swallow-wort is the predominate vegetation, when compared with nearby old-field sites where native plant species thrive (Ernst and Cappuccino, 2005). There are several indirect and secondary effects of swallow-wort on native species as well. Investigations of grassland bird populations in New York and Ontario have shown reduced breeding and nesting behavior in areas where swallow-wort has formed mono-specific stands (DiTommaso et al., 2005, Miller and Kricfalusy 2008). There is also evidence of swallow-wort adversely impacting monarch butterfly populations since these butterflies often oviposit on swallow-worts instead of native milkweed species (DiTommaso and Losey, 2003). Monarch larvae cannot survive on swallow-wort so these plants effectively act as a population sink for monarchs (Casagrande and Dacey, 2001, 2007). Swallow-wort may pose an even greater threat through competitive displacement of milkweeds as well as other important host plants of native species (DiTommaso et al., 2005).

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. Black swallow-wort was identified as the invasive plant of greatest concern to the late Leslie J. Mehrhoff, Manager of the Invasive Plant Atlas of New England (pers. comm. to R.A. Casagrande, 2003). 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”. **As documented in Appendices to this proposal, swallow-wort management is becoming an increasing problem for stakeholders in RI (Appendix letter of support from Seldom Seen Farm and Watson Farm), Massachusetts (LOS from Naushon Trust), NY (note from IPM Labs), and Michigan (two notes appended).**

NER-IPM Program goals for 2012. This project meets 13 of the 14 goals of the current NE Regional IPM program (we don't serve underrepresented audiences) and these aspects are described in the Project Description.

1. Will reduce risks to the environment by substituting biological control for herbicides.
2. Will reduce applicator health risk from herbicide application.
3. Has stakeholder support and the priority has been cited (above and see letters of support).
4. Pest found in at least five states (24 states and two Canadian Provinces.)
5. Will fill a niche. (Chemicals are relatively ineffective and costly. There are presently no effective biocontrol agents for swallow-worts and no other group is proposing releases.)
6. Involves multiple states in an active partnership (RI, NH, and collaborators in Canada)

7. Will advance IPM implementation in a few years. (We will release biocontrol agents this year or next with expected results beginning in the year of release.)
8. Is interdisciplinary (using insects to control weeds and evaluating ecology of both.)
- 9, 10, 14. Reduces dependence on conventional chemical pesticides and associated costs.
12. Addresses an emerging pest. (As described, swallow-wort populations are greatly expanding.)
13. Is likely to be adopted. (We will assist the natural spread of effective agents.)

Alternative Management Options. Current management practices are limited to manual removal of plants or seedpods, mowing, and applying herbicides (Lawlor and Raynal, 2002; DiTommaso et al., 2005; McKague and Cappuccino, 2005; Averill et al., 2008; Douglas et al., 2009). The only method to ensure long-term control of swallow-wort requires excavation of the entire plant because root crown fragments left behind can root in the soil and produce additional shoots (DiTommaso et al., 2005). Hand picking seedpods from plants to limit spread is another control measure where digging and herbicides are not an option, such as rocky habitats or protected natural areas. However removal of seedpods is only effective in reducing seed pressure, if it is repeated throughout the growing season (Lawlor, 2000). Tests conducted in Ontario revealed that repeated mowing reduced the average stem height of *V. rossicum* but did not decrease overall cover (Christensen, 1998). In a follow-up study, McKague and Cappuccino (2005) determined that mowing has no effect on plant biomass and is only slightly effective at reducing seed production if the treatment is timed following initial fruit production. In New York, Averill et al. (2008) demonstrated that clipping *V. rossicum* had no effect on stem cover, density, or seedpod production, regardless of how frequently the treatment was applied. Usually when the primary aerial stem is damaged on swallow-wort plants, the root crowns readily send up multiple auxiliary shoots which can compensate for biomass loss and possibly exacerbate infestations (e.g. increased seed production)(DiTommaso et al., 2005; McKague and Cappuccino, 2005).

