GUIDE

for the Importation and Release of

ARTHROPOD BIOLOGICAL CONTROL AGENTS

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada
Canada

Guide for the First-time Importation and Release of Arthropod Biological Control Agents in Canada[1]

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[1]Prepared for the AAFC Biological Control Working Group, May 2017

Electronic version available at www.agr.gc.ca
Catalogue No. A42-122/2017E- PDF
ISBN 978-0- 660-09498- 4
AAFC No. 12700E

Paru également en français sous le titre *Guide relatif à l'importation et dissémination au Canada d'arthropodes destinés à la lutte biologique*

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Cover image of parasitoid courtesy of A. M. Brauner, Agriculture and Agri-Food Canada, Ottawa

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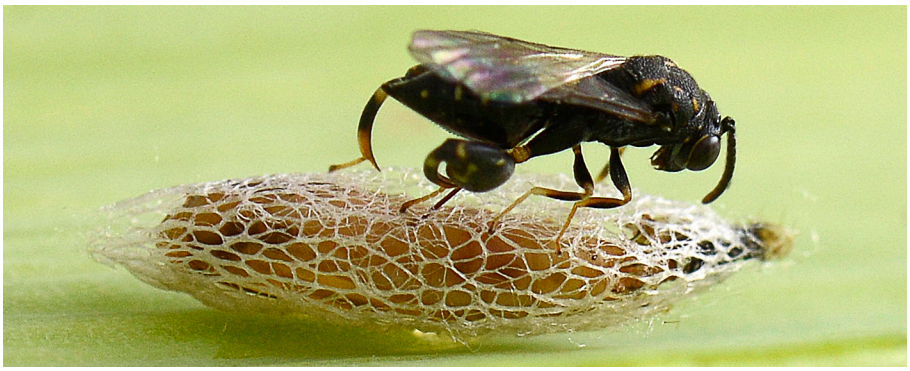


BACKGROUND

Biological control is the use of natural enemies (parasites/parasitoids, predators, pathogens, antagonists, or competitors, i.e., 'agents') to suppress a targeted pest population and often involves the use of arthropods (Van Driesche and Bellows 1996; Heimpel and Mills 2017). Common pest groups targeted with arthropod biological control agents include insects, mites, and weeds. Although lauded as a natural method of pest management on its own, biological control is but one management tool that can be integrated with other methods (e.g., cultural, chemical) for pest control. There are four main biological control strategies, depending on how the organism agents are used.

- The aim of *classical* (= introduction) biological control is the permanent establishment of a foreign, host-specific organism for a pest through one or a small number of targeted introductions.

- *Inundative* biological control involves the repeated and controlled application of large numbers of a biological agent to immediately reduce a pest population. Inundative biological control is generally not self-sustaining, unlike classical biological control, and thus is conducive to commercialization.

- *Augmentative* biological control involves the increase of an established biocontrol organism, whether native or introduced, through release of additional individuals.

- *Conservation* biological control pertains to management of habitat or environmental conditions that are conducive to increase in population size of an established biological control agent and its impact on the targeted pest.

These strategies are not mutually exclusive. For example, once a classical agent becomes established, conservation methods could be developed to facilitate population increase, or the agent could be used augmentatively with mass rearing. Similarly, the boundary between inundative and augmentative approaches is not clearly defined.

Any biological control program must consider the ecological ramifications associated with the chosen strategy, particularly because the release of a living, self-propagating organism can be a permanent, non-reversible action (e.g., the intent of classical biological control). Protection of the environment is a priority of the Canadian government, and thus there is regulatory oversight for the implementation of biological control. This takes the form of the Plant Protection Act (S.C. 1990) for importation and release of arthropod biological control agents, and the Pest Control Products Act (S.C. 2002) for the inundative use of microbial agents in Canada.

The process of finding the most appropriate arthropod biological control agents for a program, and subsequently obtaining regulatory approval for their first time release in Canada, is typically a long and complex process. It includes careful study and safety evaluation of the candidate agents by scientists. This process has been particularly rigorous for release of arthropods for classical weed biological control (e.g., up to 10 years or longer for some programs), because of public concerns over the safety of plants of both economic and environmental value.

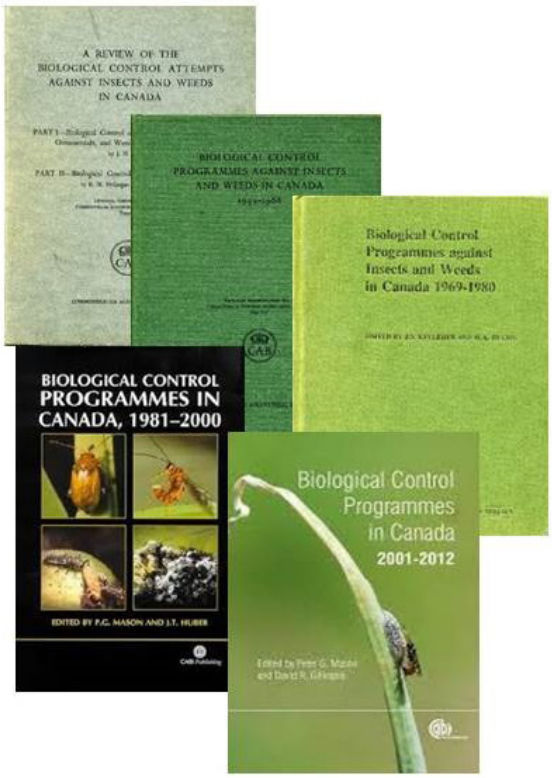
When requesting permission to release arthropod agents for control of either weed or pest arthropods, scientifically-based consideration of the potential economic and environmental risks must be demonstrated by those requesting the release.

Any test results obtained during a biological control program, together with other relevant information on the ecology and biology of a candidate agent, are presented in a petition submitted to the Canadian Food Inspection Agency (CFIA). The CFIA then solicits the recommendation of expert reviewers and makes a decision to grant or deny permission to import and release the named agent.

When requesting permission to release arthropod agents for control of either weed or pest arthropods, scientifically-based consideration of the potential economic and environmental risks must be demonstrated by those requesting the release.

INTENT OF THIS GUIDE

This guide provides petitioners, reviewers of petitions and interested Canadian citizens with information on the requirements for obtaining regulatory permission for first-time importation and release of insect and mite biological control agents in Canada. Where necessary, this information is presented separately for agents intended for use against weeds and those for arthropod pests because of slightly different protocols in the testing and petitioning of these agents.



The guide also outlines the process involved in preparing a petition of expected standardized format, and how the actual permit for importation is obtained after an agent has been approved for release. For reviewers, it outlines what information should be provided by petitioners to allow for a scientifically sound assessment of the safety of a candidate agent.

Although classical biological control has a long history of safe use in Canada (see Winston *et al.* 2014), in recent years, a more rigorous petitioning process has been implemented to ensure its continued safety. The process has moved increasingly towards a harmonized North American strategy because actions in one country may have effects on its neighbours (Mason *et al.* 2005). Petition preparation and review is firmly based on the application of known scientific principles. However, this also means that as a scientific endeavour, the evaluation of biological control agents is continuously evolving toward improved processes. This is true not only in North America but also at global scale. For example, a current and challenging topic of research in classical weed biological control is how to predict the efficacy of agents prior to their release so that only a few, effective agents need be introduced into a new geographic area to control a pest (McClay and Balciunas 2005). Ultimately, both the benefits and risks of every pest control action or non-action must be weighed when making decisions that may affect our environment. Because of its ecological focus, Canada's petitioning process for arthropod introductions provides an opportunity for close scrutiny of our pest control actions, and in the end, greater confidence in our decision-making.

CANADIAN FEDERAL LEGISLATION & REGULATORY PROCESS FOR ARTHROPOD INTRODUCTIONS

Several pieces of legislation and regulatory bodies are involved in the importation and use of classical biological control agents in Canada. Whether as a petitioner, petition reviewer, or an importer and end user of a biological control agent, awareness and compliance with respect to the applicable legislation is critical to the continuity of biological control as an accepted, safe tool in pest management.

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The following are brief descriptions of the key legislations and processes for foreign arthropod introductions.

Plant Protection Act

The main legislation concerning the importation and release of biological control agents in Canada is the *Plant Protection Act* (S.C. 1990). This Act is administered and enforced by the Canadian Food Inspection Agency (CFIA) as Canada's National Plant Protection Organization as defined by the International Plant Protection Convention of 1951 (IPPC 2016).

The stated purpose of the *Plant Protection Act* is "to protect plant life and the agricultural and forestry sectors of the Canadian economy by preventing the importation, exportation and spread of pests and by controlling or eradicating pests in Canada". To that end, the *Plant Protection Act* provides the necessary foundation for the development of requirements for preventing plant pests from entering and being released into the Canadian environment[a] . This includes measures concerning classical biological control agents, which despite their end use as

[a] "Pest" is defined in the Plant Protection Act (s. 3) as "any thing that is injurious or potentially injurious, whether directly or indirectly, to plants or to products or by-products of plants".

beneficial organisms, are regulated by the CFIA both as potential plant pests and pathways for plant pests[b] , especially prior to and during importation. In the same way, commercially produced arthropods (parasitoids and predators) used for greenhouse pest biological control are also regulated by the CFIA.

Plant protection requirements for the importation and release of biological control agents in Canada are provided in the CFIA's policy directive D-12-02: *Import Requirements for Potentially Injurious Organisms (Other than Plants) to Prevent the Importation of Plant Pests in Canada* (2012). This directive applies to a range of organisms that present a risk to plant health, including invertebrates and micro-organisms.

As set out in D-12-02, all non-indigenous biological control agents require approval from the CFIA before their first release into the Canadian environment. Approval is conditional upon the submission to the CFIA of a petition for release and the completion of the petition review process[c].

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Upon approval of a petition, a **plant protection import permit** is issued by CFIA along with a list of strict compliance conditions to be met by the designated importer.

Pest Control Products Act

The Pest Management Regulatory Agency (PMRA) is responsible for administering the *Pest Control Products Act* (S.C. 2002). With a purpose to "protect human health and safety and the environment by regulating products used for the control of pests", this Act provides for the registration of pest control products before they may be manufactured, possessed, handled, stored, transported, imported, distributed or used[c].

Biological control agents that are used inundatively as commercialized, 'off-the-shelf' products in pest control, such as microbial agents (e.g., plant or arthropod pathogens), are regulated as pest control products under

[b] This is consistent with Canada's obligations as contracting party to the International Plant Protection Convention, which recognizes the risks associated with the import and release of biological control agents. In that regard, international standards provide risk management guidance specific to these organisms: ISPM 3, Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms.

[c] "Pest control product" is defined in the Pest Control Products Act (ss. 2(1)) as:
(a) a product, an organism or a substance, including a product, an organism or a substance derived through biotechnology, that consists of its active ingredient, formulants and contaminants, and that is manufactured, represented, distributed or used as a means for directly or indirectly controlling, destroying, attracting or repelling a pest or for mitigating or preventing its injurious, noxious or troublesome effects; (b) an active ingredient that is used to manufacture anything described in paragraph (a); or (c) any other thing that is prescribed to be a pest control product. (produit antiparasitaire)

the *Pest Control Products Act*[d], and as such, must meet registration requirements before they may be imported into, sold or used in Canada. The requirements for the registration of microbial pest control agents and products in Canada are outlined in PMRA's Regulatory Directive DIR2001-02: *Guidelines for the Registration of Microbial Pest Control Agents and Products.*

The Pest Control Products Regulations further describe requirements for the experimental release of microbial pest control agents. The PMRA provides detailed guidance to researchers on Research Notifications and Authorizations on its webpage, as well as in Regulatory Proposal PRO93-05: Research Permit Guidelines for Microbial Pest Control Agents.

Species at Risk Act

Federally, threatened and endangered species are protected by the *Species at Risk Act* (SARA) (S.C. 2002) under the responsibility of Environment and Climate Change Canada (ECCC). Species considered for protection under SARA are assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). COSEWIC assessments classify the species as extinct, extirpated, endangered, threatened, of special concern, not currently at risk, or lacking sufficient information to classify the species. Lists of species in each category are updated annually. In addition to the federal list of species at risk, provinces have their own legislation and lists to protect regionally rare or threatened species. Petitioners for biological control agent introductions are advised to check whether species closely-related to the target species are protected under either federal and/or provincial legislation. Non-target feeding on such species is of particular concern and may prevent release of the agent.

Access and Benefit Sharing

Historically, exploration for natural enemies of a targeted alien pest species, their capture and preservation for identification, and culture for studies on biology and host-specificity was achieved through initiatives sponsored by the countries where the alien pest had invaded and caused economic or environmental damage (Mason and Brodeur, 2013). Access to these genetic resources (a.k.a. biological control agents) was limited only by funding levels or political conflicts that presented safety issues. However, developments such as the Convention on Biological Diversity (CBD) have presented new challenges with the potential to impede biological control. The CBD is an international, legally binding, treaty with three main objectives: 1) conservation of biological diversity; 2) sustainable use of its components; and 3) fair and equitable sharing of benefits arising from genetic resources (Convention on Biological Diversity, 2016).

[d] Microbial pest control agents are naturally occurring or genetically modified microorganisms, including bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae, and related organisms.

However, no guidance was provided on specific methods of implementation, enforcement, etc., leaving participating countries to determine how to comply with the CBD. In 2010, an agreement on Access and Benefit Sharing (ABS), the '**Nagoya Protocol**', came into effect. The Protocol is an agreement between the signatory countries of the CBD as to how to access and share the benefits of genetic resources (including biological control agents), currently and in future (United Nations, 2010). The Protocol states that each country has the responsibility to prepare its own legislation and regulations. Article 8 'Special Considerations' of the Protocol encourages each country developing ABS legislation to: create conditions to promote and encourage research, consider present or imminent emergencies that threaten or damage human, animal or plant health, and consider the importance of genetic resources in food security.

The Convention on Biological Diversity is an international, legally binding, treaty with three main objectives: 1) conservation of biological diversity; 2) sustainable use of its components; and 3) fair and equitable sharing of benefits arising from genetic.

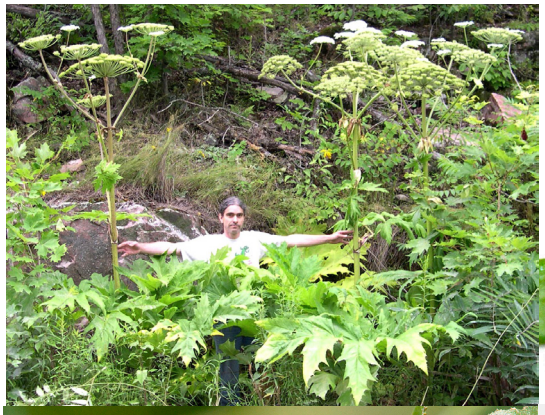
The implications of ABS on biological control could be significant. Bureaucratic procedures can potentially impede exploration for new biological control agents, prevent sending specimens to experts for identification, and create barriers for the exportation of potential agents (Cock, 2010). As with other areas of non-commercial research, such as taxonomy, ecology and general biodiversity (see Feit *et al.*, 2005), biological control is caught between the intent to prevent biopiracy and the need to understand and preserve global biodiversity. The result is that 'prior informed consent' and 'mutually agreed terms', possibly with monetary or non-monetary benefit-sharing mechanisms, will need to be developed for each biological control initiative with each country that is a source of potential agents (Cock *et al.*, 2010). The International Organization for Biological Control (IOBC) has developed Best Practices guidance for the use and exchange of biological control agents (Mason *et al.* 2017).

Currently, Canada has no official ABS system in place. Environment and Climate Change Canada is the ABS lead for Canada and a Federal/Provincial/Territorial Working Group has been created

to determine what an ABS system would be in Canada. To ensure research and development of biological control agents will continue with minimal disruption, the AAFC Biological Control Working Group drafted two documents, "Canadian Biological Control Agent and Pollinator Genetic Resources: AAFC Policy for provision of naturally-occurring beneficial genetic resources to other jurisdictions" and a "standard letter" to be included when shipments of biological control agents of Canadian origin are made (Appendix A) (AAFC Biological Control Working Group, 2009).

Awareness of and compliance with ABS policy in countries where such legislation exists will be key to ensuring that biological control in the future is successful. The International Organization for Biological Control (IOBC) and the Center for Agriculture Bioscience International (CABI) are tracking ABS developments and are sources for information on activities relevant to biological control.

Provision of biological control agents originating in Canada to other countries should follow 'Best Practices'. Documentation to be included when shipments of biological control agents of Canadian origin are made should be based on the "Canadian Biological Control Agent and Pollinator Genetic Resources: AAFC Policy for provision of naturally-occurring beneficial genetic resources to other jurisdictions" and a "standard letter".



Regulatory procedures for first-time introduction of biological control agents

This guide specifically outlines what is required and the regulatory processes that must be followed in requesting and undertaking the first-time importation of foreign arthropods to be used in biological control; whether for field releases of agents in the classical biological control of agricultural and forestry pests (arthropods or weeds), or for commercial use in enclosed environments (e.g. biological control of arthropod greenhouse pests) (Figure 1).

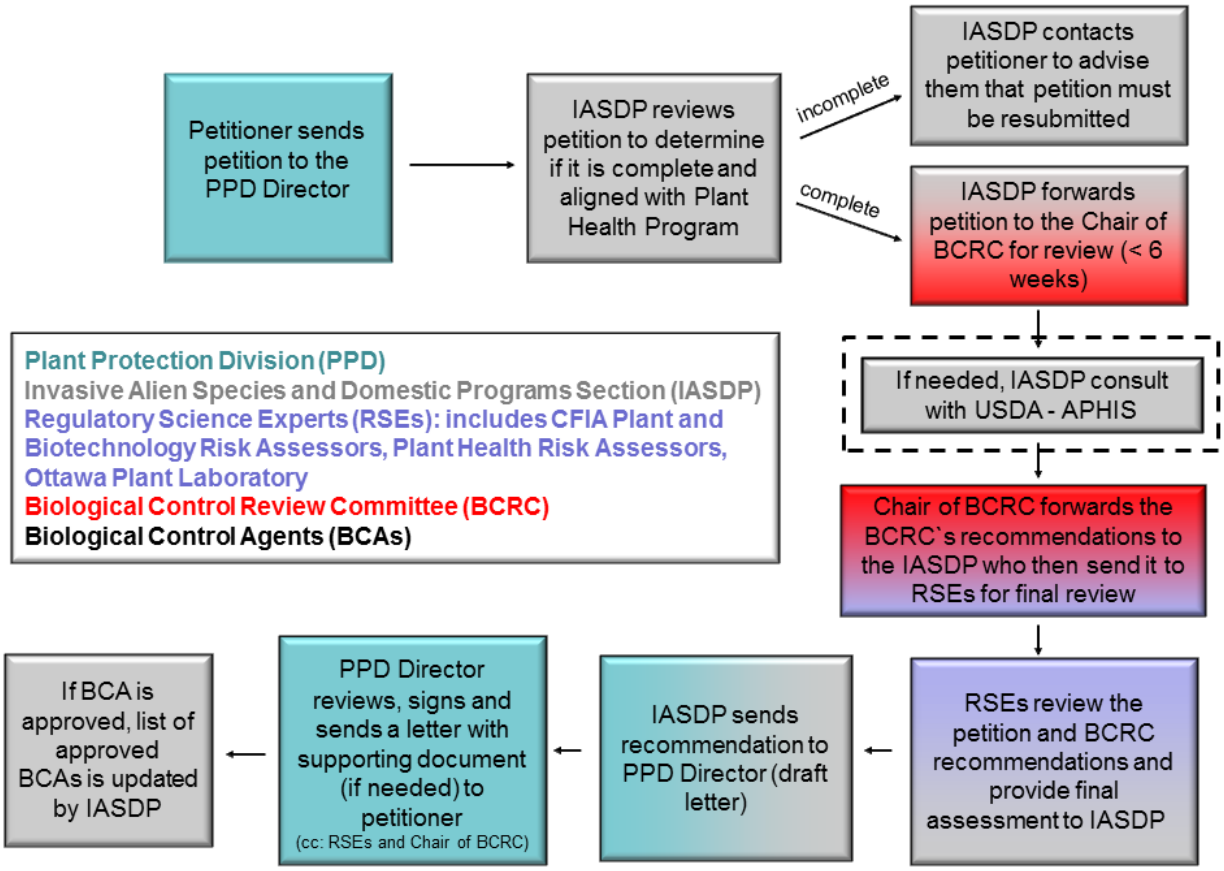


Figure 1. Review process for petitions to release a classical biological control agent in Canada.

Import permits:

Typically, all importations of biological control agents require an import permit from CFIA, whether they are being made for the first-time or not (see the CFIA's Plant Protection Policy Directive D-12-02). The permit is a legal authorization for the importation that accompanies the shipment of live arthropods, and which is inspected by customs and CFIA officials at Canadian ports of entry to ensure that the correct organism(s) is (are) being received.

Currently, there are approximately 60 arthropod biological control agents that have been historically used commercially in

Canada. Due to their safe track record of use, and if imported from CFIA approved sources, these do not have to undergo petitioning and risk assessment prior to importation. However, if these agents are to be imported from a new source, CFIA must receive voucher specimens in advance of issuing an import permit. Under these circumstances, CFIA also requires information on the host species used to propagate the commercial agent, so that additional, unauthorized species are not introduced into Canada with shipments of the biological control agent.

> Arthropods must not be released from the facility into the environment until a petition is reviewed, and official CFIA approval for the release has been granted.

Regulatory petitions:

Research conducted to fulfill the information requirements of a petition for first-time importation and release of agents may be done by the petitioner in association with scientific organizations specialized in this area of research. For example, CABI in Delémont, Switzerland, or the United States Department of Agriculture (USDA), Agricultural Research Services (ARS) European Biological Control Laboratory (EBCL) in Montpellier, France, have had a long history of foreign exploration for and testing of candidate biological control agents for North American invasive, alien pest species.

The process for submission and review of petitions for the first-time importation and release of foreign arthropod biological control agents in Canada is presented in Figure 1 and detailed below as a series of steps. Once an agent is given regulatory approval for release in Canada, then an application must be made to the CFIA for the import permit (see "Available resources", p. 29). A permit application, without full petition, also is made to the CFIA for the importation of arthropods into a Canadian containment facility that has been authorized by the CFIA for receipt and containment of these organisms. This is typically done to allow research to be conducted on candidate biological control agents (e.g., host-specificity and efficacy testing) to generate the necessary data for a petition for their release in Canada.