The effects of two non-selective herbicides, triclopyr and glyphosate, were evaluated on populations of *V. rossicum* in Ontario (Christensen, 1998). It was determined that at least two applications of glyphosate in mid-June and early August were required in order to reduce swallow-wort cover by 90% the following year. Further, after treatment with herbicides, the sites were open for successful colonization by another invasive plant, *Melilotus alba* (sweet white clover) which replaced *V. rossicum* as the dominant vegetation (Christensen, 1998). In New York, one treatment of triclopyr (1.9 kg ai/ha) reduced *V. rossicum* cover and stem density by 56% and 84% after 2 years (Averill et al., 2008). However, despite encouraging results from one application the authors cautioned that long-term control could only be sustained by repeated applications and active restoration.

All current control measures are generally only effective in the short-term, require substantial resources or labor and could have collateral impacts on native species in the surrounding habitats (DiTommaso et al., 2005; McKague and Cappuccino, 2005; Averill et al., 2008; Douglas et al., 2009). The use of biological control agents may be the only viable option for long-term reductions in swallow-wort populations.

Previous Work on this problem. Beginning in 2006, field surveys in Europe by Aaron Weed (then PhD candidate working under R.A. Casagrande) identified five potential biological control agents for *Vincetoxicum* spp. Initial research conducted at CABI EU-CH

in Delemont Switzerland allowed us to assess each species and narrow our focus to the most suitable candidates for further testing (Weed, 2010). Two chrysomelid beetles, *Chrysolina asclepiadis asclepiadis* and *Chrysochus asclepiadeus* were collected on the leaves of *Vincetoxicum* species in Europe. During impact studies, both species were shown to adversely impact both *V. rossicum* and *V. nigrum* (Weed et al., 2011a). However, under no-choice conditions, the larvae of both beetle species completed development on several native North American species and thus they are not candidates for release.

Pupae of the tephritid fly *Euphranta connexa* were collected from seedpods of *V. hirundinaria* in Moutier, Switzerland during 2006. Adults oviposit in developing seedpods and larvae feed on the developing seeds of the target weeds (Weed et al., 2011b). Given the severity of *Vincetoxicum* infestations in North America and the fact that *Vincetoxicum* spp. have adaptable reproductive systems (i.e. clonally through rhizomes, sexually or through self-pollination) we have focused our attention on agents that may directly impact plant biomass. However, work continues with *E. connexa* at CABI EU-CH to assess its potential at reducing swallow-wort spread (Gassmann et al., 2011).

The noctuid moth species *Hypena opulenta* and *Abrostola asclepiadis* were collected on *Vincetoxicum* species in Ukraine and brought to CABI EU-CH to study their life cycle and feeding habits (Weed and Casagrande, 2010). The larvae of *A. asclepiadis* feed on the leaves of *Vincetoxicum* spp. and impact studies in Europe demonstrated complete defoliation of plants at low larval densities (Weed et al., 2011a). Host-range records for *A. asclepiadis* indicate that the species is monophagous on *V. hirundinaria* in its native range (Förare 1995) and host-range testing in quarantine at URI on 73 species to date shows that this insect is apparently monophagous on *Vincetoxicum*. *Abrostola clarissima*, collected in the Russian North Caucasus region has also shown good host-specificity, developing on only one species (*Metastelma barbigerum* Schelle) of 65 species tested by Milbrath and Biazzo (pers. comm.). *M. barbigerum*, which exists only in south Texas, will be tested with *A. asclepiadis* in 2012.

Research at URI (funded by NER IPM from 2008-2010) has shown that *H. opulenta* is a multivoltine species with overlapping generations (Weed and Casagrande, 2010). Impact studies determined that all larval densities significantly reduce aboveground biomass, seedpod production and seed production in *V. rossicum* (Weed and Casagrande, 2010). Testing of 76 species, including *M. barbigerum* (Appendix 1), indicates that this species is monophagous on *Vincetoxicum* and in October, 2011 we submitted a release petition to USDA-APHIS for *Hypena opulenta* against swallow-worts (Appendix 2).

Hypena opulenta develops through five instars and pupae overwinter in the soil and leaf litter (Weed and Casagrande, 2010). Typically, females begin laying eggs on the undersides of *Vincetoxicum* leaves or petioles 2 to 5 d after emergence. The average lifespan of adult moths is 17 d and each female can lay up to 600 eggs with an average of 400 (Weed and Casagrande, 2010). Larvae feed individually on the underside of the leaf typically through the 3rd instar and then feed on the young, expanding leaves, which may suppress flowering (Weed and Casagrande, 2010). It takes 4-6 weeks to complete development and a portion of each generation undergoes pupal diapause (Weed and Casagrande, 2010).

It is expected that multiple, overlapping generations will continually defoliate and stress *Vincetoxicum* spp. throughout the season. One study demonstrated that only two larvae per plant are needed to reduce shoot biomass and plant reproduction (Weed and Casagrande, 2010). We expect that this agent will spread throughout the distribution of the target weeds in North America. Our *H. opulenta* culture came from an area in Ukraine in Plant Hardiness Zone 5 (-20 to -10F) (<http://www.ars.usda.gov/Main/docs.htm?docid=9815&page=3>). Much of the current distribution of *Vincetoxicum* spp. in the USA is also in USDA Plant Hardiness Zone 5 (<http://www.usna.usda.gov/Hardzone/ushzmap.html>) so winter temperatures should not restrict the establishment and spread of the agent. This will be further tested with the proposed releases towards the northern edge of the range in Ottawa.

The impact of *H. opulenta* is likely to be dependent on local light conditions (Milbrath, 2008), level of herbivory, and plant community composition. For example, the impact of artificial defoliation on growth and reproduction of *V. rossicum* and *V. nigrum* was significantly higher when plants were grown under shade compared to high light conditions (Milbrath, 2008). Defoliation can also decrease the competitive ability of swallow-worts (Douglass et al., 2009; Weed et al., 2011a). Cappuccino et al. (2002) demonstrated that *V. rossicum* growth is negatively affected by direct competition with monocots. Herbivory together with competition from mixed plant communities will further decrease the competitive ability of swallow-worts.

Based on the results of our stringent host-specificity testing and detailed evaluation of the biology and feeding habits of *H. opulenta*, there are no foreseeable negative impacts on non-target plants.

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.”

Outcome of No Action. If there are no agents released, we expect continued swallow-wort range expansion and environmental degradation. Wildlife and native vegetation will continue to be displaced and farmers and land managers will continue using conventional control methods that are largely ineffective.

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

Objectives and Anticipated Impacts

The overall goal of this project is to establish an effective program of classical biological control against swallow-worts in North America. Fulfillment of this goal will permanently reduce populations of black and pale swallow-wort throughout its range, thereby reducing adverse impacts of these weeds in agriculture and in managed and unmanaged landscapes. Successful biological control will also reduce the costs and risks associated with herbicide applications against these weeds.

The major objective of this project at present is to release and establish *Hypena opulenta* and evaluate the utility of this insect in controlling swallow-wort species in North America. This agent presently appears to be the most promising of all the European natural enemies of swallow-worts. Within three years, we will have evaluated it against both weed species under a range of environmental conditions, allowing robust predictions of its value in various ecosystems. We will also know which, if any, additional agents (presently under evaluation) should be released to supplement the activity of *H. opulenta*.

Supporting objectives:

1. Determine the role of *H. opulenta* in sun and shade on black and pale swallow-wort species. Experiments conducted on Naushon Island MA, with controls on nearby islands and the mainland will show us the potential utility of this herbivore against both weeds growing in various habitats.

2. Determine the role of *H. opulenta* under different climatic conditions. The climate of Naushon Island is greatly moderated by the Atlantic Ocean. By conducting essentially paired experiments in Canada, we will have effectively bracketed the range of climates where swallow-worts grow in North America. This work, done by Canadian collaborators at essentially no cost to this program, will greatly strengthen the value of research conducted in southern New England.