If the arthropod species being held in containment under a conditional CFIA permit is subsequently approved for release, then the original permit is simply revised by the CFIA so that the conditions against release are dropped.

New regulations under ECCC's New Substances Notification Regulations for Organisms, in future, will require that ECCC be notified by a 'Qualified Designated Authority' (QDA) that the candidate agent has been imported into a certified containment facility (i.e., Plant Protection Certification (PPC) approved by the CFIA). Within AAFC, a QDA would currently be the Local Containment, Biosafety and Biosecurity Committee that reports to the Associate Director, Research and Development at the Centre where the work is being conducted.

> New Environment Canada and Climate Change regulations will require that they be notified by a 'Qualified Designated Authority' that the candidate agent has been imported into a CFIA-certified containment facility.

Steps in the petition review process for release of Biological Control Agents

Step 1

Petitions for the first-time importation and release of foreign arthropods are submitted by the petitioner(s) to the CFIA Director of Plant Protection Division (see "Available resources", p. 25 for address and website information). Petitions contain host-specificity and other biological data on the agent to be imported and released (see section on "Key elements of a petition", p. 15). They also must conform to the format and substance of the North American Plant Protection Organization (NAPPO) standards for the import and release of phytophagous and entomophagous biocontrol agents; Regional Standards for Phytosanitary Measures 7 and 12, respectively (see "Available resources", p. 25).

Step 2

The petition is reviewed by the Invasive Alien Species and Domestic Programs (IASDP) Section of the CFIA to ensure it aligns with the Plant Health Program and to determine if the documentation is incomplete or incorrectly formatted according to NAPPO standards. An incomplete petition requires resubmission with appropriate corrections and/or additions.

Step 3

If documentation is complete, petitions are forwarded to the Chairperson of the Biological Control Review Committee (BCRC) for review. The BCRC is coordinated by the Science and Technology Branch of AAFC, and is composed of Canadian taxonomists in entomology and botany, ecologists, scientists and/or specialists within the Federal and Provincial Governments and Canadian universities, and field consultants. There is also Committee representation from the Pest Management Regulatory Agency (PMRA). The expertise required on the Committee for the review of each petition is determined on a case-by-case basis by the BCRC Chairperson.

Step 4

As needed, the IASDP Section consults with the United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS) on any bilateral issues related to the petition.

Step 5

Petitions received by the BCRC Chairperson are forwarded, with a deadline for review, to the appropriate BCRC members. Petitions also are circulated for comments to the Sanidad Vegetal (Mexico), and those involving classical weed biological control agents are reviewed by the USDA-APHIS Technical Advisory Group (TAG).

Step 6

When all comments have been received by the Chairperson of the BCRC, they are collated and analyzed by the Chairperson. If questions are raised concerning the safety of the introduction, the petitioner(s) may be asked directly by the Chairperson to provide more information or clarification. The Chairperson then summarizes the comments, with any additional information, and makes a recommendation to CFIA Regulatory Science Experts (RSEs) on whether release of the candidate agent into Canada should be allowed. The RSEs include Plant and Biotechnology Risk Assessors, Plant Health Risk Assessors and the Ottawa Plant Laboratory within the CFIA.

Step 7

The RSEs then review all information and the BCRC recommendation on the proposed arthropod introduction. They forward the petition, reviewers' comments, the BCRC recommendation, and their final assessment and recommendation to the IASDP Section.

Step 8

Based on the recommendations from the BCRC and RSEs, the IASDP Section drafts a letter for the Director of the Plant Protection Division (PPD) to advise the petitioner(s) of one of the following decisions; 1) authorization of the importation and release of the arthropod agent, 2) a request for more research on the agent, or 3) a decision to deny the release of the biological control organism in Canada, with reasons provided. A copy of all comments may be provided to the petitioner(s) upon request, while protecting the anonymity of the reviewers.

Step 9

Once approved for release, the organisms can be imported under permit through a CFIA-certified containment facility, where their identity and health (e.g., vigour and disease-free status) are checked prior to release into the environment by the petitioner(s). Petitioners may also opt to rear shipped arthropod agents for at least one generation in containment prior to field release to rid them of internal parasitoids and disease, or to make sure that the colony is solely composed of the approved species. If release is denied, typically there is no further action, although the petitioner(s) can opt to conduct further research and resubmit the petition. A request for further research by the Director PPD typically results in a resubmission of the petition for review. Review and CFIA-PPD Director response to petitions takes approximately 6 months. Timely review of petitions by experts is important because delays can be costly to researchers who may be rearing and/or holding candidate agents ready for potential release. Furthermore, there may be only small windows of time available for these releases to occur, and an extended delay in receipt of the PPD Director's decision may mean waiting another year before agent release. Conversely, petitioners are asked to anticipate a reasonable length of time before receiving a response from the PPD Director and should plan accordingly. Petitions that are well-prepared facilitate timely review.

THINGS TO CONSIDER BEFORE STARTING A BIOLOGICAL CONTROL PROJECT

Biological control is an important strategy for biologically-based pest management either in the protection of human-utilized resources/products (e.g., crops, rangeland, forests) or in the conservation of natural ecosystems (Van Dreische *et al.* 2010). As a living pest control tool, biological control can be self-regulating, self-dispersing and may be regarded as being more environmentally and economically sustainable over the long-term than chemical pesticide use. There also have been numerous examples of biological control's successful use in Canada (Mason and Huber 2002; Mason and Gillespie 2013) and worldwide (McFayden 2000; Wratten and Gurr 2000; Suckling and Sforz 2014; Winston *et al.* 2014) to warrant its serious consideration as a safe, viable and effective method of pest control. However in each case, before a biological control program is initiated a number of issues should be considered to ensure it is an appropriate fit as a control method for a pest (Paynter *et al.* 2015). This is because the pre-release host-specificity testing of potential agents can be a costly, long-term phase of a biological control program. Considerations may include biological issues such as relatedness of the target pest to important beneficial species, economic assessments, impacts of the target species, and social aspects, such as

conflicts of interest that arise when the pest is considered useful. A capability assessment (Van Driesche and Bellows 1996) also may assist in determining if appropriate resources are available to conduct the work. These resources may include the availability of qualified personnel, access to appropriate infrastructure, and organizational commitment to the biological control project.

The foremost thing to consider in a biological control program is whether there is a clear indication of the need to control the target species. Is the target actually a pest and by what definition? What is the economic and/or environmental impact of the target pest? Are there other means to control the target, if so what are they, do they provide effective control, and are they environmentally safe? Barbosa and Segarra-Carmona (1993) proposed criteria that could be used to choose appropriate targets for classical biological control of arthropods. Later, Peschken and McClay (2009) proposed criteria that could be used to select appropriate targets for classical biological control of weeds. They suggest that in addition to determining project priorities, such an evaluation process can identify areas of strength and weakness that need to be addressed during a project. Such a process also can serve to develop an estimate of the costs for a biological control program (e.g., Paynter *et al.* 2015). Although both Barbosa and Segarra-Carmona (1993) and Peschken and McClay (2009) proposed values for each criteria, a 'yes' or 'no' evaluation can be equally useful. Specific points that should be considered as part of a biological control program are presented in Appendix B.

Once it has been established that there are significant negative impacts of the potential target species, and that other control options are ineffective and/or environmentally unsafe, then the feasibility of biological control as a pest management tool can be explored.

The foremost thing to consider in a biological control program is whether there is a clear indication of the need to control the target species.

KEY ELEMENTS OF A PETITION FOR RELEASE OF ARTHROPOD BIOLOGICAL CONTROL AGENTS IN CANADA

Guidelines for information that is required in a petition for the first-time introduction and release of a biological control agent in Canada are available from the North American Plant Protection Organization (NAPPO) (see "Available resources", p. 25). The major elements for a petition for release of exotic phytophagous agents for the biological control of weeds (NAPPO 2015a; RSPM 7) and exotic entomophagous agents for the biological control of pest arthropods (NAPPO 2015b; RSPM 12) include;

- A statement of the proposed action
- Target pest information
- Biological control agent information
- Host-specificity test methods and results
- Environmental and economic impacts of the proposed release
- Pre-release compliance
- A plan for post-release monitoring

To illustrate the format and content of a petition, model petitions are presented (Appendices A, B and C). As well, the evaluation forms used by reviewers are provided (Appendix D and Appendix E). Following is a more detailed description of what is being sought for each of the major elements of a Canadian petition.

a) Proposed action

The information required here is identical for proposed introduction of both exotic entomophagous and phytophagous arthropods for biological control. The following questions should be addressed: a) Why does the targeted pest need to be controlled (i.e., known economic and/or environmental impacts of the pest); b) Why is the particular biological control agent being proposed for introduction (i.e., based on known host-specificity of the agent and any information/predictions on its potential impact on the targeted pest); c) Who will be involved in the releases and monitoring; d) Which rearing/containment facilities will be handling or clearing the agents, and what are the proposed methods of ensuring a pure colony will be released from the facilities; e) What are the proposed methods for mass- rearing and/or release of the agents, including timing of releases. Clear answers to these questions gives decision-makers the information needed to adequately weigh the benefits and risks of the proposed actions. This section also should give the reviewers and regulators confidence that the petitioners are experts on the issues and organisms involved and have an appropriate, clear, and well-thought-out plan for the introductions.

b) Target pest information

There are some slight differences here in the NAPPO-requested information between petitions for entomophagous and phytophagous agent introductions. However, in general, the petitioners are asked to outline known details on the following: a) The targeted pest's systematics, including full classification, taxonomic synonymies, common names and morphological description (any genetic study results also can be provided here); b) Economic impact and benefits of the pest (i.e., the latter is needed for identification of potential

conflicts of interest where some members of society find the pest valuable); c) The pest's distribution within its probable place of origin and in North America; d) The pest's regulatory status (e.g., whether the pest is on provincial or federal lists and regulated in transportation and/or control within Canada); e) Economically or environmentally important species in North America (introduced or indigenous) that are related to the target pest (Note: for entomophagous agents, the relation can be either phylogenetic or related in ecological similarity); f) Whether there are any organisms previously-introduced or indigenous to North America that are known to attack the pest.

Although not requested in the NAPPO guidelines, additional information that helps in a comprehensive risk assessment includes: g) Outline of the target's biology and ecology of relevance to its pest status and control; h) Known distributions of species of economic or environmental importance that are related to the target pest (i.e., will these overlap with the pest's distribution?); i) The pest's status in the USA if it is a joint petition between Canada and the USA, or if there is shared concern between the two countries in control of the pest; j) Whether the target pest is closely- related to one or more threatened or endangered species protected under provincial/state and (or) federal regulations.

c) <u>Biological control agent information</u>

Here the petitioner is requested to cite and/or summarize all known information on the biological control agent that is available from the literature, museums or field observations. Whether the agent is phytophagous or entomophagous, the petition is to include: a) Taxonomy of the agent, how the agent was identified and location of voucher specimens (see Section on "Preparation and importance of voucher specimens....", p. 22). As with the pest, any genetic study results on the agent can be provided here; b) The existing and expected geographic range of the agent (Note: existing range includes both native and other areas of introduction, but petitioners should also discuss any known habitat preferences, climatic requirements, and/or constraints of the agent); c) Source of the agent population that was tested and which will be used for release, with collector and identifier listed and where voucher specimens are located; d) Known host range from various records; e) Related species in proposed area of introduction; f) Life history, including dispersal capabilities and type and levels of damage to

the pest host in its place of origin; g) Known natural enemies of the agent; h) How the agent will be handled in containment (e.g., held in cages, level of security).

d) Host-specificity testing

In order that petition reviewers and regulators arrive at the best possible decision, it is important that the petitioner include the results of well-designed tests (e.g., suited to the biology of the agent and with appropriate controls and number of replicates).

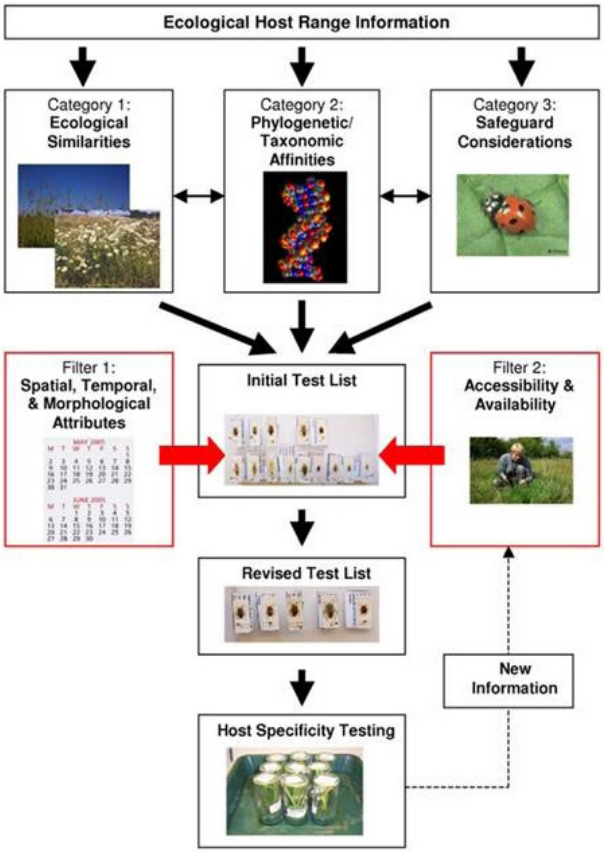
Phytophagous arthropod introductions:

To determine the physiological host range of candidate biological control agents for weeds, researchers use a variation of the "centrifugal phylogenetic method" originally proposed by Wapshere (1974) (Briese 2005). In this method, representative plant species in a progression of levels of relatedness to the target weed (e.g., genetic variations of the target weed species, plants in the same genus, family, and then closely- related families relative to the weed species) are chosen for testing. Also added to the list are plants that are recorded as hosts in the literature, collection records or reports, the reported hosts of arthropod species that are closely-related to the candidate biocontrol agent, and unrelated plant species that have physical or chemical similarities to the target weed. More recently, emphasis has been placed on testing plant species within the most closely-related groups, because if problems in host-specificity arise, they are more likely to be found within these rather than more distantly-related groupings. There also has been a distinct trend toward including more representative species that are indigenous to North America.

Although not officially requested by CFIA, it is recommended that the researchers interested in starting a biological control program on an invasive weed species for the first time prepare and submit for review by the AAFC-BCRC a proposed test plant list before starting testing. In addition to helping set the direction of what may amount to many years of testing, an approved test list may prevent unnecessary testing of species. Its preparation also is an excellent means of becoming familiar with the plant taxon involved, its biogeography, potential species and geographies of concern, and potential sources of native test plant material. Once a test plant species list has been submitted and has received feed-back from scientific experts and regulators, host-specificity testing begins either overseas where the candidate agent occurs naturally, or within a North American containment facility. Tests typically include no-choice and multiple-choice feeding, oviposition and development tests in the laboratory under caged situations and overseas field tests (i.e., either caged or in the open). No-choice results are used to determine the "physiological host range" of an arthropod (i.e., plant species acceptable for the agent's development; narrow for monophagous or oligophagous arthropods), whereas multiple-choice and field studies help predict the "ecological" or field host range of a species (i.e., a subset of the physiological host range; the plant species which are accepted after passing through the species-specific behavioural and ecological filters (Schaffner 2001).

The testing of host range of weed biocontrol agents came under scrutiny by those concerned about the documented attack of plant species indigenous to North America by some arthropod agents previously released for biological control (Louda *et al.* 1997; Strong 1997; Pemberton 2000). Although it was revealed during standard testing of the time that non-target feeding by these agents was possible (i.e., 1960s for testing of Rhinocyllus

conicus for control of introduced thistles), public concerns over the safety of indigenous species; particularly rare and endangered species, have been elevated in recent years (Hinz *et al.* 2014). The results of these concerns are that the countries that use classical weed biological control have become more risk-adverse, and their resultant regulatory policies on the importation and release of arthropod biological control agents have become more conservative (Sheppard *et al.* 2003; Hinz *et al.* 2014). In turn, this has resulted in more stringent, longer-term testing of agents, higher overall project costs, and fewer agents released per weed biological control projects (Sheppard *et al.* 2003). However, it also has encouraged researchers to improve on existing methods of testing so that the results are more predictive of the ecological host range of a candidate biological control agent (Marohasy 1998; Briese 1999), and of recent, also more predictive of the agent's efficacy on the target weed once released (McClay and Balciunas 2005; Morin *et al.* 2009). The risk of underestimating the ecological host range is that economically or environmentally valued species may be negatively affected by the proposed release. However, an overly-conservative estimation of host range may mean that a potentially effective and safe agent may be denied release (Hinz *et al.* 2014).



To ensure that regulators arrive at the best possible decision, it is important that the petitioner include the results of well-designed tests.

Entomophagous arthropod introductions:
Host-specificity testing data is a requirement for petitions for release of entomophagous invertebrates for release. Overall, it is more difficult to test entomophagous arthropods against potential arthropod hosts (i.e., compared to the testing of phytophagous species for weed biological control), because of the many challenges in obtaining, laboratory rearing, testing, and even identifying native arthropods for the tests. However, there have been growing concerns over potential direct and indirect impacts on non-target arthropod species by imported entomophagous biological control agents, and a global call to introduce some host-specificity testing of entomophagous candidates for biological

control (Lockwood *et al.* 2001; van Lenteren *et al.* 2003, 2006; Van Driesche and Reardon 2004; Bigler *et al.* 2006). Methodology for risk assessment has been developed within the EU-financed project "Evaluating Environmental Risks of Biological Control Introductions into Europe (ERBIC)" as a basis for regulation of import and release of exotic natural enemies used in inundative forms of insect pest biological control (i.e., not in classical biological control, although some of the same principles and approaches could apply) (van Lenteren *et al.* 2003, 2006). This methodology integrates information on the potential of an agent to establish, its abilities to disperse, its host range determined from laboratory and field tests, and its predicted direct and indirect effects on non-target insect species.

Determination of host range forms a central element in the whole benefit:risk evaluation process. Lack of host specificity may lead to unacceptable risk if the agent establishes and disperses widely, whereas in contrast, a monophagous (i.e., develops on a single species) biological control agent is not expected to create serious risk even when it establishes and disperses well. Ecological theory is the conceptual and unifying basis for considering non-target risk in the European system, and in determination of host range. A procedure similar to the centrifugal phylogenetic method used for evaluation of weed biological control agents has been proposed.

To determine the physiological host range of candidate biological control agents for arthropods, researchers use multiple criteria to select suitable non-target host species for testing (Kuhlmann *et al.* 2006). In this method, phylogenetic relatedness, ecological similarity, representative species in a progression of taxonomic levels of relatedness to the target pest (e.g., genetic variants of the target species, arthropod species in the same genus, family, and then closely-related families relative to the pest species) are chosen for testing. Also added to the list are arthropods that are recorded as hosts in the literature, collection records or reports, the reported hosts of arthropod species that are closely-related to the candidate biological control agent, and unrelated arthropod species that have physical or chemical similarities to the target pest.

Laboratory tests should be conducted using no-choice and, where applicable, choice designs. No-choice experiments will provide data to show the non-target species that may support development of the agent (i.e., may be at risk as part of the agent's natural host range). Choice tests will demonstrate to what extent a non-target species of concern may be preferred by the agent relative to the target (i.e., species likely to be chosen and fed on by the released agent, and thus, potentially at risk).

CFIA may now request that host range testing for candidate entomophagous agents include arthropods released for biological control of weeds.

Choice tests give an indication of the 'ecological host range' of a candidate biological control agent; which is the range of taxa the agent will actually use as hosts upon release in the new environment. Ecological host range studies may also be conducted in the region where the biological control agent and pest originated. Field collections of target and non-target

species are made in the native range and associated natural enemies are documented. Data generated will demonstrate the actual levels of attack in nature, providing evidence of potential impact on non-target species.

In Canada, potential non-target concerns when using entomophagous arthropods are not only focused on indigenous arthropod species as potential hosts, but also on previously-introduced weed biological control arthropods, particularly if they are phylogenetically close to the target host and occur in areas where releases are being proposed.

e) Environmental and economic impacts of proposed release

The purpose of biological control regulation should not be to discourage the use of biological control, but to ensure a fair, timely and scientifically-based assessment of the potential benefits and risks of the method without blunting its value as an effective tool against invasive pests (Sheppard *et al.* 2003). What must be accepted by all participants is that there is risk inherent in every method of pest control available, including biological control. However, there also is a risk (economic and/or environmental) in choosing not to control an invasive pest species. Available pest control choices and their implications must be carefully weighed during petition review using either a non-formal comparison or formal risk:benefit analysis (see Sheppard *et al.* 2003). The more information that is provided to the reviewers/regulators by the petitioner on the candidate biological control agent, including host specificity test results, host records, impact on target pest and known ecological interactions in place of origin, will help produce a fairer and more accurate assessment of potential environmental and economic impacts of the agent once released. Based on a long and sound history of scientific investigation on host- specificity, we can predict with certainty that there will be fewer direct non-target impacts if the organism is highly host- specific. Predicting the more complex indirect, ecological interactions that may occur post-release of an agent is not as easy, and may only be revealed by post-release monitoring. However, it is the duty of the petitioner to try to assess and predict some of these indirect non-target effects, and of the petition reviewer to weigh the benefit, risk and cost of a release (even if not fully known) against the benefits, risks and costs of other pest control choices. For instance, what is the cost of doing nothing or spraying broad spectrum chemical pesticides year after year versus the potential cost of a non- target impact caused by a released insect? There may be economic benefits for biological control in inaccessible terrain, in undisturbed or protected habitats, against perennial weeds, and on large tracts of land. Biological control may not be as effective against annual plants in large monocultures or in habitats that are frequently disrupted.

f) Post-release monitoring

The petitioner must demonstrate that a plan is in place for post-release monitoring of the biological control agent. The plan should be based on the baseline data collected before release of the agent and should focus on documenting establishment, increase and spread of the agent, and impacts on the target and any non-target species identified during host range testing as potentially vulnerable. Important metrics that should be documented each year at release sites for at least the first 5 years and at intervals (e.g., every 5 years) thereafter include: number of individuals present on the target species at a pre-determined point in the life-cycle of the agent for inter-year comparisons ; number of individuals present at selected distances from the release location; and number of target and non-target species attacked by the agent, where the two may co-occur. Other parameters that should be considered for monitoring include: changes in the growth, reproduction and survival of target and attacked non-target individuals; changes in the growth, persistence , and distribution of target and non-target populations; and any noticeable changes in community-level processes and structure (e.g., shift in species composition or diversity). Although it can take many years to detect an impact of the agent, and there may be no guarantee of funding for the monitoring of releases, the information in the post-release monitoring plan is important to decision-making within a specific biological control program. The information also plays an important role in further development of existing methods of agent host specificity testing or release, with the aim of continuously improving on the overall safety and efficacy of biological control.

g) Pre-release compliance

At the time of submission, the petitioner must provide proof that voucher specimens have been submitted to the Canadian National Collection of Insects Arachnids and Nematodes (CNC), from each colony or source of agents to be released. Voucher specimens are meant to verify the identity of the released specimens and provide a basis for future taxonomic research. Details are provided below on the preparation and importance of voucher specimens.