Approach and Procedures

Overall approach. Our laboratory investigations show *H. opulenta* to develop readily on both pale and black swallow-wort under light conditions mimicking full sun – open field conditions, but this is at variance with our observation in Europe that this insect is normally found in forested sites. Field releases will test the hypothesis that this species will establish in fields and forests in North America. Research will be conducted on Naushon Island, MA where we have both swallow-wort species growing in sun and shade and we have control over swallow-wort management on the entire 19 square kilometer island. Control plots are on Block Island and Conanicut Island (RI) and mainland plots in RI and CT. We anticipate APHIS approval for release of *H. opulenta* in June, 2012. If this approval is delayed, we will continue to monitor prospective release and control plots this season and initiate releases in June, 2013. We will continue to monitor release and control plots for the 2013 and 2014 field seasons.

Canadian participation. For several years, colleagues in Canada have cooperated with us and with our colleagues at CABI – Europe in Switzerland in developing a swallow-wort biological control program. They have evaluated other agents and also provided CABI with funding that has partially supported research on agents that we're evaluating. We will provide Rob Bouchier (Ag Canada) with a starter colony of *H. opulenta* which will be reared in the Insect Microbial Containment Facility (AAFC Lethbridge) in preparation for the proposed releases for release in 2012 or 2013. Canadian colleagues also submitted a release permit for *H. opulenta* in October, 2011 and anticipate approval this year. Canadian releases and evaluations (described below in Objective 2) are intended to mimic our field efforts in New England, evaluating this agent under very different climatic conditions. **No funding from the Northeast Regional IPM program will be used for this Canadian research**, but this work will greatly strengthen our work and allow a rapid determination of the potential value of *H. opulenta* under a wide range of conditions.

Objective 1.

We intend to release *Hypena opulenta* adults from our laboratory colony in early June 2012 on Naushon Island, MA into forested populations of *V. nigrum* and *V. rossicum*. We also

plan releases of *H. opulenta* into plots of both swallow-wort species in sunny sites in fields. These release sites will include our long term monitoring plots of *V. rossicum* and *V. nigrum*. We plan to release about 500 adults into each of these four sites on the island. We will monitor release sites as described below.



Fig. 3 Swallow-wort sites on Naushon Island, MA. Release sites include sun and shade plots. We'll also survey distant sites (Uncatena and Veckatimest Islands and a stand near the south end of Naushon) for agent spread and establishment.



Fig. 4. Pale swallow-wort stand on Naushon Island extending from an open field into a mature forest. This is a potential release site (labeled Pale in Fig. 3) which we have monitored since 2008.

Post Release Monitoring. We have established sites on Naushon Island and Conanicut Island and Block Island (RI) and mainland RI, and CT that have been monitored since 2008. Initially, we were using two 1m² quadrats per site collecting data on number of stems per 0.5m², number of seedlings per 0.1m², percent cover by *Vincetoxicum* spp., and percent cover of all other plant species within each quadrat. Beginning in 2009, using the same data collection procedures as described previously, we changed to using four 0.5m² quadrats per site in order to coordinate our sampling with that being conducted by Dr. Lindsey Milbrath of USDA ARS. We will continue to collect and evaluate data using the same standardized procedures for all sites. Once the agents are released, we will monitor feeding damage per plant, as well as densities of *H. opulenta* eggs and larvae per plant. These plots will be sampled through 2014 (and beyond) to determine agent establishment, spread, and impact. Changes in swallow-wort abundance (stem density and percent cover) and native plant cover in release plots will be compared to our control plots and the inland plots under study by Milbrath in New York.



Fig. 6. Forested and open field sites of black swallow-wort on Naushon Island. Both sites (labeled Black in Fig. 3) are proposed for agent release.

Objective 2 (Research in Canada). Canadian colleagues are planning to release on property owned by the University of Toronto: the Koffler Scientific Reserve north of Toronto in 2012 or 2013. This release location will be paired with *Vincetoxicum* control sites that have been mapped by the Toronto Regional Conservation Authority since 2000. The TRCA monitoring estimates will be supplemented by additional detailed quantitative density estimates (Milbrath protocol) for *V. rossicum* prior to the releases. The primary type of habitat will be forest and forest edge containing *Vincetoxicum rossicum*. Release rates (500 insects) will be the same as with the US sites to enable comparisons in establishment and impact. If suitable numbers of insects are available in 2013, we will also release at the Fletcher Wildlife Garden in Ottawa adjacent to Carleton University where collaborator N. Cappuccino has conducted previous studies of *Vincetoxicum* biology. This site, which is at the northern edge of the *Vincetoxicum* range, will further test overwintering ability of *H. opulenta*.