PETITION REVIEW GUIDELINES

The onus is on the reviewers of petitions to ensure that a petition for the first-time introduction of a foreign biological control agent provides relevant, scientifically-based information on the agent, target pest, and if known, on potential non-targets. A thorough and scientifically-sound petition allows accurate weighing of the potential benefits and risks of releasing a non-indigenous organism into our environment, and ultimately, arrival at the best possible and most appropriate decision for all involved. Following is a list of questions to help guide reviewers in their important task. Also attached is a sample Reviewer's Comment Sheet from the AAFC-BCRC (Appendix E).

1. Are the systematics of the target pest and its distribution well described, with references and taxonomic expertise identified? Have voucher specimens been deposited in the CNC in Ottawa?

2. Are the systematics of the biological control agent and its distribution well described, with references and taxonomic expertise identified? Have voucher specimens been deposited in the CNC in Ottawa?

3. Have parasites/parasitoids and pathogens of the biological control agent been identified?

4. Have the known information/references on the biology and ecology of the biological control agent been included?

5. Have any native biological control agents of the target pest been described along with their relative importance as control agents?

6. Have appropriately replicated and controlled host-specificity tests been conducted?

7. Is the location(s) and timing of release(s) included in the submission?

8. Is there any previous history of use of the biological control agent in the proposed area, other Canadian regions, in the USA or other countries?

9. Is there any testing/consideration of potential non-target effects from release of the biological control agent (i.e., both direct and indirect effects)?

10. Is a post-release monitoring program in place or planned for the biological control agent?

11. Have constraints been identified in advance of the planned release? Are any potential environmental impacts anticipated?

12. What is the environmental impact of inaction or alternative controls instead of using the biological control agent? Has a risk:benefit analysis been done?

CANADA'S CONTAINMENT SYSTEM

In Canada, there is a protocol for the verification and use of containment facilities for the importation, rearing and handling of entomophagous and phytophagous arthropods, including biological control agents that have not yet been approved for release in Canada.

Canadian Food Inspection Agency requirements

The process and requirements for importing and handling potentially injurious organisms, including biological control agents, are set out in the CFIA's Plant Protection Policy Directive D-12-02. Because of the potential for causing harm to plant health in Canada, some organisms may only be imported under containment and cannot be released into the environment. The import of these organisms is allowed under an import permit, provided that appropriate physical and operational containment requirements are in place at destination.

Importers must demonstrate that their containment facility complies with plant pest containment requirements described in the CFIA's Containment Standards for Facilities Handling Plant Pests. These standards present the requirements for the different plant pest containment (PPC) levels, which in the research context range from the

lowest PPC Basic to the highest PPC-3. The appropriate level is determined by the CFIA based on the review and assessment of an import permit application, in consideration of the proposed organism and intended use.

If PPC-1 containment, or higher is required and the destination facility has not yet been verified for compliance with the Containment Standards for Facilities Handling Plant Pests, a verification process (i.e., certification) is undertaken by the CFIA.

Once the CFIA is satisfied that appropriate containment requirements are met, a permit is issued and the import and handling of the organism is allowed. The CFIA may conduct facility inspections to assess and monitor compliance with the requirements, and as needed take enforcement action in response to situations of non-compliance.

If the construction or modification of a containment facility is planned, you are encouraged to communicate, as applicable, with the CFIA for guidance concerning the plant pest containment standards.

AAFC National Containment, Biosafety and Biosecurity Requirements

Agriculture and Agri-Food Canada (AAFC) is committed to ensuring that research activities involving human and animal pathogens, and "other organisms requiring containment" (i.e., biological control agents) are conducted in facilities respecting appropriate legislation and utilizing best practices and procedures that reduce the risks to the public at large, stakeholders, employees, animals, plants, and the environment (AAFC, 2015). To fulfill this commitment AAFC formed the **National Containment, Biosafety, and Biosecurity Committee** (NCBBC) to assist the Department in developing a containment and biosafety program. The program ensures that the Department's research activities comply with a uniform set of operational and design requirements with the goal of ensuring that all facilities handling plant pests and pathogens (and human/animal pathogens) meet the appropriate containment standards set by CFIA (and Public Health Agency of Canada).

The minimum operational and scientific design requirements to achieve an appropriate level of work-site containment associated with handling pests are set out in "Containment, Biosafety, and Biosecurity Guidelines for AAFC Research Facilities".

The NCBBC developed "Containment, Biosafety, and Biosecurity Guidelines for AAFC Research Facilities" to set out the minimum operational and scientific design requirements to guide researchers toward achieving an appropriate level of work-site containment associated with handling pests (AAFC, 2015).

The NCBBC also provides guidance and training to Associate Directors, Research, Development and Technology and local containment/ Biosafety, and Biosecurity Committees (LCBBC) with respect to risk assessments, project review, and approval.
When required the NCBBC reviews research proposals and makes recommendations regarding project approval to the responsible local manager, the Associate Director, Research, Development and Technology regarding project approval.

Part III of the Guidelines outlines the containment standards required for handling plant pests and other organisms. Essentially, CFIA's *Containment Standards for Facilities Handling Plant Pests* govern work by AAFC scientists on plant pests and other organisms requiring containment. In the case of biological control agents, International Standards developed under the auspices of the North American Plant Protection Organization (NAPPO) take precedence: e.g., NAPPO Regional Standard for Phytosanitary Measures (RSPM) 22, *Guidelines for the Construction and Operation of a Containment Facility for Insects and Mites used as Biological Control Agents*, takes precedence over the CFIA *Containment Standards For Facilities Handling Plant Pests*.

PREPARATION AND IMPORT OF VOUCHER SPECIMENS/ ROLE OF TAXONOMISTS

In biological control, accurate identification of both the agent and target species is essential to ensuring that the correct species is tested and released. Misidentifications may result in unintended harm to non-target species and ecosystems. To ensure that identifications are accurate, a series of voucher specimens should be deposited in the CNC for every biological control project. Voucher series should be made from colonies used for biological control releases and also any wild populations used for pre-release biological studies. Written records supported by well preserved and properly labelled voucher specimens allow for repetition of scientific studies associated with a biological control project (Huber 1998). A voucher series also will enable misidentifications to be corrected and the taxonomic names to be updated as systematic reviews and revisions are made. Voucher series can also provide material for taxonomists to study for revisions. If properly preserved and labelled, voucher specimens are essentially permanent and can be studied repeatedly as new techniques become available to verify their identity (Huber 1998).

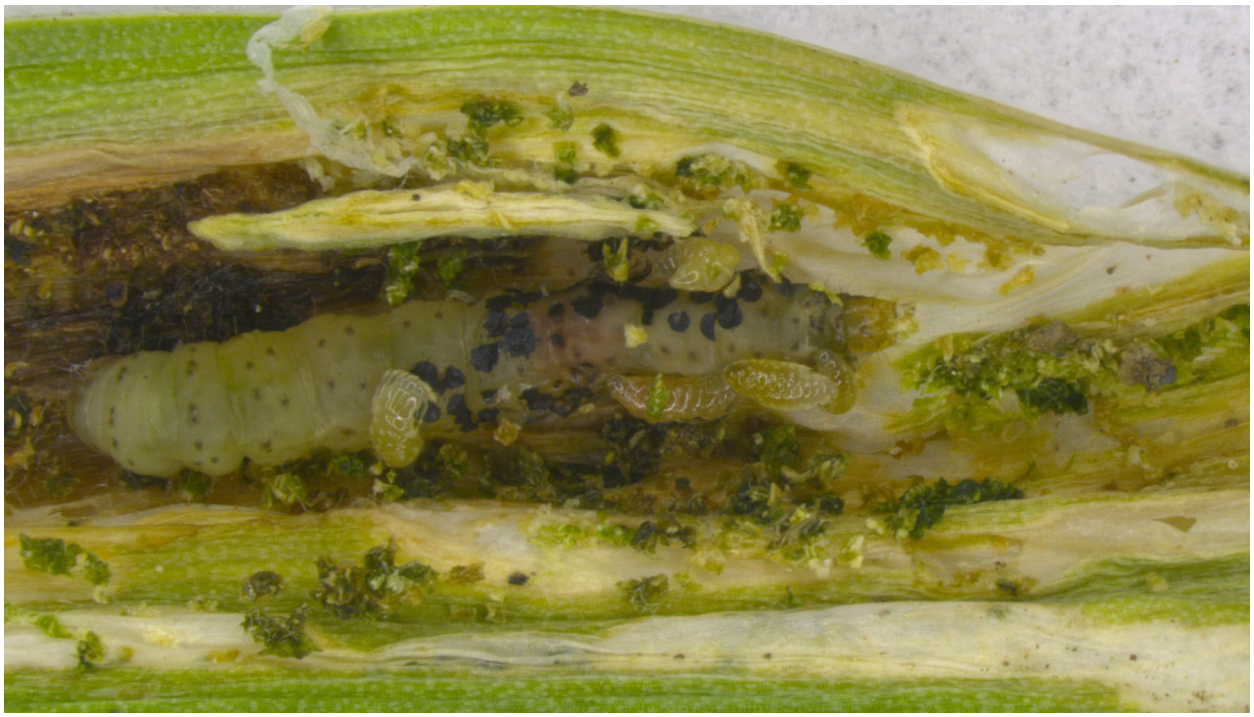
Relevant experts have sometimes misidentified the voucher specimens used to identify biological control agents. This can happen because of inaccuracies in the taxonomic literature. To reduce the possibility of this recurring, a subset of the voucher specimens should be 'DNA barcoded' (Hebert *et al.* 2003). Barcode mitochondrial DNA sequences (Cytochrome oxidase, subunit-I) should be compared with (and deposited) in public databases (e.g., Barcode of Life Database (BOLD), The EMBL Nucleotide Sequence

Database, National Center for Biotechnology Information (NCBI), and results included in the petition document. Barcode sequences are used to test morphological species' determinations, and can be obtained from tissue from one leg, leaving the remainder of the voucher specimen intact. The value of barcodes is that they provide a quick and inexpensive method to detect 'cryptic' species and (or) increase confidence in morphological determinations. Barcodes deposited in public databases are assigned a unique identifying number; i.e., the accession number. This number should be included in the petition, and on a label on the voucher specimen from which it came.

Noyes (1994) and Huber (1998) provide examples of the consequences associated with misidentification of candidate biological control agents. These include: 1) individuals believing that they are working with the same species when in fact they are not, leading to conflicting information being generated (e.g., a known solitary parasitoid being newly reported as a gregarious parasitoid); 2) individuals believing they are working on different species when they are actually working with the same species generates duplicate data; 3) individuals believing that they are working with a single species that turns out to be a complex of closely-related species or unrelated species that are difficult to recognize. For example, Trichogramma was long thought to include only three Nearctic species (Pinto 1998). Once it was discovered that many more existed (68 are described by Pinto (1998)) the information contained in the hundreds of publications for which no voucher specimens were available has become virtually useless. In contrast, Gibson *et al.* (2005) examined voucher specimens deposited in accessible collections of parasitoids reared from pest management projects on cabbage seedpod weevil and determined that the specimens had been incorrectly identified as species introduced as biological control agents. In fact, the specimens belonged to native congeners and the introduced species had not established. Not only was the taxonomy of these groups clarified and updated but the conclusion that biological control of cabbage seedpod weevil had failed was refuted and a new initiative was begun.

Misidentifications of potential agents in background studies can also wrongly eliminate candidate biological control agents. Such errors can incorrectly indicate that the host range (i.e., specificity) of the potential agent is too broad for biological control use. Many published host-parasitoid lists are unreliable because of misidentifications (Noyes 1994). Taxonomic experts should be consulted or involved in biological control research because such flawed catalogues are often the starting point for biological control projects.

Similarly, taxonomic experts, literature and vouchers (arthropod or plant) are necessary for verifying identifications of host species used in host-specificity testing (arthropods and plants). Host plant vouchers should be taken wherever biological research is done



on plant species for which identification is difficult. Noyes (1994) provides useful recommendations including: 1) in publications, only mention host and agent names that are verified by a taxonomist; 2) consult appropriate taxonomists before starting a project; 3) isolate hosts and preserve their remains with the parasitoids that emerge; 4) include locality and host information with any material submitted for identification; 5) deposit voucher material in a well-maintained major collection (e.g., the CNC or DAO herbarium in Ottawa) where it is available to regulatory personnel and to scientists conducting taxonomic reviews/ revisions; 6) state in any publication who identified the material and where the voucher series are deposited.

Proper preservation and labelling of arthropod voucher material is as important as ensuring that a series is retained. Huber (1998) provides guidance on these procedures for arthropods. Biological control workers should be particularly careful to fix specimens in the appropriate manner for further processing and storage by someone else. The voucher material should be: 1) correctly labelled; 2) clean; 3) complete and intact, i.e., no body parts missing; 4) fully developed adults; and 5) properly preserved (Huber 1998). Details for preserving specimens are outlined in Martin (1977) and Huber (1998). Standards for preparing labels are provided by Huber (1998) and Wheeler *et al.* (2001) http://biologicalsurvey.ca/briefs.

The order of information that should be included on the arthropod specimen label is:

1. Country;
2. Lesser political unit (e.g., province, state);
3. County (if applicable);
4. Exact locality that can be found on a map;
5. Distance and compass point from map locality, if needed;
6. Altitude or depth, if needed;
7. Latitude and longitude, to the nearest second (critical information);
8. Date of collection (day, month in Roman numerals, year);
9. Emergence date, if different fromcollection date;
10. Habitat or, for parasitic and phytophagous insects, the host;
11. Collector's name;
12. Collection technique.

Proper preservation and labelling of plant voucher material is also important. Carter *et al.* (2007) give information for properly preparing herbarium specimens. The voucher material should be: a) entire plants or all plant parts; b) mature; c) in flower if possible. Standards for preparing labels vary among herbaria. For the Department of Agriculture Ottawa (DAO) collection, the order of information that should be included on the herbarium label is:

1. Plant identification (genus, species, authority, family)
2. Collection locality (country; lesser political unit (e.g., province, state); county (if applicable); exact locality (critical information); altitude or depth, if needed; latitude and longitude, to the nearest second or .001 decimal degrees
3. Habitat and description of plant
4. Collector and reference number
5. Collection date (day, month, year)

RELEASE AND REDISTRIBUTION OF ARTHROPOD BIOLOGICAL CONTROL AGENTS

There are no federal regulations in place that restrict the movement of arthropod biological control agents once they are authorized by CFIA for introduction and use in Canada. Only Newfoundland and Labrador has legislation that restricts movement of agents from other regions of Canada into their province (see also Mason *et al.* 2013). However, it is in the best interests of biological control for practitioners to demonstrate due diligence in the movement of any arthropod, regardless of whether it has been given regulatory approval for use as a biological control agent or not. For instance, periodically either native or accidently introduced arthropods may be found using invasive pest species and then be considered for use in biological control.

If mass produced or collected as biological control agents and moved into regions where they do not naturally occur, these species may cause harm. Canada is a very large and geoclimatically diverse country, made up of a mosaic of ecologically distinct regions.

Obviously, not all introduced or indigenous species are appropriate for release within all Canadian ecosystems, depending on what vulnerable species or ecological communities may be present. The obvious problems associated with moving and releasing poor choices of species into inappropriate regions include the inadvertent reduction of biodiversity as well as the possible reassignment of previously non-pest species to economic pest status.

It is recommended that before any redistribution of a proposed arthropod agent is made to a different ecoregion of Canada that the potential risks be carefully assessed first using existing information. If pre- notified of intent, a government expert in biological control could help guide and inform those seeking to relocate an agent; e.g. based on host specificity test results from an existing petition for agent release, or other pertinent literature. If a significant potential risk to relocating an arthropod exists, it may be recommended to conduct additional studies on the arthropod and submit a dossier of information to regulatory authorities notifying them of and justifying the proposed action.

ACKNOWLEDGEMENTS

We thank Chandra Moffatt (AAFC, Fredericton) and Paul Abram (AAFC, Agassiz) for reviewing this guide. Thanks also to Stephen Darbyshire for advice on preparing host plant voucher specimens. Thanks to Jessica Hsiung (AAFC, Ottawa) for layout and graphics. The support of the Biodiversity and Bioresources program and the Pest Management Centre are also acknowledged. This guide was prepared by members of the AAFC Biological Control Working Group.

SOURCE OF PHOTOS

Cover/sections, *Diadromus pulchellus* attacking leek moth pupa – A.M. Brauner, AAFC Ottawa, ON

Page 1, top, *Conura albifrons* parasitizing leek moth – A.M. Brauner, AAFC Ottawa, ON; right, *Argiope aurantia* 'spinning' a grasshopper – A.M. Brauner, AAFC Ottawa, ON

Page 2, *Tetrastichus setifer* ovipositing in Lilioceris lilii larva – A.M. Brauner, AAFC Ottawa, ON

Page 3, Biological Control Programmes in Canada book covers

Page 4, *Hypena opulenta* larva on *Vincetoxicum rossicum* – A.M. Brauner, AAFC Ottawa, ON

Page 6, *Eumorpha achemon* larva with *Cotesia congregata* parasitoid cocoons – A.M. Brauner, AAFC Ottawa, ON

Page 7, *Rhinusa pilosa* gall on *Linaria vulgaris* toadflax – R.A. De Clerck-Floate, AAFC Lethbridge, Alberta

Page 8, clockwise from top left, *Heracleum mantegazzianum* - B. Flahey, AAFC Ottawa, ON; *Lilioceris lilii* ovipositing on Lilium – A.M. Brauner, AAFC Ottawa, ON; *Cirsium arvense* – S. Darbyshire Brauner, AAFC Ottawa, ON *Agrilus planipennis* mating – A.M. Brauner, AAFC Ottawa, ON

Page 11, *Mecinus janthiniformis* on *Linaria dalmatica* – R.A. De Clerck-Floate, AAFC Lethbridge, AB

Page 12, *Calophasia lunula* on *Linaria vulgaris* – R.A. De Clerck-Floate, AAFC Lethbridge, AB

Page 14, top, Asilidae fly – A.M. Brauner, AAFC Ottawa, ON; bottom, *Bagous nodulosus* on *Butomus umbellatus* – Ivo Tovseski, CABI, Serbia

Page 16, left, *Aulacidea pilosellae* on *Pilosella officinarium* – T. Haye, CABI, Switzerland; right, gregarious parasitoids on *Fraxinus* gall – A.M. Brauner, AAFC Ottawa, ON

Page 18, multi-critera non-target host selection method – T. Haye, CABI, Switzerland

Page 20, *Telenomus podisi* on *Halyomorpha halys* eggs – P. Abram, AAFC Agassiz, BC

Page 21, top, *Phymata pennsylvanica* male on female feeding on bee – A.M. Brauner, AAFC Ottawa, ON; bottom, *Diadromus collaris* on pupae of *Plutella xylostella* – T. Haye, CABI, Switzerland

Page 22, left, *Popilla japonicas* with eggs of *Istocheta aldrichi* – A.M. Brauner, AAFC Ottawa, ON; right, *Anastatus bifasciatus* parasitizing *Halyomorpha halys* eggs – Photo T. Haye, CABI, Switzerland

Page 23, *Mogulones crucifer* on *Cynoglossum officinale* – R.A. De Clerck-Floate, AAFC
Page 24, containment laboratory – R.A. De Clerck-Floate, AAFC Lethbridge, Alberta
Page 26, *Trichomalus perfectus* on silique – T. Haye, CABI, Switzerland
Page 27, top, *Habrobracon* sp. larvae feeding on *Acrolepiopsis assectella* larva – A.M. Brauner, AAFC Ottawa, ON; bottom, *Balaustium* sp. feeding on eggs of *Halyomorpha halys* – W. Wong, AAFC Agassiz, BC
Page 28, left, *Vincetoxicum rossicum* herbarium specimen – S. Darbyshire, AAFC Ottawa, ON; right, *Diadromus pulchellus* and *Gambrus ultimus* pinned museum specimen – A.M. Brauner, AAFC Ottawa, ON
Page 29, top, field release of *Diadromus pulchellus* ; bottom, sentinel pupae of *Acrolepiopsis assectella* – A.M. Brauner, AAFC Ottawa, ON
Page 30 *Mantis religiosa religiosa* – A.M. Brauner, AAFC Ottawa, ON

AVAILABLE RESOURCES

a) List of key departments/organizations involved in the importation process.

Canadian Food Inspection Agency
Plant Protection Division, Invasive Alien Species and Domestic Programs Section,
59 Camelot Drive, Floor 2, Ottawa, Ontario K1A 0Y9 CANADA
http://www.inspection.gc.ca/

Application to Import Plants and other Things Under the Plant Protection Act
http://www.inspection.gc.ca/DAM/DAM-plants-vegetaux/STAGING/text-texte/c5256_1331652913719_eng.pdf

D-12-02: Import Requirements for Potentially Injurious Organisms (Other than Plants to Prevent the Importation of Plant Pests in Canada)
http://www.inspection.gc.ca/plants/plant-pests-invasive-species/directives/imports/d-12- 02/eng/1432586422006/1432586423037

Containment Standards for Facilities Handling Plant Pests - First Edition
http://inspection.gc.ca/plants/plant-pests-invasive-species/biocontainment/containment- standards/eng/1412353866032/1412354048442?chap=0

Agriculture and Agri-Food Canada
Ottawa Research and Development Centre,
960 Carling Avenue, Ottawa, Ontario K1A 0C6 CANADA
http://www.agr.gc.ca/eng/science-and-innovation/research-centres/ontario/ottawa-research-and-development-centre/?id=1180546650582

Lethbridge Research and Development Centre,
5403 1st Avenue South Lethbridge, Alberta T1J 4B1 CANADA
http://www.agr.gc.ca/eng/science-and-innovation/research-centres/alberta/lethbridge-research-and-development-centre/?id=1180547946064

b) Locations and contacts for major insect and botanical collections in Canada that may be of use to petitioners and petition reviewers:

National Identification Services
Agriculture and Agri-Food Canada, Ottawa Research and Development Centre,
960 Carling Avenue, Ottawa, Ontario K1A 0C6 CANADA

Canadian National Collection of Insects, Arachnids and Nematodes
http://www.agr.gc.ca/eng/science-and-innovation/research-centres/ontario/ottawa-research-and-development-centre/the-canadian-national-collection-of-insects-arachnids-and-nematodes/?id=1270047992811

Canadian National Collection of Vascular Plants
http://www.agr.gc.ca/eng/science-and-innovation/research-centres/ontario/ottawa-research-and-development-centre/the-agriculture-and-agri-food-canada-collection-of-vascular- plants/?id=1251393521021

c) Useful references including web sites:

International Plant Protection Convention
International Standards for Phytosanitary Measures – ISPM 3 Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms; ISPM 5 Glossary of Phytosanitary Terms
https://www.ippc.int/en/core-activities/standards-setting/ispms/

North American Plant Protection Organization
NAPPO Regional Standards for Phytosanitary Measures – RSPM 7 Preparation of Petitions for First Release of a Non-indigenous Phytophagous or Phytopathogenic Biological Control Agent; RSPM 12 Preparation of Petitions for First Release of a Non-indigenous Entomophagous Biological Control Agent; RSPM 22 Guidelines for Construction and Operation of a Containment Facility for Insects and Mites used as Biological Control Agents
http://www.nappo.org/english/standards-and-protocols/regional-phytosanitary-standards- rspms

Insect Liberations Database
Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, 960 Carling Avenue, Ottawa, Ontario K1A 0C6 CANADA

Reviewer's Manual for the Technical Advisory Group for Biological Control Agents of Weeds
USDA-APHIS-PPQ
https://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/tag-bcaw_manual.pdf

GLOSSARY

Unless cited differently, the following terms follow the FAO definitions ISPM 5 'Glossary of Phytosanitary Terms 2007' (FAO 2007), and the NAPPO RSPM 5 'Glossary of Phytosanitary Terms 2013' (NAPPO 2013).

AAFC – Agriculture and Agri-Food Canada

antagonist – an organism (usually pathogen) which does no significant damage to the host but its colonization of the host protects the host from significant subsequent damage by a pest [ISPM No.3, 1996]

ARS – Agricultural Research Service of the United States Department of Agriculture

BCRC – Biological Control Review Committee. Canadian committee of experts that reviews petitions for first-time release of arthropods for biological control

biological control agent – a natural enemy, antagonist or competitor, and other organism used for pest control [ISPM No.3, 1996; revised ISPM No. 3, 2005]

biological control (biocontrol) – pest control strategy making use of living natural enemies, antagonists or competitors and other self-replicating biotic entities [ISPM No.3, 1996]

CABI – Centre for Agriculture Bioscience International. International non-profit organization specializing in biological control

CFIA – Canadian Food Inspection Agency

CFS – Canadian Forest Service of Natural Resources Canada

classical biological control – the intentional introduction and permanent establishment of an exotic biological agent for long-term pest control [ISPM No.3, 1996]

competitor – an organism which competes with pests for essential elements (e.g., food, shelter) in the environment [ISPM No.3, 1996]

containment (of a biological control agent) – official confinement of regulated organisms for observation and research or for further inspection, testing and/or treatment

containment facility – a building for safe storage and/or propagation of biological control agents

control (of a pest) – suppression, containment or eradication of a pest population [FAO, 1995]

ECCC – Environment and Climate Change Canada

ecoarea – an area with similar fauna, flora and climate and hence similar concerns about the introduction of biological control agents [ISPM No.3, 1996]

ecological (field) host range (of a biological control agent) – host organisms that a natural enemy actually accepts and uses in the environment, under natural field conditions (Van Klinken, 2000).

ecosystem – a dynamic complex of plant, animal and micro-organism communities and their abiotic environment interacting as a functional unit [ISPM No. 3, 1996; revised ICPM, 2005]

entomophagous – organisms that eat insects [NAPPO Doc. 001-001-01]

establishment – perpetuation, for the foreseeable future, of an organism within an area after entry [FAO 1995; IPPC 1997; formerly 'established']

establishment (of a biological control agent) – the perpetuation, for the foreseeable future, of a biological control agent within an area after entry [ISPM No. 3, 1996]

exotic – not native to a particular country, ecosystem or ecoarea (applied to organisms intentionally or accidentally introduced as a result of human activities). As this Code is directed at the introduction of biological control agents from one country to another, the term "exotic" is used for organisms not native to an area or country [ISPM No.3, 1996]

FAO – Food and Agriculture Organization of the United Nations

habitat – part of an ecosystem with conditions in which an organism naturally occurs or can establish [ICPM, 2005]

host – the organism in or on which a parasite lives; the plant on which an insect [mite] feeds; maker of a cell or other structures in which guest insects [mites] take up their abode (Nichols, 1989)

host range – species of plants (or animals) capable, under natural conditions, of sustaining a specific pest [FAO, 1990]

host-specificity (see specificity)

IASDP – Invasive Alien Species and Domestic Programs section of the Canadian Food Inspection Agency

ICPM – Interim Commission on Phytosanitary Measures, Food and Agriculture Organization, Rome, Italy

import permit (of a biological control agent) – official document authorizing importation (of a biological control agent) in accordance with specified requirements [FAO, 1990; revised FAO, 1995; ICPM, 2005]

introduction (of a biological control agent) – the release of a biological control agent into an ecosystem where it did not exist previously (see also "establishment") [ISPM No. 3, 1996]

inundative release – the release of large numbers of a mass-produced biological control agents or beneficial organisms with the expectation of achieving a rapid effect [ISPM No.3, 1996; revised ISPM No. 3, 2005]

IPPC – International Plant Protection Convention as deposited in 1951 with FAO in Rome and as subsequently amended [FAO, 1990; revised ICPM, 2001]

ISPM – International Standard for Phytosanitary Measures [CEPM, 1996; revised ICPM, 2001]

legislation – any act, law, regulation, guideline or other administrative order promulgated by a government [ISPM No.3, 1996]

monitoring – an official ongoing process to verify phytosanitary situations [CEPM, 1996]

monophagous – feeding on a single species or genus of hosts (Bernays and Chapman, 1994)

multiple-choice tests - host range tests in which candidate hosts are presented in groups of several species to the organism being evaluated (Van Driesche and Bellows, 1996)

NAPPO – North American Plant Protection Organization

National Plant Protection Organization – official service established by a government to discharge the functions specified by the IPPC [FAO, 1990; formerly Plant Protection Organization (National)]

natural enemy – an organism which lives at the expense of another organism in its area of origin and which may help to limit the population of that organism. This includes parasitoids, parasites, predators and pathogens [ISPM No.3, 1996; revised ISPM No. 3, 2005]

no-choice tests – host range tests in which candidate host species are presented separately to the organism being evaluated (Van Driesche and Bellows, 1996)

oligophagous – feeding on a restricted number of species, usually from within a family or subfamily of plant classification for phytophagous organisms (Bernays and Chapman, 1994)

organism – any biotic entity capable of reproduction or replication, in its naturally occurring state [ISPM No. 3, 1996; revised RSPM No. 3, 2005]

oviposition – the act of depositing eggs (Nichols, 1989)

parasite – an organism which lives on or in a larger organism, feeding upon it [ISPM Pub. No.3, 1996]

parasitoid – an insect parasitic only in its immature stages, killing its host in the process of its development, and free-living as an adult [ISPM No.3, 1996]

pathogen – micro-organism causing disease [ISPM No.3, 1996]

pest – any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products [FAO, 1990; revised FAO, 1995; IPPC, 1997]

petition – a formal, written application to a regulatory agency seeking approval to release a non-native biological control agent or pollinator into the envrionment [RSPM 12, 2008; RSPM 29, 2008]

PHD – Plant Health Division of the Canadian Food Inspection Agency

phylogenetic – relating to phylogeny (i.e., evolutionary relationships among organisms) (Nichols, 1989)

physiological host range (of a biological control agent) – the range of host organisms that a natural enemy is capable of accepting and/or using for its survival. Typically the physiological host range is broader than the ecological host range for a specialist species (Van Klinken, 2000)

phytophagous – pertaining to organisms that eat plants

PMRA – Pest Management Regulatory Agency, federal agency within Health Canada that is responsible for the regulation of pest control products in Canada

predator – a natural enemy that preys and feeds on other animal organisms, more than one of which are killed during its lifetime (ISPM No. 3, 1996]

quarantine (of a biological control agent) – official confinement of biological control agents subject to phytosanitary regulations for observation and research, or for further inspection and/or testing [ISPM No.3, 1996] (see containment of a biological control agent)

regional standards – standards established by a regional plant protection organization for the guidance of the members of that organization [IPPC, 1997]

Regulatory Experts – individuals responsible for assessing applications for import and release of insects, mites and terrestrial mollusks under the authority of the Plant Protection Act and Regulations of the Canadian Food Inspection Agency

release (into the environment) – intentional liberation of an organism into the environment (see also "introduction" and "establishment") [ISPM No. 3, 1996]

RSPM – Regional Standards for Phytosanitary Measures

specificity – a measure of the host range of a biological control agent on a scale ranging from an extreme specialist only able to complete development on a single species or strain of its host (monophagous) to a generalist with many hosts ranging over several groups of organisms (polyphagous) [ISPM No.3, 1996]

standard – document established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context [FAO, 1995; ISO/IEC GUIDE 2:1991 definition]

suppression – the application of phytosanitary measures in an infested area to reduce pest populations [FAO, 1995; revised CEPM, 1999]

synonymy – the relationship between synonyms (i.e., each of 2 or more scientific names of the same rank used to denote the same taxon); a list of synonyms (Nichols 1989)

systematics – the study of biological classification (Nichols, 1989)

TAG – Technical Advisory Group of the USDA-APHIS which reviews petitions for the release of organisms for the classical biological control of weeds

taxonomic synonymies – lists of synonyms (see synonyms) relating to scientific names (Nichols, 1989)

USDA-APHIS– United States Department of Agriculture-Animal and Plant Health Inspection Service

voucher specimens – a series of individuals from a specific population deposited in the National Collection(s) of the country [RSPM 5, 2013]

REFERENCES

AAFC (2015) Containment, Biosafety, and Biosecurity Guidelines for AAFC Research Facilities. AAFC National Containment, Biosafety, and Biosecurity Committee, Science and Technology Branch, Ottawa, Canada, 127p. Available at: http://intranet.agr.gc.ca/agrisource/AgriDoc.do?id=2185108&lang=eng

Barbosa, P. and Segarra-Carmona, A. (1993) Criteria for the selection of pest arthropod species as candidates for biological control. *In* Van Driesche, R.G. and Bellows, T.S. (eds.). *Steps in Classical Arthropod Biological Control*. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, Maryland, USA, pp.5-23.

Bernays, E.A., and Chapman, R.F. (1994) *Host-plant selection by phytophagous insects*. Contemporary topics in entomology 2. Chapman and Hall Inc., New York, USA.

Bigler, F., Babendreier, D., and Kuhlmann, U. (eds.) (2006) *Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment.* CABI Publishing, Wallingford, UK.

Briese, D.T. (1999) Open field host-specificity tests: is 'natural' good enough for risk assessment? *In* Withers, T.M., Barton Browne, L. and Stanley, J. (eds.) *Host Specificity Testing in Australasia: Towards Improved Assays for Biological Control*. CRC for Tropical Pest Management, Brisbane, Australia, pp.44-59.

Briese, D.T. (2005) Translating host-specificity test results intothe real world: The need to harmonize the yin and yang of current testing procedures. *Biological Control* 35: 208– 214.

Carter, R., Bryson, C.T, and Darbyshire, S.J. (2007) Preparation and use of voucher specimens for documenting research in weed science. *Weed Technology* 21: 1101-1108.

Cock, M.J.W. (2010) Biopiracy rules should not block biological control. *Nature* 467, 369.

Cock, M.J.W., van Lenteren, J.C., Brodeur, J., Barratt, B.I.P., Bigler, F., Bolckmans, K., Consoli, F.L., Haas, F., Mason, P.G. and Parra, J.R.P. (2010) Do new access and benefit sharing procedures under the Convention on Biodiversity threaten the future of biological control? *BioControl* 55: 199–218.

Convention on Biological Diversity (CBD) (2016) List of Parties. Available at: http://www.cbd.int/convention/parties/list/

De Clerck-Floate, R., Plue, P. and Lee, T. (eds.) (2004). *A Tour of the Lethbridge Research Centre Insect Microbial Containment Facility*. AAFC, Lethbridge, AB.

FAO (2013) ISPM 5 Glossary of Phytosanitary Terms Available at: https://www.ippc.int/sites/default/files/documents/20140214/ispm_05_en_2014-02-14cpm-8_201402141055--559.25%20KB.pdf

Feit, U., von den Driesch, M. and Lobin, W. (eds.) (2005) Access and Benefit-Sharing of Genetic Resources: Ways and means for facilitating biodiversity research and conservation while safeguarding ABS provisions. Report of an international workshop in Bonn, Germany held in 2005, 8-10 November. Convened by the German Federal Agency for Nature Conservation. Available at: https://www.bfn.de/fileadmin/MDB/documents/service/skript163.pdf

Gibson, G.A.P., Baur, H., Ulmer, B., Dosdall, L. and Muller, F. (2005) On the misidentification of chalcid (Hymenoptera: Chalcidoidea) parasitoids of the cabbage seedpod weevil (Coleoptera: Curculionidae) in North America. *The Canadian Entomologist* 137: 381– 403.

Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R. (2003) Biological identification through DNA barcodes. *Proceedings of the Royal Society of London Series B: Biological Sciences* 270: 313–321.

Heimpel, G.E. and Mills, N.J. (2017) Biological Control: *Ecology and Applications*. Cambridge University Press, Cambridge, UK.

Hinz, H.L., Schwarzländer, M., Gassmann, A. and Bourchier, R.S. (2014) Successes we may not have had: A retrospective analysis of selected weed biological control agents in the United States. *Invasive Plant Science and Management* 7: 565–579.

Huber, J.T. (1998) Importance of voucher specimens. *Journal of Natural History* 32: 367–385.

IPPC (2016) International Plant Protection Convention – revised text 1997. Available at: https://www.ippc.int/en/publications/131/

Lockwood, J.A., Howarth, F.G. and Purcell, M.F. (eds.) (2001) *Balancing Nature: Assessing the Impact of Importing Non-native Biological Control Agents.* Thomas Say Publications in Entomology: Proceedings, Entomological Society of America, Lanham, Maryland.

Louda, S.M., Kendall, D., Connor, J. and Simberloff, D. (1997) Ecological effects of an insect introduced for the biological control of weeds. *Science* 277: 1088–1090.

Marohasy, J. (1998) The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. Biocontrol News and Information 19:13N-20N.

Martin, J.E. (1977) *Collecting and Preserving Insects, Mites and Spiders. The Insects and Arachnids of Canada Part 1*. Research Branch, Canada Department of Agriculture Publication1643, 182p.

Mason, P.G. and Gillespie, D.R. (eds.) (2013) *Biological Control Programmes in Canada 2001-2012*. CAB International Publishing, Wallingford, UK.

Mason, P.G. and Huber, J.T. (eds.) (2001) *Biological Control Programmes in Canada, 1981-2000.* CABI Publishing, Wallingford, UK.

Mason, P.G., Flanders, R.G. and Arrendondo-Berna, H.A. (2005) How can legislation facilitate the use of biological control of arthropods in North America? *In* Hoddle, R.G. (Compiler) *Proceedings of the 2nd International Symposium on Biological control of Arthropods, Davos, Switzerland, 12-16 September 2005*, United States Department of Agriculture, Forest Service, Morgantown, WV, FHTET-2005-08, Vol II, pp. 701-714.

Mason, P.G., Kabaluk, J.T., Spencer, B. and Gillespie, D.R. (2013) Regulation of biological control in Canada. *In* Mason, P.G. and Gillespie, D.R. (eds.) *Biological Control Programmes in Canada 2001-2012*. CAB International Publishing, Wallingford, UK, pp.1-5.

Mason, P.G., Cock, M.J.W., Barratt, B.I.P., Klapwijk, J., van Lenteren, J.C., Brodeur, J., Hoelmer, K.A. and Heimpel, G.E. (2017) Best practices for the use and exchange of invertebrate biological control genetic resources relevant for food and agriculture. *BioControl* DOI 10.1007/s10526-017-9810-3

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

McFadyen, R.E.C. (2000) Successes in biological control of weeds. pp 3-14. *In* Spencer, N.R. (ed.). *Proceedings of the X International Symposium on Biological Control of Weeds 4-14 July 1000*, Montana State University, Bozeman, Montana, USA. Forest Health Technology Enterprise Team, FHTET-99-1, Morgantown, West Virginia, USA, 95p.

Morin, L., Reid, A.M., Sims-Chilton, N.M., Buckley, Y.M., Dhileepan, K., Hastwell, G.T., Nordblom, T.L., Raghu, S., (2009) Review of approaches to evaluate the effectiveness of weed biological control agents. *Biological Control* 51: 1–15.

NAPPO (2011) *Guidelines for Construction and Operation of a containment Facility for Insects and Mites used as Biological Control Agents*. RSPM 22. Available at: http://www.nappo.org/english/standards-and-protocols/regional-phytosanitary-standards- rspms/rspm-22/

NAPPO (2013) *Glossary of Phytosanitary Terms.* RSPM 5. Available at: http://www.nappo.org/english/standards-and-protocols/regional-phytosanitary-standards- rspms/rspm-05/

NAPPO (2015a) *Guidelines for Petition for Release of Exotic Phytophagous Agents for the Biological Control of Weeds.* NAPPO, RSPM 7. Available at: http://www.nappo.org/english/standards-and-protocols/regional-phytosanitary-standards- rspms/rspm-07/

NAPPO (2015b) *Guidelines for Petition for Release of Exotic Entomophagous Agents for the Biological Control of Pests.* NAPPO, RSPM 12. Available at: http://www.nappo.org/english/standards-and-protocols/regional-phytosanitary-standards- rspms/rspm-12/

Nichols, S.W. (compiler) (1989) *The Torre-Bueno Glossary of Entomology.* The New York Entomological Society and American Museum of Natural History, New York, NY, 840p.

Noyes, J.S. (1994) The reliability of published host-parasitoid records: a taxonomist's view. *Norwegian Journal of Agricultural Sciences*, Supplement No. 16: 59–69.

Paynter, Q., Fowler, S.V., Hayes, L., and Hill, R.L. (2015) Factors affecting the cost of weed biocontrol programs in New Zealand. *Biological Control* 80: 119–127.

Pemberton, R.W. (2000) Predictable risk to native plants in weed biological control. *Oecologia* 125: 489-494.

Peschken, D.R. and McClay, A.S. (2009) Picking the target: A revision of McClay's scoring system to determine the suitability of a weed for classical biological control. [reprint of contribution] *In* Delfosse, E.S. and Scott, R. R. (eds.) *Proceedings of the Eighth International Symposium on the Biological Control of Weeds February 2-7, 1997*. Lincoln University, Canterbury, New Zealand (1995), pp. 137–143.

Pest Control Products Act (2002) Available at: http://laws-lois.justice.gc.ca/PDF/P-9.01.pdf

Pinto, J.D. (1998) Systematics of the North American species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae). *Memoirs of the Entomological Society of Washington* Number 22, 287p.

Plant Protection Act (1990) http://laws-lois.justice.gc.ca/PDF/P-14.8.pdf

Schaffner, U. 2001. Host range testing of insects for biological weed control: How can it be better interpreted? *Bioscience* 51(11): 951–959.

Sheppard, A.W., Hill, R., De Clerck-Floate, R.A., McClay, A., Olckers, T., Quimby, P.C. Jr. and Zimmerman, H. (2003) A global review of risk-benefit-cost analysis for the introduction of classical biological control agents against weeds: a crisis in the making? *Biocontrol News and Information* 24(4): 77N-94N.

Strong, D.R. (1997) Fear no weevil? *Science* 277: 1058–1059.

Suckling, D.M. and Sforza, R.F.H. (2014) What magnitude are observed non-target impacts from weed biocontrol? PLoS ONE 9(1): e84847, 12p.

Van Driesche, R.G. and Bellows, T.S. (1996) *Biological Control*. Chapman and Hall, New York, NY, USA.

Van Driesche, R. G. and Reardon, R. (eds.) (2004) *Assessing Host Ranges for Parasitoids and Predators Used For Classical Biological Control: A Guide to Best Practice*. USDA, Forest Health Technology Enterprise Team, FHTET-2004-03, Morgantown, West Virginia, USA, 243p.

Van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D.L., Blossey, B., Brancatini, V., Casagrande, R., Causton, C.E., Coetzee, J. A., Cuda, J., Ding, J., Fowler, S.V., Frank, J.H., Fuester, R., Goolsby, J., Grodowitz, M., Heard, T.A., Hill, M.P., Hoffmann, J.H., Huber, J., Julien, M., Kairo, M.T.K., Kenis, M., Mason, P., Medal, J., Messing, R., Miller, R., Moore, A., Neuenschwander, P., Newman, R., Norambuena, H., Palmer, W.A., Pemberton, R., Perez Panduro, A., Pratt, P.D., Rayamajhi, M., Salom, S., Sands, D., Schooler, S., Sheppard, A., Shaw, R., Schwarzländer, M., Tipping, P.W., van Klinken, R.D. 2010. Classical biological control for the protection of natural ecosystems: past achievements and current efforts. *Biological Control* 54: S2–S33.