Vincetoxicum monitoring at Canadian sites has included primarily mapping using GPS within the Toronto Regional Conservation Authority. There are some *Vincetoxicum* density data using quadrats, taken since 2000, at multiple locations. In 2012 we will establish additional standard monitoring quadrats based on the Milbrath protocol to enable comparisons between the Canadian and US release sites and the sites being studied by Milbrath. At all sites, data on pre-release swallow-wort densities will be compared to post-release data while monitoring for insect damage such as feeding per plant, larval densities, number of eggs per plant, and adults observed. We will also compare attack rates between forested and open field populations of swallow-wort in order to determine the habitat preference of *H. opulenta* in North America. This will help us to evaluate establishment and the impact of agents released at each site. We will also monitor other areas near the release sites where swallow-wort is present in order to determine if *H. opulenta* has established and how far it has spread. These findings will allow us to make decisions on future release sites.

Research Timetable

Research conducted during the 2012 season.

Objective 1:

Ship *Hypena opulenta* to Lethbridge quarantine lab in Canada for rearing.

Ship *H. opulenta* to quarantine lab at Cornell for impact research by Lindsey Milbrath (USDA/ARS).

Rear *H. opulenta* in URI quarantine lab

If release petition approved in time, field-release agents in June.

(With or without release) sample release and control plots on Naushon, Conanicut, and Block Islands and mainland RI and CT as described in Approach & Procedures to determine agent establishment, spread, and defoliation. If released, evaluate possible parasitism of *H. opulenta*.

Objective 2:

Rear *H. opulenta* in Canadian quarantine lab, preparing for release in 2012 or 2013.

Sample release and control plots as described in Approach & Procedures to determine agent establishment, spread, and defoliation.

If released, evaluate possible parasitism of *H. opulenta*.

Additional Activities in support of Overall Goal:

Between seasons: discuss results on agent establishment, spread, and defoliation with Canadian and USDA colleagues.

Compare URI testing results with *Abrostola asclepiadis* with Milbrath's results on *A. clarissa* and jointly decide if a release petition should be prepared for either species.

Impact Assessment: We will initiate a survey whereby an undergraduate student will visit a set of growers to collect data on swallow-wort management costs.

Research conducted during the 2013 season.

Objective 1:

If agents were not released in 2012, field-release agents in June.

Sample release and control plots on Naushon, Conanicut, and Block Islands and mainland RI and CT as described in Approach & Procedures to determine agent establishment, spread, and impact. Evaluate possible parasitism of *H. opulenta*.

If agents do not establish from prior release, re-release into selected plots.

Objective 2:
 If agents were not released in 2012, field-release agents in Canada in June.
 Sample release and control plots as described in Approach & Procedures to determine agent establishment, spread, and impact. Evaluate possible parasitism of *H. opulenta*.
 If agents do not establish from prior release, re-release into selected plots.
 Rear *H. opulenta* for additional releases if necessary.

Additional Activities in support of Overall Goal:
 Between seasons: discuss results on agent establishment, spread, and impact with Canadian and USDA colleagues.
 If *H. opulenta* establishes in sun and shade in US and Canada, delay preparation of *Abrostola* release petition. If not, proceed with this second biocontrol agent.

Impact Assessment: We will compile baseline costs for swallow-wort management, adjust survey if needed, and collect data on this year's on swallow-wort management costs.

Research conducted during the 2014 season

Objective 1:
 Sample release and control plots on Naushon, Conanicut, and Block Islands and mainland RI and CT as described in Approach & Procedures to determine agent establishment, spread, and impact.

Objective 2:
 Sample release and control plots in Canada as described in Approach & Procedures to determine agent establishment, spread, and impact.

Additional Activities in support of Overall Goal:
 After season: discuss results on agent establishment, spread, and defoliation with Canadian and USDA colleagues.
 If *H. opulenta* establishes in sun and shade in US and Canada and demonstrates potential to adversely impact plants in the field, prepare for further distribution of this agent in USA and Canada.
 If agent does not establish under some conditions, consider *Abrostola* species and other agents under investigation at URI, Canada/CABI, and USDA/ARS.

Impact Assessment: After collecting a third season of swallow-wort management costs, we will summarize costs for various agricultural enterprises and extrapolate state-wide.

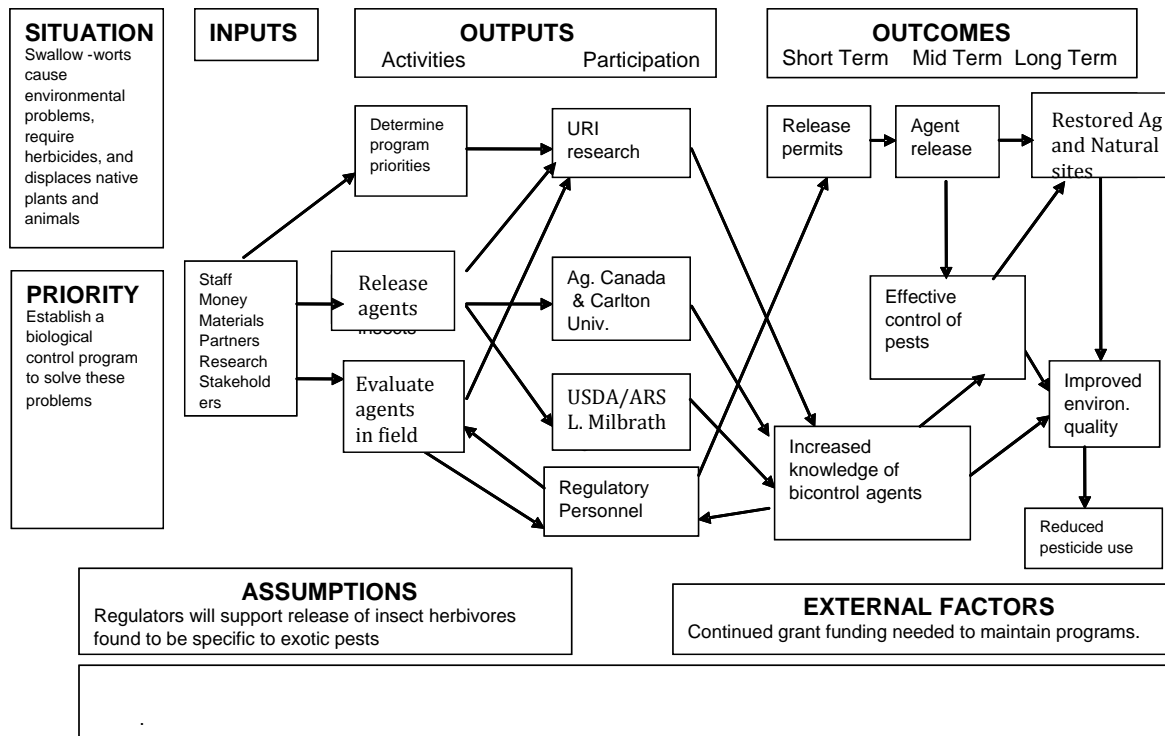
Evaluation of Project Impacts

There are several levels of impacts from this research project. The first level is the impact of the released biological control agent against the target weeds. This impact will be measured by evaluating the post-release status of the swallow-worts in long-term plots where we have already conducted three years of pre-release studies. As described in research approach, we will be monitoring cover, biomass, and reproduction of swallow-worts and other plant species in these plots. Changes in release plots relative to controls will be attributed to the impact of the biological control agent. This impact will be measured through this proposal.

Successful biological control of swallow-worts will have myriad beneficial impacts including increased diversity of plants, arthropods, birds, and even more monarch butterflies as

described earlier in this proposal. Some background data already exist on these ecosystems and changes are not expected over the next three years.