Van Klinken, R.D. (2000) Host specificity testing: Why do we do it and how we can do it better. Briese, D.T. *In* R. Van Driesche, R., Heard, L.Y., McClay, A. and Reardon, R. (eds.) *Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Agents – The Biological Basis for Improvement in Safety.* X International Symposium on Biological Control of Weeds, Bozeman, Montana, USA, July 4-14, 1999. Forest Health Technology Enterprise Team, Morgantown, West Virginia, USA, FHTET-99-1, pp. 54-68.

van Lenteren, J.C., Babendreier, D., F. Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., Van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G. and Zeng, Q.-Q. (2003) Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl* 48:3–38.

Wapshere, A.J. (1974) A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* 77: 201–211.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cock, M.J.W. and Julien, M.H. (eds.) (2014) *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*, 5th edition. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia, USA, FHTET-2014-04, 838 p.

Wheeler, T.A., J.T. Huber, J.T., and Currie, D.C. (2001) *Label Data Standards for Terrestrial Arthropods*. Biological Survey of Canada (Terrestrial Arthropods), Document Series No. 8, 1- 20.

Wratten, S. and Gurr, G. (eds.) (2000) *Measures of Success in Biological Control*. Kluwer Academic Publishers. Dordrecht, The Netherlands.

APPENDIX A
Access and Benefits Sharing Best Practices Policy

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Canadian Biological Control Agent and Pollinator Genetic Resources: AAFC Policy for provision of naturally-occurring beneficial genetic resources to other jurisdictions

The use of naturally-occurring beneficial genetic resources (NBGR) as agents for the biological control of pest species is a long-standing and environmentally-friendly strategy to reduce damage to organisms of economic importance. The provision of NBGR from jurisdictions where the pest is native to those where the pest has invaded is a common and standard practice. The NBGR is introduced to provide some level of suppression of the pest species and the public is provided multiple benefits such as reduced economic losses, reduced pesticide use, a healthier environment, etc. Provision of NBGR for pollination of crops from where they are native to areas where they are not native has also been practiced with the goal to increase yields.

In Canada, provision of NBGR to governments of other countries and/or non-government organizations (NGOs) is made by Agriculture and Agri-Food Canada (AAFC). The NBGR are provided upon request by individuals representing governments, other institutions, or private enterprise and without formal documentation except where AAFC or the receiving party may have an administrative policy for material transfer or other relevant agreements.

Agriculture and Agri-Food Canada is the major supplier of NBGR for uses in agriculture, including native ecosystems that may be linked to agricultural practices such as rangelands and riparian zones. In particular, AAFC supplies NBGR for use as biological control agents to suppress pest species that may or may not be naturally distributed in Canada.

Conditions under which provision of NBGR is made include:

- NBGR provided by AAFC are done so with the understanding that the receiving individual, company or organization has met all regulatory requirements of their country for receipt of the material

- Upon receipt of a NBGR, a letter acknowledging that the material has been received in good order must be signed, dated and returned to the AAFC supplier.

- All uses benefit the public and that new knowledge generated is made widely available.

- Live material, and data supplied by AAFC as exchange or in response to requests, is provided for research purposes and, if approved by local regulatory officials release into nature. AAFC provides no warranty and accepts no responsibility or liability for the suitability of such material or data for any use or study. Assessment of suitability of such material and data for intended use is the responsibility of the receiving institutions or researchers and it is expected that due assessment will be carried out to ensure that the use of the material will not endanger non-target organisms.

- Voucher specimens of biological control agents and associated data supplied by AAFC as exchange or, in response to requests, are provided for research purposes. These specimens are subject to loan agreements if borrowed from the Canadian National Collections.

- If an NBGR provided by AAFC is the subject of a publication or referenced in a publication, Agriculture and Agri-Food Canada should be acknowledged as the source of the live material, dead specimens and/or data. We appreciate receiving notice and re-prints or electronic copies of all publications that include material provided by AAFC. These can be sent to the contact person who supplied the NBGR.

Conditions under which provision of NBGR is not appropriate and should be denied include:

- If there are research studies indicating that there is a significant threat that harmful organisms, such as parasites and pathogens associated with the NBGR, will be introduced into the target jurisdiction and harm non-target organisms or reduce the effectiveness of the NBGR.

- Where there is a significant threat demonstrated that collection of the Canadian population(s) of the NBGR will have a detrimental effect on those populations or may cause disruption of ecosystem services in Canada.

- Where the NBGR is a federally or provincially listed species or under consideration for species at risk status by the COESWIC.

Note: The consequences of any lack of attention to these conditions, for example, leading to damage to non-target organisms or loss of NBGR populations, will result in limited future access to Canadian NBGR.

APPENDIX B
Considerations When Developing a Biological Control Project

Considerations before starting an arthropod biological control project*.

A. Economic/Environmental Aspects	
Level of damage/crop losses:	
Severe	[ ]
Moderate	[ ]
Light	[ ]
Nature of pest problem:	
Persistent	[ ]
Most years	[ ]
Some years	[ ]
Pest of public concern:	
Yes	[ ]
No	[ ]
Pest with alternative societal uses/value (i.e., potential for conflict of interest if control successful):	
Yes	[ ]
No	[ ]
Pest invasiveness in natural habitats:	
High level of threat to native species biodiversity and/or ecosystem function	[ ]
Medium threat	[ ]
Low threat	[ ]
Designated uniqueness or sensitivity of natural habitat under threat from invading pest:	
Priority for protection	[ ]
Medium	[ ]
Low	[ ]
Losses ($$) or environmental impact if no control:	
Low	[ ]
Moderate	[ ]
High	[ ]
Pest status in crop/production or environmental system:	
Sole key pest	[ ]
No key pests	[ ]
Other key pests exist	[ ]
Type of pest:	
Indirect losses	[ ]
Direct/indirect	[ ]
Direct	[ ]

Potential for rapid and extensive spread of pest:	
Low	[ ]
Moderate	[ ]
High	[ ]
Other active biological control agents targeting pest in system:	
Yes	[ ]
No	[ ]
Other methods of pest control being used (e.g., cultural, chemical):	
Yes	[ ]
No	[ ]
If chemical control, environmental effects:	
Groundwater contaminant	[ ]
Broad-spectrum	[ ]
Narrow-spectrum	[ ]
Existing IPM program in crop/production system:	
Yes	[ ]
No	[ ]

B. Biological Aspects	
Pest origin:	
Introduced	[ ]
Native	[ ]
Crop/habitat stability (if applicable to production system):	
Perennial	[ ]
Annual	[ ]
Pest feeding habits/location:	
Exposed habitat	[ ]
Concealed habitat	[ ]
Pre-introduction studies: occurrence of natural enemies:	
Comprehensive knowledge (taxonomy, distribution)	[ ]
Limited knowledge	[ ]
No knowledge	[ ]
Pre-introduction studies: impact of natural enemies:	
Life table studies available	[ ]
Some data on mortality/impact caused by natural enemies	[ ]
No data available on mortality/impact	[ ]
Status of biological control in other areas/countries:	
Establishment/increase phase	[ ]
Release phase	[ ]
Testing phase	[ ]
Exploration phase	[ ]
Initiation phase	[ ]
No program	[ ]
Efficacy of biological control in other areas of release using same agent or species closely-related to the agent being considered:	
Pest controlled	[ ]
Pest partially controlled	[ ]
No control	[ ]

Availability of biological control agents:	
Local/commercial source	[ ]
National source	[ ]
Foreign source	[ ]
No known source	[ ]

* Adapted from Barbosa and Segarra-Carmona (1993)

Considerations for ranking target weeds according to their suitability for classical biological control*.

A. Economic aspects	
Economic losses:	
Very severe	[ ]
Severe	[ ]
Light	[ ]
Infested area:	
Very large	[ ]
Large	[ ]
Small	[ ]
Expected spread:	
Extensive	[ ]
Small	[ ]
Toxicity (health problems caused to humans and/or livestock):	
Very severe	[ ]
Severe	[ ]
None or small	[ ]
Available means of control:	
Environmental damage High	[ ]
Medium	[ ]
Low	[ ]
Economic justification Low or not justified	[ ]
Medium	[ ]
High	[ ]
Beneficial aspects:	
None or small	[ ]
Major	[ ]
Very major	[ ]

B. Biological aspects	
Infraspecific variation:	
Small (asexual, selfing, vegetative breeding system)	[ ]
Medium (sexual, outcrossing breeding system)	[ ]
Extensive (sexual, outcrossing breeding system)	[ ]
Geographical area where the weed is native:	
Native only outside North America	[ ]
Native to North America and other regions	[ ]
Cosmopolitan or origin unknown	[ ]
Relative abundance:	
More abundant/aggressive in area where it is to be controlled than in area of origin	[ ]
Possibly more so	[ ]
Not so	[ ]

Success of biological control elsewhere:	
Under full biological control elsewhere	[ ]
Under partial biological control elsewhere	[ ]
Biological control not attempted	[ ]
Biological control attempts failed elsewhere	[ ]

Number of known promising biological control agents:	
One score for each promising species 0-?	[ ]
Habitat stability:	
High (i.e., rangeland, permanent pastures)	[ ]
Moderate (perennial crops, extensive sources of infestation on waste land, roadsides)	[ ]
Low (damage virtually restricted to annual cropland)	[ ]
Number of economic species in the same genus:	
0	[ ]
1	[ ]
>1	[ ]
Number of economic species in the same tribe:	
0	[ ]
1 to 3	[ ]
4 to 8	[ ]
>8	[ ]
Number of ornamental species in same genus:	
0	[ ]
1 to 5	[ ]
>5	[ ]
Number of ornamental species in same tribe:	
0	[ ]
1 to 15	[ ]
>15	[ ]
Number of native North American species in same genus:	
0	[ ]
1 to 20	[ ]
>20	[ ]
Number of native North American species in same tribe:	
0	[ ]
1 to 41	[ ]
41 to 120	[ ]
>120	[ ]

* Adapted from Peschken and McClay (2009)

APPENDIX C
Comment Sheet for Review of a Petition for either an Entomophagous or Phytophagous Species Intended for Classical or Inundative/Augmentative Biological Control within Canada

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

REVIEWER'S COMMENT SHEET
PETITION FOR INTRODUCTION OF A BIOLOGICAL CONTROL AGENT

Proposed agent (genus, species, family):	Petition Designation:
	Canada CAN
	Mexico
Target species (scientific and common names):	U.S.A.
	Petition Date:
	Date sent to Reviewers:
Proposed release area(s) are in:	Review due date:
Canada (X) U.S.A. ( ) Mexico ( )	Return to: Ms. Andrea Brauner, Secretary CAN BCRC Fax: 613-759-1926 Agriculture and Agri-Food Canada Research & Development Centre, K.W. Neatby Building 960 Carling Avenue Ottawa, ON K1A 0C6 CANADA
Petitioner(s) and organization(s):	

REVIEWER RECOMMENDATIONS

	Acceptable	Unacceptable*	Did not assess	Insufficient information
Taxonomy of target	( )	( )	( )	( )
Test species list	( )	( )	( )	( )
Taxonomy of agent	( )	( )	( )	( )
Biology of agent	( )	( )	( )	( )
Host range tests	( )	( )	( )	( )
Impact on non-target species	( )	( )	( )	( )
Post-release monitoring	( )	( )	( )	( )
Other	( )	( )	( )	( )

APPROVAL FOR RELEASE IS:

(a) recommended without reservations ( )

(b) recommended with reservations* ( )

(c) not recommended* ( )

COMMENTS: required for any item marked with an asterisk (*)

Reviewer #

Reviewer name:	Telephone:
Organization:	Fax #:
	Email:
Signature:	

APPENDIX D
Comment Sheet for Non-target Host Test List for Assessment of a Biological Control Agent

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

REVIEWER'S COMMENT SHEET
NON-TARGET HOST TEST LIST FOR ASSESSMENT OF A BIOLOGICAL CONTROL AGENT

Proposed agent (genus, species, family):	Petition Designation:
	Canada CAN
	Mexico
Target species (scientific and common names):	U.S.A.
	Petition Date:
	Date sent to Reviewers:
Proposed release area(s) are in:	Review due date:
Canada (X) U.S.A. ( ) Mexico ( )	Return to:
Petitioner(s) and organization(s):	Ms. Andrea Brauner, Secretary CAN BCRC Fax: 613-759-1926 Agriculture and Agri-Food Canada Research Centre, K.W. Neatby Building 960 Carling Avenue Ottawa, ON K1A 0C6 CANADA

REVIEWER RECOMMENDATIONS

	Acceptable	Unacceptable*	Did not assess	Insufficient information
Taxonomy of target	( )	( )	( )	( )
Test species list	( )	( )	( )	( )
Proposed Host range tests	( )	( )	( )	( )
Other	( )	( )	( )	( )

APPROVAL FOR RELEASE IS:

(a) recommended without reservations	( )
(b) recommended with reservations*	( )
(c) more information needed*	( )
(d) not recommended*	( )

COMMENTS: required for any item marked with an asterisk (*)

Reviewer:

Reviewer name:	Telephone:
Organization:	Fax #:
	Email:
Signature:	

APPENDIX E
Model Petition for Release of a Phytophagous Species for Classical Biological Control of a Weed

A PETITION FOR EXPERIMENTAL OPEN–FIELD RELEASE OF *HYPENA OPULENTA* A POTENTIAL BIOLOGICAL CONTROL AGENT OF SWALLOW-WORTS (*VINCETOXICUM NIGRUM* AND *V. ROSSICUM*) IN NORTH AMERICA[a]

Submitted by

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A. Gassmann[2], and R. Bourchier[3]

NOVEMBER, 17, 2011

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***Disclaimer**: This sample petition is provided for information purposes and as a general template for required information, at the time of publication of this guide. Prospective petitioners should consult with the Canadian Food Inspection Service, at the time of petition preparation, for any revisions to the information needed and current petition requirements.*

[a] The release and establishment of a biocontrol agent in Canada is the culmination of 7 to 10 years of work to identify promising agents, conduct host-range testing for safety, impact testing and then to draft and submit a petition for a release to regulatory authorities (see Appendix 1). This work involves extensive international collaborations in the countries of origin for the biocontrol agents and between the countries, states and provinces with the pest populations in North America. Invasive species are usually trans-border problems; depending on the scope of the pest problem, release petitions may be submitted simultaneously for review in Canada, the United States and Mexico (see NAPPO RSPM 7, 2015 (https://www.nappo.org/english/standards-and-protocols/regional-phytosanitary-standards-rspms). As Canada has a shared border with the United States and thus often shared invasive pests, historically joint petitions for release of weed biological control agents have often been submitted to regulators. In these cases, the petition contains similar information to the NAPPO guideline but in a slightly different format outlined in the USDA-APHIS Technical Advisory Group on Biological Control of Weeds Manual (USDA-APHIS 2017, https://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/tag-bcaw_manual.pdf.). This is an example of a joint petition submitted in 2011.

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Abstract

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. The use of conventional control methods has largely been unsuccessful in managing established infestations, and biological control appears to be the most promising alternative. The European leaf-feeding moth, *Hypena opulenta* has demonstrated potential for successful biological control of swallow-worts. We propose to release this species against invasive swallow-wort species in North America.

Host range testing using an approved TAG list of 76 potential host plants using no-choice larval development has shown that the larvae of *H. opulenta* are monophagous on *Vincetoxicum* spp. There are only three species of *Vincetoxicum* in North America: the two target weeds and *V. hirundinaria*, another European species which has not naturalized in North America. This proposed biological control agent does not present a risk to any native North American plant species or any other species of economic importance. This insect species caused 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.

The population of *H. opulenta* used in host testing and proposed for release originates from an area of Ukraine with a continental climate and is thought to be well adapted to climatic conditions in the areas of North America that are currently invaded by swallow-worts.

We petition 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. The proposed US release location is on Naushon Island MA. It has several stands of *V. rossicum* and *V. nigrum* with both species growing in open fields, in the forest, and on the forest edge. We have four years of data from pre-release plots in these stands and several other control plots in RI. This experimental field release into shaded and full-sun plots will allow us to determine herbivore impact on both plant species under variable light conditions. Release on this island will allow us to assess efficacy on both weed species in sun and shade and enhance the success of any subsequent redistribution throughout North America. The primary proposed Canadian release site will be north of Toronto on property owned by the University of Toronto (Koffler Scientific Reserve) and it will be paired with control sites that have been monitored by the Toronto Regional Conservation Authority. The site type will include primarily *V. rossicum* growing in forest and forest edge habitats as this is the dominant form in the area. The Toronto Regional Conservation authority has been mapping and monitoring *Vincetoxicum* in the greater Toronto area since 2000. Additional detailed pre-release quadrats will be established in 2012 for post-release monitoring and paired with mapped control plots.

Contrasting the known ecological and economic risks of the continued spread and impact of *Vincetoxicum* spp. in North America, with the potential risks/benefits of the release of *H. opulenta*, we are requesting the release of this moth in the US and Canada. The insect is specific to the target *Vincetoxicum* species and has potential to have a significant impact on the spread, seed production and biomass of these invasive plants.

I Introduction

a. 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 species of concern, *Vincetoxicum nigrum* and *Vincetoxicum 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* was from BC in 1885, however this species has not persisted in that province; the earliest record for Ontario was 1889 (DiTommaso et al. 2005). *Vincetoxicum nigrum* is less common in Canada; early records were often cofused with *V. rossicum.* It has however 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).

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). It is also listed in the USDA Plants database as being in British Columbia. However, this is a historic record of a possible garden planting and it is not currently known to be present in the province (DiTommaso et al. 2005). *Vincetoxicum nigrum* has a greater distribution and has become established in 21 states from Maine through Kansas and in California. In Canada it has also naturalized in Ontario and Quebec but is more of a problem in localized patches rather than on a large regional scale (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).

b. Proposed Action

Initial fieldwork in Europe (Weed, 2010) identified five potential insect biological control agents of *Vincetoxicum* spp. Extensive research in quarantine at the University of Rhode Island (URI) and open field cage experiments in Europe has demonstrated that the leaf feeding noctuid moth, *Hypena opulenta* is monophagous and has potential to control swallow-worts growing in the shade. Two other noctuids, *Abrostola asclepiadis*, and *A. clarissa* also offer promise for swallow-wort control in open sites (Weed, 2010, Milbrath pers. comm.). Host range testing and evaluation is also underway on these two species.

In the United States we have established long-term monitoring plots on Naushon Island, MA since 2009, where both *Vincetoxicum rossicum* and *Vincetoxicum nigrum* exist under a range of light conditions, to assess population dynamics of *Vincetoxicum* prior to and following agent releases. We have similar plots in Rhode Island located on Conanicut Island, Block Island, and in Charlestown.

We propose to release *H. opulenta* as a biological control agent for *V. rossicum* and *V. nigrum* on Naushon Island in 2012. This island will be monitored for agent establishment, impact, and spread. Naushon has several stands of *V. rossicum* and *V. nigrum* with both species growing in open fields, in the forest, and on forest edge. We have four years of data from pre-release plots in these stands and this experimental field release will allow us to determine herbivore impact on both plant species under variable light conditions. We are able to direct swallow-wort management practices for the entire 19 km^2 island. In Europe *H. opulenta* is found in forested sites on *V. rossicum* (Weed 2010). We do not know if it will attack *V. rossicum* and *V. nigrum* growing in the sun.

We will continue to collect data on our other long-term sites, which will serve as controls, and we will evaluate other sites for additional releases. The goal is to create self-sustaining populations of *H. opulenta* to reduce swallow-wort infestations, decrease the use of herbicides, and restore native plant communities and ecosystem health.

We propose releasing this species on a New England island on the extreme northeastern edge of swallow-wort distribution. With a prevailing fair weather wind from the southwest, it will likely take several years before these insects move significantly west into the mainland of North America. This will allow us time to determine the effectiveness of this agent against both swallow-wort species and decide whether this species will need to be supplemented by other biological control agents in controlling inland swallow-wort populations.

In Canada the proposed release sites will provide alternative habitats and climatic conditions and increase the chances of establishment and impacts of *H. opulenta. Vincetoxicum rossicum* growing on shaded sites is a more serious problem than *V. nigrum* in Canada. Thus we are proposing to release *H. opulenta* in shaded *V. rossicum* sites towards the northern edge of its potential range to test establishment under colder overwintering conditions. We have selected two locations for initial releases. The primary site is on property owned by the University of Toronto: the Koffler Scientific

Reserve at Jokers Hill in King Township north of Toronto, This release location will be paired with *Vincetoxicum* control sites that have been mapped by the Toronto Regional Conservation authority since 2000. A secondary site if numbers of insects are available will be at the Fletcher Wildlife Gardens in Ottawa. The Toronto site is more similar to the proposed Naushon site in the US, as proximity to Lake Ontario will provide a more moderate maritime winter. The Ottawa site is close to the current northern edge of the *Vincetoxicum* invasion and thus provides a test of the cold tolerance of *H. opulenta.* It is perhaps most similar of the proposed release locations to the original collection sites for *H. opulenta* near Donetsk Ukraine. At the proposed study sites there has been qualitative and limited quantitative monitoring (mapping since 2000 by TRCA, studies by N. Cappuccino at Ottawa) of *Vincetoxicum* populations. These estimates will be supplemented by additional detailed quantitative density estimates for *V. rossicum* prior to the releases.

II Target Weed Information

a. Taxonomy of the Target Weeds

1. Classification

Common name: Black swallow-wort
Scientific name: *Vincetoxicum nigrum* (L.) Moench
Synonyms: *Cynanchum louiseae* Kartesz & Gandhi, *Cynanchum nigrum* (L.) Pers.
Class: Magnoliopsida
  Subclass: Asteridae
    Order: Gentianales
      Family: Apocynaceae
        Subfamily: Asclepiadoideae
          Tribe: Asclepiadeae
            Subtribe: Tylophorinae
              Genus: *Vincetoxicum*
                Species: *nigrum* (L.) Moench

Common name: Pale swallow-wort, dog-strangling vine
Scientific name: *Vincetoxicum rossicum* (Kleopow) Barbarich
Synonyms: *Cynanchum rossicum* (Kleopow) Borhidi
Class: Magnoliopsida
  Subclass: Asteridae
    Order: Gentianales
      Family: Apocynaceae
        Subfamily: Asclepiadoideae
          Tribe: Asclepiadeae
            Subtribe: Tylophorinae
              Genus: *Vincetoxicum*
                Species: *rossicum* (Kleopow) Barbarich

2. Problems in Identification or Taxonomy

There have been discrepancies in the generic placement of swallow-worts in European and North American literature (Tewksbury et al., 2002; DiTommaso et al., 2005). Some taxonomists use the generic names *Cynanchum* and *Vincetoxicum* interchangeably; however, recent molecular and chemical analysis has shown that the two target weeds in North America are distinct from the genus *Cynanchum. Vincetoxicum* is actually a sister genus to the Old World genus *Tylophora* and is placed in a different subtribe than *Cynanchum* (Liede, 1996). *Vincetoxicum hirundinaria* is the only other member of this subtribe present in North America. While this species has been reported in Michigan, New York, and Ontario Canada (USDA PLANTS database, 2011) it has not yet naturalized (Gleason and Cronquist, 1991). Thus it is not considered a primary target of this petition but it was included in the test-plant list as an additional control.

3. Origin and Location of the Herbarium Containing Voucher Specimens, Date of Depository, and Taxonomist who identified the Target Weeds

Target weed voucher specimens were identified by Stephen Darbyshire and deposited in 2006 into the Agriculture and Agri-Food Canada Central Experimental Farm, Saunders Building #49 in Ottawa, Ontario K1A 0C6 Canada (Milbrath and Biazzo, 2007).

b. Description of the Target Weeds

DiTommaso et al. (2005) described both species of swallow-worts as herbaceous, perennial twining vines that have erect stems ranging from 40 to 200 cm long. Subterranean buds produce one to several stems from a semi-woody rootstock that forms a short rhizome with roots that are thick, pale, and fleshy. The leaves are opposite and generally oval with a pointed tip and smooth margins. Both species have leaves that are glossy green until they turn golden yellow while senescing in late summer. Flowers are five-parted and 5-8mm in diameter and are produced in the axils of the leaves throughout the plant. Seedpods are slender to plump green follicles with one to two formed per flower. After ripening, the pods split open lengthwise to release numerous, polyembryonic seeds (Sheeley and Raynal, 1996). The dark brown seeds are ovoid, flattened and have apical tufts of hair that aid in dispersal. It is easiest to distinguish the two species when in bloom because *V. nigrum* has dark purple to black flowers with pubescent petals while *V. rossicum* has pale pink to maroon flowers with hairless petals. Both species display hairs on the stem; however, the hairs of *V. rossicum* are denser and in more distinct bands than *V. nigrum* (DiTommaso et al., 2005). Further, the seeds of *V. rossicum* are generally half the size of the seeds of *V. nigrum.*

c. Distribution of the Target Weeds

1. Native Range

Vincetoxicum nigrum is native to Mediterranean regions of France, Italy, Portugal and Spain (Tutin et al, 2006) (Fig. 1). *Vincetoxicum rossicum* is naturally distributed in Ukraine and southeastern Russia (Markgraf, 1972). In Ukraine it is found in the Ternopil, Kharkiv, Dniprppetrovsk, and Lugansk regions. It is found in the European part of USSR on the slopes of ravines among the shrubby vegetation, occurring in the Trans-Volga, Vloga-Don, Lower Volga, and Black Sea regions. The type specimen is stored in Kyiv, KW herbarium (pers comm. Andrii Mosyakin, M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev, Ukraine.) It is now naturalized in Norway (Lauvanger and Borgen, 1998).

Figure 1: Native range of *Vincetoxicum nigrum.* Flora Italiana. 2011.

2. North American Distribution

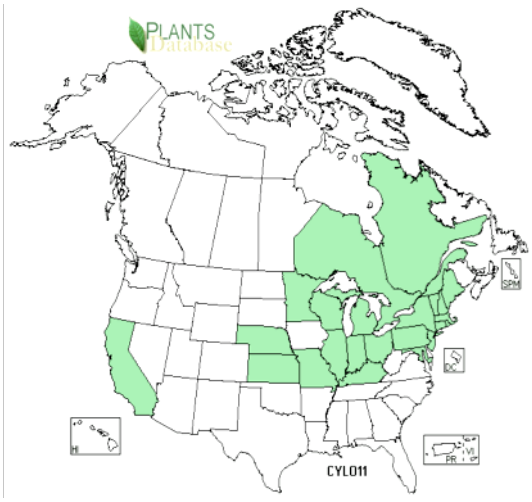


Figure 2: *Vincetoxicum nigrum* distribution in North America. USDA Plants database, 2011.

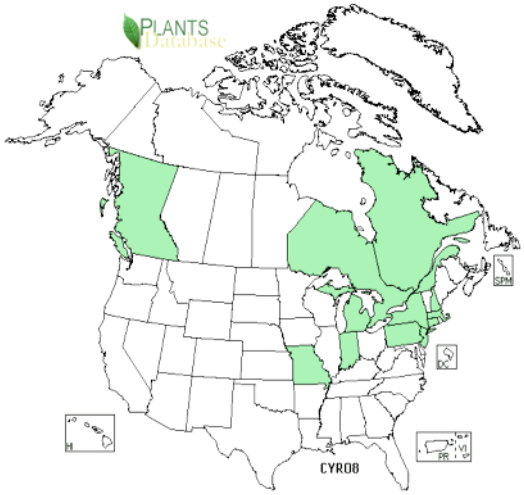


Figure 3: *Vincetoxicum rossicum* distribution in North America. USDA Plants database, 2011.

3. Genetic Variability

Populations of *V. nigrum* in Italy and France were reported as tetraploid with a chromosome count of 2n=44 (Moore, 1959; Geurmache et al., 2010). Populations of *V. nigrum* in Spain were described as diploid with a chromosome count of 2n=22 (DiTommaso et al., 2005). However, the discrepancy in ploidy level within the native

range was most likely due to a misidentification of specimens rather than karyotypic variation (Geurmache et al., 2010). *Vincetoxicum nigrum* populations in North America (Ottawa and New York) are also tetraploid (Geurmache et al., 2010). *Vincetoxicum rossicum* has been confirmed as diploid (2n=22) in the invasive (Ontario and New York) and native ranges (Ukraine) (Moore, 1959; Geurmache et al., 2010). All studies to date indicate that there has been no change in ploidy level since introduction into North America.

4. Habitats or Ecosystems where these Weeds are found in North America
In its native range of Ukraine, *Vincetoxicum rossicum* is usually distributed in small, scattered populations growing in forested sites, often close to rivers, while *Vincetoxicum nigrum* is generally found in dry, stony slopes within its native range in France (Weed, 2010). However, with a long history of establishment and a lack of herbivore pressure (Milbrath, 2010), both species have subsequently displayed a much greater tolerance to a diverse array of habitats and climates within their North American range.

In North America, both species are hardy colonizers in a wide variety of primarily upland habitats including but not restricted to pastures, old fields, hillsides, shores, flood plains, roadsides, and forest margins, where they have been associated with alkaline, calcareous, and acidic soils (DiTommaso et al., 2005). Both species can endure a broad range of moisture regimes and flourish in either full sun or partially shaded areas; however, *V. rossicum* also establishes within forest understories.

d. Taxonomically Related Plants

There are no *Vincetoxicum* species that are native to North America. The genus *Vincetoxicum* consists of around 70 species that are strictly Eurasian (Liede, 1997). Other than the target species, *Vincetoxicum hirundinaria* is the only other species that is reported to exist in North America. *Vincetoxicum hirundinaria* was first recorded in North America in Gray's Manual (Robinson and Fernald, 1908) as *Cynanchum vincetoxicum*. The USDA Plants Database shows this species (listed as *Cynanchum vincetoxicum*) as existing in NY, MI, and Ontario, Canada but unlike *V. nigrum* and *V. rossicum*, it has not become naturalized (Gleason and Cronquist, 1991). In fact, it may have very limited existence outside of botanical gardens. At least 5 of the 6 herbarium records that Sheeley and Raynal (1996) were able to locate were from or very near gardens and the most recent specimen was collected in 1956. If this plant currently exists in the North America, it is rare and not naturalized (pers. comm. A DiTommaso, Cornell University). There is no evidence that *V. hirundinaria* is of any economic importance as an ornamental plant in North America. To the contrary; it is listed as a Class A Noxious weed in VT. The closest relatives of *Vincetoxicum* spp. in North America belong to other subtribes within the tribe Asclepiadeae.

e. Distribution of Taxonomically Related Plants

Vincetoxicum nigrum, *V. rossicum*, and *V. hirundinaria* are the only species in the subtribe Tylophorinae (tribe Asclepiadeae) present in North America. Further, there are only four genera in the tribe Asclepiadeae present in North America that presently overlap in distribution with swallow-wort populations. These include three *Funastrum* species, forty *Asclepias* species, six *Matelea* species and one *Cynanchum* species (Milbrath and Biazzo, 2007). Representatives of all of these genera have been included in our host-range testing.

f. Life History of the Target Weeds

Vincetoxicum nigrum and *V. rossicum* are long-lived herbaceous, perennial vines that overwinter as seeds or rootstalks. At the start of each spring shoots expand from the root crown (DiTommaso et al., 2005). Stems are approximately 5 cm long when leaves begin to open up. Sheeley (1992) found that the mean stem length of *V. rossicum* grown in the shade was significantly longer than those grown in the sun; however, the stem weights were not significantly different.

In New York, which is relatively central to the distribution of *V. rossicum* flowering occurs from late May until late July, typically peaking in mid-June (Lumer and Yost, 1995). A similar phenology is reported in Ontario for sites in the Don Valley and Ottawa (DiTommaso et al. 2005). Seedpods begin developing in the end of June and continue through the middle of August. Individual seedpods contain around 20 seeds with apical tufts of hair that facilitate wind-dispersal in late summer and into fall (DiTommaso et al., 2005). In heavily shaded forest understories, populations of *V. rossicum* do not usually reproduce, but they can persist for decades until gaps in the canopy create improved conditions for successful reproduction (Sheeley, 1992).

Swallow-wort can reproduce sexually, clonally, and through self-pollination. Adding to this adaptable breeding system, the seeds of *V. nigrum* do not need a period of dormancy to germinate and in some areas can produce an estimated 2,000 seeds per square meter (Lumer and Yost, 1995). These characteristics allow swallow-wort to thrive in a variety of climates in both disturbed and intact ecosystems.

g. Impacts of the Target Weeds

1. Beneficial Uses

Several *Vincetoxicum* spp. are utilized in folk medicine within their native range where plant parts have been used as laxatives, diuretics, and anti-tumor agents (DiTommaso et al., 2005). However, there is no documentation of similar uses in North America.

2. Social and Recreational Uses

Vincetoxicum nigrum and *V. rossicum* were originally brought into North America as minor ornamentals and were recorded as escaping cultivation in botanical gardens (Sheeley, 1992). There is currently no known cultivation in North America.

3. Impact on Threatened and Endangered Species

Vincetoxicum rossicum has been documented out-competing the US federally endangered Hart's tongue fern, *Phyllitis scolopendrium*, near Onodaga, New York (Lawlor, 2000) and it is also a threat to displace the sole New England population of *Asclepias viridiflora* which is an endangered species in Connecticut (Tewksbury et al., 2002).

In Canada, the principal distribution of invasive *Vincetoxicum* spp overlaps completely with the Carolinian forest region in southern Ontario. This relatively small habitat area contains at least 125 plants species that are either federally or provincially listed as endangered, threatened or of concern (NHIC 2011). *Vincetoxicum* spp are invading globally rare alvar habitats which occur across the limestone plains of Ontario, and are considered one of the province's most ecologically significant habitats. Federally listed (COSIWIC; S1-S3) plant species from these alvars include: *Agalinis gattingeri, Carex juniperorum, Celtis tenuifolia, Cirsium hillii, Hymenoxys herbacea, Iris lacustris, Rosa setigera, Solidago houghtonii (*Oldham and Brinker 2009). *V. rossicum* is common in one of the two areas in the Rouge Valley where the bashful bulrush (*Trichophorum planifolium* was recorded in the 1970s. The only extant location of this federally-endangered species in Canada is Hamilton Royal Botanic Gardens (Miller and Kricfalusy 2008).

Vincetoxicum nigrum is currently overgrowing the US federally listed Jessop's milkvetch, *Astragalus robbinsii* populations located around Windsor, Vermont (DiTommaso et al., 2005). Additional endangered species may be affected as swallow-wort range expansion occurs.

4. Economic Impact

Swallow-worts negatively affect farming practices, livestock, and ornamental landscapes. Open pastures create ideal conditions for swallow-wort establishment and growth because grazing reduces competition from other plants. 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 planted at restoration sites (Christensen, 1998**)** and pine plantations in Ontario (DiTommaso et al., 2005).

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 the environment, and it may result in resistance among target weeds (Lawlor, 2000).

5. Regulatory Status

In the United States *Vincetoxicum nigrum* is a prohibited plant in Massachusetts, a banned invasive plant in Connecticut, a Class B Noxious Weed in Vermont and a Prohibited Invasive Species in New Hampshire (USDA PLANTS database, 2011). *Vincetoxicum rossicum* is listed as a prohibited plant in Massachusetts, a banned invasive plant in Connecticut, and a Prohibited Invasive Species in New Hampshire (USDA PLANTS database, 2011). In Canada neither *Vincetoxicum* species is listed provincially as a noxious weed (DiTommaso et al 2005) possibly because these lists tend to be agriculturally based. *Vincetoxicum rossicum* has been listed as a noxious weed in at least one township in Ontario as a local bylaw referencing the Ontario weed control act (DiTommaso et al 2005).

6. 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).

7. Impact of Weed Control on Non-target Plants

Swallow-wort is often established in natural areas with native plant communities or near economically important crops where digging can have negative effects during manual removal (Lawlor and Raynal, 2002). Similarly, herbicides are often used against large infestations of swallow-wort, but the fact that swallow-wort is often intertwined with other plants, makes foliar application a major risk to non-target plants or crops in the surrounding area (Lawlor and Raynal, 2002; DiTommaso et al., 2005).

h. 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 frequent 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 exacerbate infestations (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 (Lawlor, 2000). The use of biological control agents may be the only viable option for long-term reductions in swallow-wort populations.

III Biological Control Agent Information

a. Taxonomy

1. Classification

Common name: None
Scientific name: *Hypena opulenta* (Christoph)

Synonyms: None
Order: Lepidoptera
Family: Noctuidae[1]
Subfamily: Hypeninae
Tribe: Hypenini
Genus: *Hypena*
Species: *opulenta* (Christoph)

[1.] Lafontaine and Fibiger (2006) suggested changing the family name to Erebidae

2. Description

Adults of *H. opulenta* have dull, light brown forewings with a dark brown band in the middle and pale orange hindwings. The body length is about 1 cm and the wingspan is approximately 3 cm. Neonates are white and as they develop, larvae become green, obtain black spots and their head capsule turns yellow. Pupae are reddish to dark brown and about 1.2 cm long (Weed and Casagrande 2010).

3. Reason for Choosing the Agent

Beginning in 2006, field surveys in Europe 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. Both adults and larvae of *C. a. asclepiadis* were found to feed on the leaves of *Vincetoxicum* plants. *Chrysochus asclepiadeus* adults were observed feeding on the leaves of *Vincetoxicum* while larvae developed in the soil and fed on the roots. During impact studies, *C. asclepiadeus* displayed promising results with root herbivory negatively affecting the entire plant biomass as well as shoot height and reproductive capacities of both *V. rossicum* and *V. nigrum* (Weed et al., 2011a). However, under no-choice conditions, the larvae of both beetle species were able to complete development on several native North American species outside of the genus *Vincetoxicum.* *Chrysochus asclepiadeus* was able to develop on several native *Asclepias* species while *C. a. asclepiadis* was able to develop on *Asclepias tuberosa* and several members of the Asteraceae family (Weed, 2010). Based on these results, further testing on these beetles was discontinued at URI; although some additional research on adult oviposition choices is still being conducted on *C. asclepiadeus* at CABI EU-CH.

Pupae of the tephritid fly *Euphranta connexa* were collected from seedpods of *V. hirundinaria* in Moutier, Switzerland during 2006. Adults oviposit in developing seedpods of *V. hirundinaria.* Larvae feed on the developing seeds, bore out of the seedpod at maturity and pupate in the surrounding soil (Solbreck, 2000). It was later discovered that *E. connexa* successfully attacks and completes development on seedpods 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 decided to focus our attention on agents that may directly impact plant biomass. However, work

has continued with *E. connexa* at CABI EU-CH to assess its potential at reducing swallow-wort spread (Gassmann et al., 2011).

The noctuid moth species *H. opulenta* and *A. 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 clarisssa*, 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 southern Texas, will be tested with *A. asclepiadis* in 2012.

Research at URI has shown that *H. opulenta* is a multivoltine species with overlapping generations (Weed and Casagrande, 2010). This indicates that *H. opulenta* will have sustained attack rates throughout the growing season for *Vincetoxicum*. Initial no-choice larval development testing at URI demonstrated that *H. opulenta* is specific to *Vincetoxicum* warranting completion of host-range testing (Weed, 2010). Impact studies determined that all larval densities significantly reduce aboveground biomass, seedpod production and seed production in *V. rossicum* (Weed and Casagrande, 2010). The positive results from these studies in Europe encouraged us to continue host-range testing of *H. opulenta* in quarantine at URI. Testing of 76 species, including *M. barbigerum* (Table 1), has shown that this species is monophagous on *Vincetoxicum*.

4. Taxonomist who identified the Agents
The culture of *Hypena opulenta* was identified by Dr. Michael Fibiger in Denmark (Weed, 2010).

5. Location of Voucher Specimens
Voucher specimens of *Hypena opulenta* are held in the collection of Insect Biological Control Laboratory at the University of Rhode Island in Kingston, RI and at CABI EU-CH.

6. Problems in Identification or Taxonomy of the Genus
There were no problems in verifying the identity of *Hypena opulenta* (Weed, 2010).

b. Geographic Range

1. Native Range
Hypena opulenta is native to Eastern Europe where it is reported in Ukraine, Iran, Turkey, and Turkmenistan (Weed and Casagrande, 2010).

2. Expected Range in North America
We expect the host range of *H. opulenta* will be limited to *Vincetoxicum* and that this agent will spread throughout the distribution of the target weeds in North America. Our

culture of *H. opulenta* originated from Ukraine where there is generally a temperate continental climate with Mediterranean climates along the coast in the south. Most of Ukraine, including our collection sites, is in Plant Hardiness Zone 5 (-20 to -10F, -28.9 to – 23.4C) (*http://www.ars.usda.gov/Main/docs.htm?docid=9815&page=3*). Much of the current distribution of *Vincetoxicum* spp. in the northeastern and midwestern United States 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 either agent. This will be further tested with the proposed releases towards the northern edge of the range in Ottawa.

c. Known Host Range

1. Literature

Prior to 2006 field surveys, the host for *Hypena opulenta* was not documented (Weed and Casagrande, 2010).

2. Field Observations

Hypena opulenta was found feeding on the leaves of *V. rossicum* near Donetsk, Ukraine during surveys in 2006 (Weed, 2010). Larvae readily feed and complete development on leaves of *V. hirundinaria, V. rossicum* and *V. nigrum* in quarantine at URI.

3. Literature on the Host Range of Closely Related Species

There are 29 reported species in the genus *Hypena* in North America north of Mexico; at least two of which are considered pests (Arnett, 2000). The complete host range of most species is unknown, but in general these species are considered to be monophagous or oligophagous (McCabe and Vargas; 1998). McCabe and Vargas (1998) list the tree genera *Acer*, *Alnus*, *Cornus*, *Corylus*, *Juglans*, *Quercus*, *Tilia*, and *Ulmus* as hosts for some species. Other species attack a variety of herbaceous plants. The green cloverworm moth, *Hypena scabra*, feeds on the leaves of strawberries *(Fragaria*), raspberries (*Rubus*), ragweed (*Ambrosia*), and many economic legumes (Pedigo et al., 1973; Roberts and Douce, 1999). *Hypena humuli*, commonly known as the hop vine moth or hop looper, feeds on the leaves of most hop varieties (*Humulus lupulus*) (Grasswitz and James, 2008) and has been known to develop on stinging nettles (*Urtica* spp.) (Grimble et al., 1992). Other species (*H. manalis*, *H. lividalis*, and *H. obsitalis*) are reported to attack a variety of nettles (Urticaceae) (McCabe and Vargas, 1998; Kravchenko et al. 2006). *Hypena laceratalis* was introduced in Australia from Kenya to control the invasive *Lantana camara* (Verbenaceae), where it causes localized defoliation (Broughton, 2000). The only reported members of the Apocynaceae (milkweeds) attacked by *Hypena* belong to the Palearctic subtribe Tylophorinae (Sridhar and Rani 2003, Kravchenko et al. 2006), which is comprised exclusively of the genera *Vincetoxicum* (target weeds) and *Tylophora* (Liede 1996). No North American Apocynaceae species are confirmed hosts plants of *Hypena.*

d. Life History

1. Biology

Hypena opulenta is a multi-voltine species that develops through five instars and overwinters as pupae 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 between 4 to 6 weeks for larvae to complete a life cycle and a portion of each generation undergoes pupal diapause (Weed and Casagrande, 2010). A greater proportion of pupae enters diapause when larvae are reared under fall conditions indicating that diapause induction is affected by variations in seasonal plant quality and photoperiod (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).

2. Known Mortality Factors

Although there are no documented mortality factors for *H. opulenta*, we believe that the species is under some form of natural enemy pressure within its native range based on initial field surveys. Larvae of *H. opulenta* were only discovered on shaded forested populations of *V. rossicum* and *V. scandens*, even though stands of *V. hirundinaria* were readily available at sites in sunny, open fields about 100 meters away (Weed et al., 2011b). Further, in quarantine *H. opulenta* performs well on *V. hirundinaria, V. rossicum*, and *V. nigrum* grown in full sun and tested under conditions of high light intensity (Weed at al., 2011b). We do not presently know why *H. opulenta* is only found in forests in Europe. It is possible that natural enemies are one factor excluding *H. opulenta* from open field populations, but there is no direct evidence to support this. At all release locations, the performance of the *H. opulenta* will be evaluated on *V. rossicum* growing in both open meadows and in the forest.