A successful biological control program will reduce the need for management efforts against swallow-wort species. Our growers manage swallow-wort by mowing to prevent seed spread and through the use of herbicides. However, we are not unique in not knowing their labor and herbicide costs for swallow-wort control. Through our regular IPM programming, we are in touch with managers of nurseries, Christmas tree plantations, pastures, orchards, and vineyards in Rhode Island and in many cases; we are already using their pesticide records to evaluate IPM programming. If funded through this proposal, we will extend our data collecting to include herbicide use and other costs for managing swallow-worts for a representative subset of Rhode Island growers and land managers. This will allow us to establish at least a partial baseline cost for these weeds. These data will provide the only cost data available (to our knowledge) on swallow-worts as well as serving as a baseline – against which to measure economic impact of swallow-wort biological control several years from now.



Key Personnel

Project Director: R.A. Casagrande, URI Professor of Entomology, initiated research on swallow-wort biological control in 2000 and 2001, working with A. DiTommaso (Cornell), A. Gassmann (CABI, Delémont Switzerland), and L. Tewksbury of URI. The initial publication on this topic (Tewksbury et al. 2002) set the stage for limited CABI surveys of

swallow-worts and their natural enemies and European research by Aaron Weed (Casagrande's PhD student) in 2006. Casagrande has directed URI research and partially funded and coordinated CABI research on swallow-worts for over a decade. He will oversee all aspects of this project.

Co-PD: Aaron Weed, PhD, Research Associate, Dartmouth College, discovered and evaluated European natural enemies of swallow-worts for his PhD dissertation and he has actively participated in this research since 2005. Aaron will assist in directing sampling, agent release, and evaluation while working from New Hampshire as well as several visits to the URI Biological Control Laboratory and field sites. He will assist in data analysis, reporting, publication, and presentations. We will pay his travel costs for these efforts.

Co-PD: Lisa Tewksbury, MS, directs the URI Biological Control laboratory where she serves as Quarantine Officer and supervises the activities of students and other personnel working with that facility. Lisa has overseen the production and testing of swallow-wort biological agents and she will produce, distribute, and release the agents as described in this proposal. Lisa established and monitored the swallow-wort pre-release sites and she will be responsible for post-release sampling as described. A portion of Lisa's salary will be covered through this proposal.

Heather Faubert has worked with RI growers since 1980, serving as our IPM scout and liaison with the fruit growers, vegetable growers, nursery producers, and Christmas tree growers. She has also worked with pasture management for invasive weeds. Heather will direct a student (shared with L. Tewksbury) who will survey these agricultural enterprises to determine their swallow-wort management methods and evaluate costs.

Undergraduate Student: full time summer, will assist in rearing agents in quarantine and assist Lisa Tewksbury in field studies associated with this research. This student will also work with Heather Faubert to develop baseline data on swallow-wort costs. This student labor will be funded through this proposal.

Collaborative Agreements

Dartmouth College, Hanover New Hampshire

Dr. Aaron Weed will continue to advise us in this research and serve as URI's "public face" for this project which he initiated in 2005. He is among the world's experts on the taxonomy and ecology of the swallow-worts and their insect herbivores and he will lend this expertise to this project in addition to his other research tasks at Dartmouth. Aaron will assist in preparing publications (he and Casagrande are editing one at present) and he will continue to give major presentations on this work (most recently at the USDA Annapolis Invasive Species Forum January, 2012.) Dr. Weed will participate in this work from his home base in New Hampshire and make 2-3 visits per year to the URI research lab and field sites. Aaron's letter of support for this project is appended.

Agriculture Canada/Carleton University

Our primary collaborator in Canada on this project is Rob Bouchier of Ag. Canada, Lethbridge, who is working on this project with Sandy Smith and Naomi Cappuccino of Carleton University. Our Canadian colleagues have already committed to this project as evidenced by their release petition for *Hypena opulenta* (letter appended) and letters of support (also appended). The Objective 2 activities described in this proposal for Canada are taken from our joint release petition, submitted in November, 2011. The facilities, personnel, and funding for the Canadian activities described in this proposal are in place.

Additional Collaboration

We have the enthusiastic support of land managers on Naushon Island for initial agent release as well as other potential release sites in Rhode Island and other states (letters appended.) We have agreed to share our colony of *Hypena opulenta* with Dr. L. Milbrath, USDA/ARS (note appended) and we will continue to share test plants and results with his laboratory.

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