The biology and feeding habits of *H. opulenta* are similar to the native North American species *Hypena humuli* (Weed and Casagrande, 2010) which has been associated with at least nine generalist parasitoids at various life stages (Grimble et al., 1992). Two species of *Trichogramma* attack eggs with documented parasitism rates of 20-70%; larvae are targeted by five species of tachinids, including *Compsilura concinnata*, and pupae are parasitized by the ichneumonids *Pimpia sanguinipes* and *Vulgichneumon brevicinctor* (Grimble et al., 1992). In addition to parasitism, larvae are often consumed by generalist predators such as yellowjackets and rove beetles (Grimble et al., 1992). *Hypena laceratalis* has been introduced to control *L. camara* in 14 countries and it is thought that parasites might be limiting its impact (Day et al., 2003). It is unclear how *H. opulenta* will be affected by natural enemies in North America.

3. Impact on the Target Plants

In 2006, Weed and Casagrande (2010) studied the effects of herbivory by *H. opulenta* on *Vincetoxicum* spp. grown from seeds collected in Rhode Island and Connecticut. Individual plants were exposed to densities of two, four, and eight larvae per plant and monitored until larvae completed development. Half of the plants received no larvae and served as controls. No plants were killed as a result of feeding and levels of defoliation varied between the plant species. *Vincetoxicum rossicum* plants with eight larvae

experienced 100% defoliation as did some plants with four larvae. However, eight larvae only caused 30% defoliation to *V. nigrum*. All larval densities significantly reduced aboveground biomass and increased the production of axillary branching of *V. rossicum.* Despite the increase in axillary branching, the plants were unable to fully compensate for the loss of aboveground biomass caused by herbivory. Larval feeding did not affect any measure of *V. nigrum* growth. While larval feeding did not affect stem growth of either species, it did significantly reduce flowering, seedpod mass, seedpod production, and the number of seeds of *V. rossicum*, but not *V. nigrum.* Based upon the results of a diapause induction experiment, *H. opulenta* will produce multiple, overlapping generations (Weed and Casagrande, 2010). These generations may continually limit the smothering growth of *V. rossicum* in forested sites and ultimately enable native species to regenerate. If compensation to herbivory by *V. rossicum* under field conditions is similar to laboratory results, continual defoliation is likely to lead to reductions in root mass (Weed and Casagrande, 2010).

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 could also decrease the competitive ability of swallow-worts in North America (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. It is possible that herbivory together with competition from mixed plant communities will further decrease the competitive ability of swallow-worts.

4. Potential Impact on Non-target Plants
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.

e. Host Specificity Studies

1. Populations Studied
All testing with *Hypena opulenta* was conducted using a population that was collected near Donetsk, Ukraine, in 2006. The test population started from four pupae that were gathered from tied leaves of *Vincetoxicum rossicum* in a forested ravine (N 47° 34.497′ E 37° 46.168′) along with 32 larvae collected on *Vincetoxicum rossicum* and *Vincetoxicum scandens* within a nearby forest (N 47° 48.681′ E 38° 32.738′) (Weed, 2010).

2. How Pest-Free Populations Were Obtained
Field-collected *Hypena opulenta* were initially brought to CABI EU- CH and monitored for any parasitoids. After several generations with no contaminants being discovered, the colonies were shipped to the URI quarantine lab. The current cultures of both species have been reared in quarantine since 2008. Dead larval specimens of both species from

our colonies were examined in August, 2011 by Leellen Solter, insect pathologist at the Illinois Natural History Survey, University of Illinois. She reported finding "nothing that is detectable under light microscopy... no nematodes, fungi, microsporidia, other protist spores, or baculovirus" in the specimens that were sent to her. The insects used for the proposed releases will come from this colony.

3. Site of Field and Lab Studies
Most larval feeding and impact testing of *H. opulenta* was done at the common garden and laboratory at CABI EU- CH in Delèmont, Switzerland. Host-range testing on *H. opulenta* took place in the insect quarantine facility at the University of Rhode Island in Kingston, RI, USA.

IV Experimental Methodology and Analysis

a. Test Plant List

An initial test-plant list for swallow-wort biological control agents, drafted with a North American perspective that included Canada and Mexico, was submitted and approved by the USDA Technical Advisory Group (TAG) on Biological Control of Weeds in 2007 (Milbrath and Biazzo, 2007), The approved TAG list included 36 native North American species and 21 introduced species of economic importance for screening (Milbrath and Biazzo, 2007). As we learned more about the host ranges of other species in the genus *Hypena*, we added 8 test plants within the families Urticaceae and Cannabaceae. An additional 15 plant species were added to increase the number of different representatives in several TAG plant categories and increase the power of our testing Seven additional plant species (in Asteraceae and Convulvulaceae) that were procured to address specific requirements of host range testing on other potential biocoontrol control agents of *Vincetoxicum* were also tested with *H. opulenta* After all additions were made to the initial TAG list, the final list (Table 1) contained 83 species to potentially test in addition to the two target weeds.

In 2006 an initial subset of the TAG list was screened by Dr. Aaron Weed to determine if testing the full list was warranted. Over two years he completed testing on the modified list of 34 species distributed over 25 genera and 4 plant families. All evidence from the preliminary testing showed that the species was likely monophagous on *Vincetoxicum* spp. From 2009 to 2011 Alexander Hazlehurst completed the host-range testing of *Hypena opulenta* for all plant species that could be obtained. To date we have tested *Hypena opulenta* on 76 species out of our total potential list of 83 plant species. From the original test list (Milbrath and Biazzo 2007) 50 out of 57 species were screened. Species from the original list that were not tested were not available and we found suitable representative substitutes. Additional specifics of the test-plant list and the rationales for substitutions are included with the results and discussion by test-plant category.

b. North American Test Plants by TAG Category

Category 1: Genetic types of the target weed species

Studies of the genetics of *Vincetoxicum spp.* are ongoing (Guermache et al. 2010, Bon et al. 2011). For the target weeds: *Vincetoxicum nigrum* plants were collected from local populations in Rhode Island and Massachusetts. *V. rossicum* plants were field collected from sites in New York, Connecticut and Massachusetts and several plants were also sent to us from areas in Europe. With the range of sources of the target plants on which *H. opulenta* has been reared, we do not expect that there will be any problem with the establishment or impact of the insect arising because of a unique genotype in the field.

Category 2: Species in the same (or closely related) genus as the target weed, including environmentally and economically important species

The only other species in the genus and sub-tribe present in North America is *V. hirundinaria* Collected populations came from Switzerland and Gottingen and Leipzig, Germany. This species is reported from Michigan, New York, and Ontario (USDA PLANTS database, 2011) but it has not yet naturalized (Gleason and Cronquist, 1991)

Category 3: Species in other genera in the same family as the target weed, divided by Subfamily and tribes, including environmentally and economically important species

We tested 48 species within the family Apocynaceae including 30 native species and 28 non-natives species. There were no North American species in category 3a in the same sub-tribe as the target species. In Category 3b (plants in other sub-tribes but same subfamily) we emphasized testing for species in sub-tribe Asclepiadinae (15 species) because this was the closest taxonomic group to the target plants and some of them were T and E species or species at risk (Table 2, Milbrath and Biazzo 2007). We added *Asclepias hirtella, A. purpurascens, A. sullivanti, A. verticillata* and *A. viridis* to the original testing list because they were listed as species of special state or provincial (Ontario) concern. We were also able to test the US federally listed *A. meadii.* Given that there was no development of *Hypena opulenta* on any of the 15 species in the genus *Asclepias* that were tested, we are confident we have adequate representation of this genus and that there is a very low risk to any rare *Asclepias* in North America. This includes a second US federally listed species *A. welshii* and *A. quadrifolia* which is a threatened species in Rhode Island and New Hampshire and a recently federally listed species in Canada (USDA PLANTS database, 2011, COSEWIC 2010). *Asclepias welshii* was not tested because obtaining live plants of this species is forbidden and to get seeds, for which there was no guarantee of germination, required extensive permitting and time. This species is only found in a few remote, desert environments in Arizona and Utah where there is no potential for V*incetoxicum* species to spread. *Asclepias quadrifolia* was only recently (2010) added to the Canadian federal list and is on the northern edge of its range in Ontario. The sites where *Asclepias quadrifolia has* been recorded are very vulnerable to *Vincetoxicum rossicum* invasion within the next 10 to 30 years (COSEWIC 2010). Given no attack on any *Asclepias spp.* during host-range testing, invasive *Vincetoxicum* is a much larger threat to *A. quadrifolia* than the potential biocontrol agent.

Also within 3b: We added *Cynanchum ascyrifolium* and *C. marnierianum* within the sub-tribe Cynanchinae to the original test-plant list for a total of four species in the genus *Cynanchum,* including *C. laeve* which is a species of concern in Pennsylvania and Ontario (Table 2). We could not locate a source for *Cynanchum acutum* which was on the original test list; however it is an Old World species distributed within Europe, Asia and Africa. It is not present anywhere in North America and there is no documentation of commercial use. There was no feeding or development on the representative *Cynanchum* species tested (Table 1).

The original test list had 8 species within the remaining sub-tribes: Gonolobinae, Metastelmatinae and Oxypetaline in the same subfamily as the target weeds. We added *Matelea carolinensis* and *M. decipiens* within the sub-tribe Gonolobinae for a total of 5 species from this sub-tribe. The representatives of the sub-tribe *Metastelmatinae* in the genera *Metastelma and Funastrum* have distinct distributions with a very limited overlap with *Vincetoxicum.* We tested *Metastelma barbigerium* as representative of the two *Metastelma* species on the original list, both of which are found in restricted habitats in Texas, outside of the potential range of *Vincetoxicum* species. *Metastelma barbigerium* was of interest as it has been found to support some development of *Abrostola clarissa* (Milbrath et al. 2011), another noctuid collected from *Vincetoxicum* that is being considered as a biocontrol agent. In the genus *Funastrum* we tested *Funastrum angustifolium* (AL, FL, GA, LA, MS, NC, SC, TX) and *Funastrum cynanchoides,* which is only found in AZ, NV, NM, UT, TX and CA. With the exception of a Californian population of *V. nigrum, Vincetoxicum* species are not found in any of the other states listed for this species. We tested the single species from sub-tribe Oxypetaline that was included in Milbrath and Biazzo (2007).

There was no development on any of the 3b plants: plant species in the same sub-family and tribe but different sub-tribe as the target plants (Table1).

Considering plant species in same subfamily but different tribes (3c) there were only 3 species on the original test plant list (Milbrath and Biazzo 2007). As representative of the tribe Marsdenieae we substituted *Marsdenia floribunda* for *Marsdenia edulis* which is only found in Mexico and some parts of the Caribbean; we also tested *Hoya carnosa* as a 2nd representatives of this tribe for a total of 4 species in two Tribes: Ceropegieae and Marsdenieae. There was no development on any of the 3c. plants: species in the same sub-family but different tribes as the target plants (Table1).

For 3d: plants in other subfamilies within the Apocynaceae, Milbrath and Biazzo (2007) proposed thirteen species from 3 subfamilies Periplocoideae, Apocynoideae and Rauvolfioideae. We tested a total of fifteen species in 3d including the single species from the Periplocoideae that was proposed. Within the Apocynoideae we tested 8 species. We added *Trachelospermum jasminoides* and *T. mandianum* within the tribe Apocyneae.

We tested a total of six species within the Rauvolfioideae. As was proposed, *Amsonia tabernanaemontana* and *Vinca minor* were tested for the subfamily tribe Vinceae and we added *Catharanthus roseus* as a third representative. We were unable to find a source of *Amsonia kearneyana,* which is only found in AZ. In contrast the three tested species in the Vinceae have a much larger distributions in North America that overlap with *Vincetoxicum* and provide adequate representation of the tribe.

There was no development on any of the 3d. plants: species in other subfamilies from the target plants (Table1).

Category 4: Threatened and endangered species in the same family as the target weed

There were 8 US federally listed species reported by Milbrath and Biazzo (2007, Table 2), 4 of which were in Hawaii and thus not at risk. There were also 33 other species of concern for differing states. At the time of the original test plant list (2007) there were no federally listed species in Canada (Milbrath and Biazzo 2007).

Of the 209 vascular plant species listed on the Federal Canadian Species at Risk List (http://www.sararegistry.gc.ca/sar/index/default_e.cfm) in 2011 there is only 1 species in the Apocyanaceae, *Asclepias quadrifolia,* which was listed in 2010 (as described above under category 3b). For the provinces where *Vincetoxicum* occurs, several species of *Asclepias* are of concern, for Ontario (*Asclepias hirtella, A. ovalifolia, A. purpurascens, A. sullivantii, A. variegata* and *Asclepias viridiflora,* NHIC 2009) and *Asclepias tubersosa* is a S2 species in Québec (Centre De Données Sur Le Patrimoine Naturel Du Québec. 2008). For the provinces outside of the current range of *Vincetoxicum* the following species are on watch lists: *A. lanuginose* (MB), *A. verticillata* (MB, SK), *A. viridiflora* (MB), *A. syriaca* (SK) (SK Conservation Data Centre 2011, Manitoba Conservation Data Centre 2009).

There are no threatened or endangered species in either the US or Canada in the same genus or sub-tribe as the target plants.

Thirty-one of the species of concern listed by Milbrath and Biazzo (2007) were in the same tribe as the target species, located in 3 genera: *Asclepias* (20 species), Cynanchum (1) and *Matelea* (10).

Asclepias: 15 species were tested including 6 (Table 1) that were on the original list of endangered species (Milbrath and Biazzo; table 2). Four of the species of concern for Ontario, *Asclepias tubersosa* for Québec and 3 of the species for MB and SK were tested with *H. opulenta* (Table 1). There was no development on any *Asclepias* species tested, confirming that there is a very low risk to any *Asclepias* specie from *H. opulenta*.

Cynanchum: the single species *C. laeve* that is of concern in Pennsylvania and Ontario was tested and there was no larval development.

Matelea: This genus is not found in Canada and several of the species are confined to southern states outside the current *Vincetoxicum* distribution (USDA Plants 2011). Five species in this genus were tested, one of which (*Matelea obliqua)* is of multiple state concern. There was no development on any *Matelea* species tested.

The remaining six species of concern listed by Milbrath and Biazzo (2007) were more distant from the target species and located in 2 different sub-families: the Apocynoideae and Rauvolfioideae of the Apocynaceae. We tested seven species in Apocynoideae including two species of concern : *Apocynum cannabinum* and *Trachelospermum difforme.* For the US federally listed *Cycladenia humilis* Benth. var. *humilis,* we were successful at acquiring seeds, but we could not get them to germinate despite trying several methods. A search of the literature indicated that there is no record of successful germination of this species (Sipes et al., 1994). *Cycladenia* is a monophyletic genus that is restricted to desert regions of California, Utah, Nevada and Arizona where *Vincetoxicum* species are not found.

For the Rauvolfioideae we tested 6 species, including the species of concern *Amsonia tabermaemontana* Walt. var. *gattingeri* . We were unable to find a source for the US listed *Amsonia kearneyana.* However, none of the species in this subfamily or the three tested representative species in the same tribe as *A. kearneyana* (Vinceae) supported development of *H. opulenta.*

There was no development on any of the threatened or endangered species of concern or their surrogates that were tested (Table1).

Categories 5 and 6 – North American or introduced species in other families (Group 5) or orders (Group 6) that have some phylogenetic, morphological, or biochemical relationship to the target weed, including economically and environmentally important plants

For the original test plant list there were 14 species from 4 families Gentianaceae, Loganiaceae, Gelsemiaceae and Rubiaceae in Group 5 (Milbrath and Biazzo 2007). We tested 13 of the 14 proposed species (Table 1) and added two additional species *Hedyotis purpurascens* and *Houstonia longifolia* within the family Rubiaceae. *Bartonia* virginica (L.) Britton, Sterns, & Poggenb. is a member of the family Gentianaceae from the original list with a wide distribution of fragmented populations throughout the east coast. We finally located this plant in Kingston and West Greenwich, RI and tested it with *H. opulenta*. We also tested three other representatives of this family, *Centarium erythraea, Gentiana andrewsii,* and *G. quinquefolia*. We tested *Spigelia marilandica* as one of the two proposed species as a representative from the Loganiaceae. This species has a similar range as *Mitreola petiolata* which we were not able to obtain. It is an annual species reported from AL, AR, FL, GA, LA, MS, MO, NC, OK, SC, TN, TX, and VA. A source in Texas informed us that populations of this species were found in remote areas of the state and were very difficult to access.

For species in other orders (group 6), we tested the two species *Buddleja davidii* (introduced ornamental from Northeast US and present in British Columbia) and the

native *Polypremum procumbens* that were proposed in the original test plant list (Milbrath and Biazzo 2007).

There was no development on any category 5 or 6 plants.

Category 7 - Any plant on which the biological control agent or its close relatives (within the same genus) have been previously recorded to feed and/or reproduce

The original TAG list did not include Category 7 plants because potential agents had not yet been determined. After choosing the agents and reviewing North American literature, we chose six species of the family Urticaceae: *Boehmeria cylindrica, Laportea canadensis, Parietaria floridana, Pilea microphylla, Pipturus albidus* and *Urtica dioica* based on previous host records for other species in the genus *Hypena*. We also added two varieties of hops, *Humulus lupulus,* in this category because of the known host range of the noctuid, *Hypena humuli.*

Six additional species: A*rtemisia absinthium, A. caudata, A. ludoviciana, A. stelleriana, A. vulgaris Tanacetum vulgare* and *Calystegia sepium* had been selected as Category 7 plants for host-range testing with the beetle *Chrysolina a. asclepiadis.* As we had these species in cultivation, we decided to test *Hypena opulenta* on them as well.

There was no development on any of the tested category 7 plants (Table 1).

We believe that we have tested an appropriate number of species distributed over a variety of genera included in the TAG list to evaluate the safety of *Hypena opulenta*. This insect displayed extremely minimal feeding and never completed development on any species outside of the genus *Vincetoxicum* during testing. Table 1 displays our complete list of test plants divided into TAG categories as well as the results of all no-choice larval development tests.

c. Design and Methods of No-choice Larval Development Tests

Since 2008, we have exercised consistent experimental procedures and methods of data analysis during all host range testing at the URI quarantine facility. At the end of each summer pupae of *Hypena opulenta* are sexed and placed in plastic cups (473ml) containing sterilized vermiculite and covered with plastic lids. The pupae are then placed in a 10°C incubator until September when they are moved to a 4°C overwintering chamber. Annually beginning in May, pupae are taken out of the overwintering chamber and placed at room temperature. The quarantine laboratory is maintained about 25°C and cages were held under light fixtures set on a 16:8 (L:D) h photoperiod with additional natural light coming from windows within each room. The light fixtures contained four GE High Output Daylight (F48T12-D-HD) fluorescent bulbs that were hung from racks approximately 10 cm above oviposition cages containing adults and plants and about 50 cm above cups of larvae used in no-choice development tests.

As adults emerged, they were moved to screened cages containing potted plants of *V. nigrum, V. rossicum* or *V. hirundinaria* as well as a source of honey-water for

sustenance. Each cage contained several females and males depending on the number of adults available – generally about 5 females and 5 males per cage. For testing and rearing purposes it was beneficial to have more adults in each cage for increased oviposition in order to maintain colonies of each species. From 2008 through 2010, we used 40X40X40cm screen cages containing plants in 2-liter pots. During the 2011 testing period we switched to taller (40X40X76cm) oviposition cages with four plastic sides and a screen top. We also added a tray of moistened soil (Metro 510 mix) to the bottom of the cage and used larger plants in 4-liter pots. The additional space, more plant biomass, and increased humidity levels may have been factors in our observations of increased numbers of eggs laid in comparison with previous years.

Eggs were removed from the host plants daily using a soft, fine-tip brush and then placed in 90 mm Petri dishes lined with filter paper. As eclosion occurred, individual larvae were placed in plastic cups (473 ml) lined with moistened filter paper. In every no-choice larval development test, a single excised leaf of a test plant species was added to each cup with a single larva and cups were sealed with a clear, plastic lid. Whenever possible, leaves were taken from the top three nodes of test plants species because neonates tend to feed on newly expanded leaves in the field (Weed, 2010). This was repeated using ten cups for each test plant species. The dates and number of larvae set up on each test plant species was recorded. The test plant cups were monitored daily and any feeding damage, frass production, larval survival, development and pupation was recorded. After all larvae in each test replicate died, the contents were discarded and the corresponding test plant was considered outside of the agent's physiological host range.

Throughout the testing periods, every three days an additional ten cups were set with a single larva and an excised leaf of *Vincetoxicum* spp. to serve as controls which were handled and examined similar to other treatments. Fresh leaves and clean filter paper were replaced in all cups as needed. The survival rates, development time, and pupation rates of controls were recorded for all testing. At various times throughout the testing period the pupal weights of controls were recorded as a reference point for the health of populations from year to year.

1. Sources of Plants Tested

We collected *Vincetoxicum nigrum* plants from local populations in Rhode Island and Massachusetts. *V. rossicum* plants were field collected from sites in New York, Connecticut and Massachusetts and several plants were also sent to us from areas in Europe. In addition to the target weeds, we also obtained plants of *V. hirundinaria* from populations in Switzerland and Gottingen and Leipzig, Germany. All test plant species were either collected in the field locally or obtained through reliable sources from around North America, including from colleagues in other regions or commercial and native plant nurseries. Any species that we collected in the field were identified by our lab personnel with support from local botanists.

d. Positive Control

As described under the heading Design and Methods of No-choice Larval Development Tests, we set up controls every three days with each batch of test plants. The *Vincetoxicum spp.* controls consistently averaged 75-82% survival (Table 1) and it was never necessary to discard a series of tests because of poor survival of controls.

e. Reasons for Decisions

Our decision to focus efforts on completing host-range assessment through no-choice larval development tests was determined by using the "reverse-order" method (Wapshere, 1989) in which larval acceptance of the host is initially tested. These tests confine the agents to a test plant species until feeding and development occurs or death takes place. These tests may produce false positives (wrongly identifying a plant as a host), but plant species not attacked under these test conditions are considered outside of the agent's physiological host range. If complete development had occurred on any test plant species we would next set up no-choice oviposition tests to check for host acceptance. However, no larvae completed development on any species outside the genus *Vincetoxicum* so these tests were not necessary.

V Results and Discussion

a. Summary of Results

Table 1. Categorized TAG List and Results of no-choice larval development testing for *Hypena opulenta* on the target weeds and test plants.

FAMILY Subfamily *Tribe:* Subtribe	TAG Cat. [A]	Species	Orig.[B]	US Distribution[C]	Canada Dist. [C]	Reps	% survival[D]
APOCYNACEAE Asclepiadoideae *Asclepiadeae* Tylophorinae	Target	*Vincetoxicum nigrum* (L.) Moench (black swallow-wort)*	I	CT, NY (for testing), CA, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, NE, NH, NJ, OH, PA, RI, VT, WI	ON, (for testing), QC	190	80.0
	Target	*Vincetoxicum rossicum* (Kleopow) Barb (pale swallow-wort)*	I	CT, NY (for testing), IN, MA, MI, MO, NH, NJ, PA	ON (for testing), QC	120	75.4
	2	*Vincetoxicum hirundinaria* (Medic.) (white swallow-wort)*	I	European species not found in N. America		40	78.9
	3a	Species in the same subtribe as target weeds: **None in North America**				N/A	N/A
APOCYNACEAE Asclepiadoideae *Asclepiadeae* Asclepiadinae	3b	*Asclepias asperula* (Dcne.) Woods. (spider milkweed)*	N	AZ, CA, CO, ID, KS, NE, NV, NM, OK, TX, UT	None	10	0
	3b	*Asclepias curassavica* L. (bloodflower)*	N	CA, FL, HI, LA, PR, TN, TX, VI	None	10	0
	3b	*Asclepias fascicularis* Dcne. (Mexican whorled milkweed)*	N	CA, ID, NE, WA, OR, UT	None	10	0
	3b	*Asclepias fruticosa* L.	I	CA	None	10	0

FAMILY **Subfamily** ***Tribe:*** **Subtribe**	**TAG Cat.** [A]	**Species**	**Orig.** [B]	**US Distribution** [C]	**Canada Dist.** [C]	**Reps**	**% survival** [D]
		(white swan milkweed)*					
	3b	*Asclepias incarnata* L. (swamp milkweed)*	N	AL, AK, AS, AR, CO, CT, DE, DC, FL, GA, HI, ID, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MO, MT, NE, NV, NH, NJ, NM, NY, NC, ND, OH, OK, PA, PR, RI, SC, SD, TN, TX, UT, VT, VI, VA, WV, WI, WY	MB, NB, ON, PE, QC	10	0
	3b,4	*Asclepias hirtella* (Pennell) Woodson (tall green milkweed)	N	AL, AR, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, OH, OK, TN, WI, WV	ON	10	0
	3b,4	*Asclepias meadii* Torr. Ex Gray (Mead's milkweed)*	N	IL, IN, IA, KS, MO, WI	None	10	0
	3b,4	*Asclepias purpurascens* L. (purple milkweed)	N	AR, CT, DC, DE, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, NE, NH, NJ, NY, OH, OK, PA, RI, SD, TN, TX, VA, WI, WV	ON	10	0
	3b	*Asclepias rubra* L. (red milkweed)	N	AL, AR, DC, DE, FL, GA, LA, MD, MS, NC, NJ, NY, PA, SC, TX, VA	None	Not Tested	N/A
	3b,4	*Asclepias speciosa* Torr. (showy milkweed)*	N	AZ, CA, CO, ID, IL, IA, KS, MI, MN, MT, NE, NV, NM, ND, OK, OR, SD, TX, UT, WA, WI, WY	AB, BC, MB, SK	10	0
	3b,4	*Asclepias sullivanti* Engelm. Ex Gray (prairie milkweed)	N	AR, IA, IL, IN, KS, MI, MN, MO, ND, NE, OH, OK, SD, WI	ON	10	0
	3b	*Asclepias syriaca* L. (common milkweed)*	N	AL, AR, CN, DE, GA, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MS, MO, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, SD, TN, TX, VT, VA, WV, WI	MB, NB, NS, ON, PE, QC, SK	10	0
	3b,4	*Asclepias tuberosa* L. (butterfly milkweed)*	N	AZ, AR, AL, CA, CO, CN, FL, GA, IL, IN, IA, KS, KY, LA, MI, MN, MS, MO, NE, NC,NM, NY, OH, OK, PA, SC, SD, TN, TX, UT, WV, WI	None	10	0
	3b	*Asclepias verticillata* L.	N	AL, AR, AZ, CT, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO,	MB, ON, SK	10	0

FAMILY Subfamily *Tribe:* Subtribe	TAG Cat. [A]	Species	Orig. [B]	US Distribution [C]	Canada Dist. [C]	Reps	% survival [D]
		(linear-leaved milkweed)		MS, MT, NC, ND, NE, NJ, NM, NY, OH, OK, PA, RI, SC, SD, TN, TX, VA, VT, WI, WV, WY			
	3b,4	*Asclepias viridiflora* Raf. (green milkweed)*	N	AL, AZ, AR, CO, CN, DE, FL, GA, IL, IN, IA, KS, KY, LA, MD, MI, MN, MS, MO, MT, NE, NJ, NM, NY, NC, ND, OH, OK, PA, SC, SD, TN, TX, VA, WV	AB, BC, MB, ON, SK	10	0
	3b	*Asclepias viridis* Walt. (green antelope horn)	N	AL, AR, FL, GA, IL, IN, KS, KY, LA, MO, MS, NE, OH, OK, SC, TN, TX, WV	None	10	0
	3b,4	*Asclepias welshii* N. & P. Holmgren (Welsh's milkweed)*	N	AZ, UT	None	Not Tested	N/A
APOCYNACEAE **Asclepiadoideae** ***Asclepiadeae*** **Cynanchinae**	3b	*Cynanchum acutum* L. (stranglevine)*	F	None	None	Not Tested	N/A
	3b	*Cynanchum ascyrifolium* Matsumura (Mosquito trap plant)	F	None	None	10	0
	3b,4	*Cynanchum laeve* (Michx.) Pers. honeyvine)*	N	Al, AR, DE, FL, GA, ID, IL, IN, IA, KS, KY, LA, MD, MS, MO, NE, NY, NC, OH, OK, PA, SC, TN, TX, VA, WV	ON	10	0
	3b	*Cynanchum marnierianum* Rauh	F	None	None	10	0
	3b	*Cynanchum racemosum* (Jacq.) Jacq. (talayote)*	N	TX	None	10	0
APOCYNACEAE **Asclepiadoideae** ***Asclepiadeae*** **Gonolobinae**	3b	*Matelea carolinensis* (Jacq.) Woods. (maroon Carolina milkvine)	N	AL, AR, DC, DE, GA, KY, LA, MD, MS, NC, SC, TN, TX, VA	None	10	0
	3b	*Matelea decipiens* (Alexander) Woods.	N	AR, GA, IL, IN, KS, KY, LA, MD, MO, NC, OK, SC, TN, TX, VA	None	10	0
	3b	*Matelea gonocarpos* (Walt.) Shinners (angularfruit milkvine)*	N	AL, AR, FL, GA, IL, IN, MD, MS, MO, NC, OK, SC, TN, TX, VA	None	10	0

FAMILY **Subfamily** ***Tribe:*** **Subtribe**	**TAG Cat.** [A]	**Species**	**Orig.** [B]	**US Distribution** [C]	**Canada Dist.** [C]	**Reps**	**% survival** [D]
	3b,4	*Matelea oblique* (Jacq.) Woods. (climbing milkvine)*	N	AL, GA, IL, IN, KY, MD, MS, MO, NC, OH, PA, TN, VA, WV	None	10	0
	3b	*Gonolobus stephanotrichus* Griseb. (anglepod)*	N	PR	None	20[E]	0
APOCYNACEAE **Asclepiadoideae** ***Asclepiadeae*** **Metastelmatinae**	3b	*Funastrum angustifolium* (Pers.) Liede & Meve (gulf coast swallow-wort)*	N	AL, FL, GA, LA, MS, NC, SC, TX	None	10	0
	3b	*Funastrum cynanchoides* (Dcne.) Schlechter (fringed twinevine)*	N	AZ, CA, NV, NM, TX, UT	None	10	0
	3b	*Metastelma barbigerum* Schelle (bearded swallow-wort)*	N	TX	None	10	0
	3b	*Metastelma palmeri* S. Watson (MacCart's swallow-wort)*	N	TX	None	Not Tested	N/A
APOCYNACEAE **Asclepiadoideae** ***Asclepiadeae*** **Oxypetalinae**	3b	*Araujia sericifera* Brot. (white bladderflower)*	I	CA	None	10	0
APOCYNACEAE **Asclepiadoideae** ***Ceropegieae***	3c	*Ceropegia woodii* Schltr. (rosary vine)*	I	Cultivated	cultivated	10	0
	3c	*Stapelia gigantea* N.E. Br. (zulu giant)*	I	HI	None	10	0
APOCYNACEAE **Asclepiadoideae** ***Marsdenieae***	3c	*Hoya carnosa* (L. f.) R. Br. (porcelain-flower)*	I	PR	None	10	0
	3c	*M. floribunda* for	N		None	10	0

FAMILY **Subfamily** ***Tribe:*** **Subtribe**	**TAG Cat.** [A]	**Species**	**Orig.** [B]	**US Distribution** [C]	**Canada Dist.** [C]	**Reps**	**% survival** [D]
		Marsdenia edulis Wats*					
APOCYNACEAE **Periplocoideae**	3d	*Periploca graeca* L. (silkvine)*	I	CN, KS, NJ, NY, OK, PA, RI, TN, TX	None	10	0
APOCYNACEAE **Apocynoideae** ***Wrightieae***	3d	*Nerium oleander* L. (oleander)*	I	AL, CA, FL, GA, LA, MS, NC, PR, SC, TX, UT, VI	None	10	0
APOCYNACEAE **Apocynoideae** ***Malouetieae***	3d	*Pachypodium lamerei* Drake (Madagascar palm)*	I	Cultivated	cultivated	10	0
APOCYNACEAE **Apocynoideae** ***Apocyneae***	3d,4	*Apocynum androsaemifolium* L. (spreading dogbane)*	N	AL, AK, AZ, AR, CA, CO, CT, DE, DC, GA, ID, IL, IN, IA, ME, MD, MA, MI, MN, MO, MT, NE, NV, NH, NJ, NM, NY, NC, ND, OH, OK, PR, PA, RI, SD, TN, TX, UT, VT, VA, WA, WV, WI, WY	AB, BC, MB, NB, NL, NS, NT, ON, PE, QC, SK, YT	10	0
	3d,4	*Apocynum cannabinum* L. (Indianhemp)*	N	AL, AK, AZ, AR, CA, CO, CT, DE, DC, FL, GA, ID, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MS, MO, MT, NE, NV, NH, NJ, NM, NY, NC, ND, OH, OK, PR, PA, RI, SC, SD, TN, TX, UT, VT, VA, WA, WV, WI, WY	AB, BC, MB, NT, NB, NL, NS, ON, QC, SK	10	0
	3d,4	*Trachelospermum difforme* (Walt.) Gray (climbing dogbane)*	N	AL, AR, DE, FL, GA, IN, IN, KY, LA, MD, MS, MO, NC, OK, SC, TN, TX, VA	None	10	0
	3d	*Trachelospermum jasminoides* (Lindl.) Lem. (confederate jasmine)	I	FL, LA	None	10	0
	3d	*Trachelospermum mandianum* (yellow confederate jasmine)	I	Ornamental	Ornamental	10	0
APOCYNACEAE **Apocynoideae**	3d,4	*Cycladenia humilis* Benth. var. *humilis* (Sacramento	N	CA	None	Not Tested	N/A

FAMILY **Subfamily** ***Tribe:*** **Subtribe**	**TAG Cat.** [A]	**Species**	**Orig.** [B]	**US Distribution** [C]	**Canada Dist.** [C]	**Reps**	**% survival** [D]
Echiteae		waxy dogbane)*					
APOCYNACEAE **Rauvolfioideae** ***Vinceae***	3d	*Amsonia illustris* Woodson (Ozark bluestar)	N	AR, KS, MO, OK, TX	None	10	0
	3d, 4	*Amsonia tabernaemontana* Walter (eastern bluestar)*	N	AL, AR, DE, FL, GA, IL, IN, KS, KY, LA, MD, MA, MS, MO, NJ, NY, NC, OK, OH, PA, SC, TN, TX, VA	None	10	0
	3d	*Vinca minor* L. (common periwinkle)*	I	AL, AR, CT, DE, GA, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MS, MO, NE, NH, NJ, NY, NC, OH, PA, OH, RI, SC, TX, TN, UT, VT, VA, WA, WV, WI	BC, NB, NS, ON, QC	10	0
	3d	*Catharanthus roseus* (L.) G. Don.	I	CA, FL, GA, HI, LA, MS, NC, SC, TX, PR, VI	None	10	0
	3d,4	*Amsonia kearneyana* Woods. (Kearney's bluestar)*	N	AZ	None	Not Tested	N/A
APOCYNACEAE **Rauvolfioideae** ***Plumerieae***	3d	*Allamanda cathartica* L. (golden trumpet)*	I	FL, PR, VI	None	10	0
	3d	*Plumeria rubra* L. (frangipani)*	I	PR, VI	None	10	0
APOCYNACEAE **Rauvolfioideae** ***Carisseae***	3d	*Carissa macrocarpa* (Eckl.) A.DC. (natal plum)*	I	FL, PR	None	10	0
GENTIANACEAE	5	*Bartonia virginica* (L.) B.S.P. (yellow screwstem)*	N	AL, CT, DC, DE, FL, GA, IL, IN, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, NH, NJ, NY, OH, PA, RI, SC, TN, TX, VA, VT, WI, WV	NB, NF, NS, ON, QC	10	0
	5	*Centaurium erythraea* Rafn. (European centaury)*	I	CA, GA, HI, ID, IN, MD, MA, MI, NY, NC, OH, PA, RI, VT, VA, WA	BC, NS, ON, QC	10	0
	5	*Gentiana andrewsii* Griseb.	N	CO, CN, DE, IL, IN, IA, KY, MD, MA, MI, MN, MO, MH, NE, NJ, NY, ND, OH,	MB, ON, QC, SK	10	0

FAMILY Subfamily *Tribe:* Subtribe	TAG Cat. [A]	Species	Orig. [B]	US Distribution [C]	Canada Dist. [C]	Reps	% survival [D]
		(closed bottle gentian)*		PA, RI, SD, VT, VA, WV, WI			
	5	*Gentianella quinquefolia* (L.) Small (agueweed)*	N	AR, CN, GA, IL, IN, IA, KS, KY, ME, MI, MD, MA, MS, MO, NH, NJ, NY, NC, PA, OH, SC, TN, VA, VT, WI, WV	ON, QC	10	0
LOGANIACEAE	5	*Mitreola petiolata* (J.F. Gmel.) Torr. & Gray (lax hornpod)*	N	AL, AR, FL, GA, LA, MS, MO, NC, OK, PR, SC, TN, TX, VA	None	Not Tested	N/A
	5	*Spigelia marilandica* (L.) L. (woodland pinkroot)*	N	AL, AR, FL, GA, IL, IN, KY, LA, MD, MS, MO, NC, OK, SC, TN, TX, VA	None	10	0
GELSEMIACEAE	5	*Gelsemium sempervirens* (L.) St. Hil. (yellow Jessamine)*	N	Al, AR, FL, GA, LA, MS, NC, SC, TN, TX, VA	None	10	0
RUBIACEAE	5	*Cephalanthus occidentalis* L. (common buttonbush)*	N	AL, AZ, AR, CA, CN, DE, FL, GA, IL, IN, IA, KS, KY, ME, MD, MA, MI, MN, MS, MO, NE, NH, NJ, NY, NC, OH, OK, PA, RI, SC, TN, TX, VT, VA, WV, WI	NB, NS, ON, PE, QC	10	0
	5	*Coffea arabica* L. (coffee)*	I	HI, PR, VI	None	10	0
	5	*Galium boreale* L. (northern bedstraw)*	N	AK, AZ, CA, CO, CN, DE, ID, IL, IN, IA, KY, ME, MD, MA, MI, MN, MO, MT, NE, NV, NH, NJ, NM, ND, OH, OR, PA, RI, SD, TN, TX, UT, VT, VA, WA, WV, WI, WY	AB, BC, MB, NT, NB, NS, ON, QC, SK, YT	10	0
	5	*Gardenia jasminoides* J. Ellis. (cape-jessamine)*	I	PR	None	10	0
	5	*Hedyotis purpurascens*			None	10	0
	5	*Houstonia caerulea* L. (azure bluet)*	N	AL, AR, CN, DE, GA, IL, IN, KY, LA, ME, MD, MA, MI, MS, MO, NH, NJ, NY, NC, OH, PA, RI, SC, TN, VT, VA, WV, WI	NB, NS, ON, QC	10	0
	5	*Houstonia longifolia* (longleaf bluets)		AL, AR, CT, DC, FL, GA, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, NC, ND, NH, NJ, NY, OH, OK, PA, RI,	AB, MB, ON, QC, SK	10	0

FAMILY **Subfamily** ***Tribe:*** **Subtribe**	**TAG Cat.** [A]	**Species**	**Orig.** [B]	**US Distribution** [C]	**Canada Dist.** [C]	**Reps**	**% survival** [D]
				SC, TN, VA, VT, WI, WV			
	5	*Mitchella repens* L. (partridgeberry)*	N	AL, AR, CN, DE, FL, GA, IL, IN, IA, KY, LA, ME, MD, MA, MI, MN, MO, NH, NJ, NY, NC, OH, OK, PA, RI, SC, TN, TX, VT, VA, WV, WI	NB, NL, NS, ON, PE, QC	10	0
	5	*Rubia tinctoria* L. (madder)*	I	CA, MA, NV, OR, PA, UT	None	10	0
SCROPHULARIACEAE	6 Ornam.	*Buddleja davidii* Franch. (butterfly-bush)*	I	CA, CN, GA, HI, KY, MA, MD, MI, NC, NY, NJ, OH, PA, TN, SC, VA, WA, WV, PR	BC	10	0
	6	*Polypremum procumbens* L. (juniper leaf)*	N	NY, NY, PA, DE, MD, TX, IL, MO, LA, FL, OK, TN, AL, GA, NC, SC, MS, AK, KY, IN	None	10	0
ASTERACEAE	7	*Artemisia absinthium* L. (wormwood)	I	CO, CT, IA, ID, IL, IN, KS, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NY, OH, OR, PA, RI, SC, SD, TN, UT, VT, WA, WI, WY	AB, BC, MB, NB, NF, NS, ON, PE, QC, SK	10	0
	7	*Artemisia caudata* (Michx.) H.M. Hall & Clem. (wild wormwood)	N	AL, AZ, CO, CT, FL, IA, IL, IN, KS, MA, ME, MI, MN, MO, MS, MT, ND, NE, NH, NJ, NM, NY, OH, OK, PA, RI, SC, SD, TX, VA, VT, WA, WI, WY	LB, MB, NB, NF, NS, NU, ON, QC, SK	10	0
	7	*Artemisia ludoviciana* Nutt. (white sagebrush)	N	AR, AZ, CA, CO, CT, DE, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WY	AB, BC, MB, NB, NT, ON, PE, QC, SK	10	0
	7	*Artemisia stelleriana* Besser (dusty miller)	I	AK, CT, DC, DE, FL, HI, LA, MA, MD, ME, MI, MN, NC, NH, NJ, NY, OH, PA, RI, VA, VT, WA, WI, WV	NB, NF, NS, ON, PE, QC	10	0
	7	*Artemisia vulgaris* L. (mug-wort)	I	AK, AL, CA, CT, DC, DE, FL, GA, HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MT, NC, NH, NJ, NY, OH, OR, PA, RI, SC, TN, VA, VT, WA, WI, WV	AB, BC, MB, NB, NF, NS, ON, PE, QC, SK	10	0

FAMILY Subfamily *Tribe:* Subtribe	TAG Cat. [A]	Species	Orig. [B]	US Distribution [C]	Canada Dist. [C]	Reps	% survival [D]
	7 Weed	*Tanacetum vulgare* L. (common tansy)	I	AK, AR, AZ, CA, CO, CT, DC, DE, HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SD, TN, UT, VA, VT, WA, WI, WV, WY	AB, BC, MB, NB, NF, NS, NT, ON, PE, QC, SK, YT	10	0
CANNABACEAE	7 Crop	*Humulus lupulus* var. "Newport" (hop plant)	**I**	Crop	Crop	10	0
	7 Crop	*Humulus lupulus* var. "Golden Nugget" (hop plant)	**I**	Crop	Crop	10	0
CONVULVULACEAE	7	*Calystegia (Convulvulus) sepium* R. Br. (larger bindweed)	I	AK, AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	AB, BC, MB, NB, NF, NS, ON, PE, QC, SK	10	0
URTICACEAE	7	*Urtica dioica* L. (stinging nettle)	I	AK, AL, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	AB, BC, LB, MB, NB, NF, NS, NT, ON, PE, QC, SK, YT	20	0 [E]
	7	*Boehmeria cylindrica* (L.) Sw. (smallspike false nettle)	N	AL, AR, AZ, CA, CT, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, NE, NH, NJ, NM, NY, OH, OK, PA, RI, SC, SD, TN, TX, UT, VA, VT, WI, WV, PR	NB, ON, QC	10	0 [F]
	7	*Laportea canadensis* L. (wood nettle)	N	AL, AR, CT, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, ND, NE, NH, NJ, NY, OH, OK, PA, RI, SC, SD, TN, VA, VT, WI, WV	MB, NB, NS, ON, PE, QC, SK	10	0
	7	*Parietaria floridana* Nutt. (Florida pellitory)	N	AL, DE, FL, GA, KY, LA, MD, MS, NC, NH, SC, TX	None	20	0
	7	*Pilea microphylla* (L.)	N	AL, AR, FL, GA, HI, LA, MI, MS, NC, SC, TN, TX, PR, VI	None	10	0

FAMILY **Subfamily** ***Tribe:*** **Subtribe**	**TAG Cat.** [A]	**Species**	**Orig.** [B]	**US Distribution** [C]	**Canada Dist.** [C]	**Reps**	**% survival** [D]
		Liebm. (artillery plant)					
	7	*Pipturus albidus* (Mamaki)	N	HI	None	10	0

[A] TAG Test Plant Categories: **1.** Genetic types of target weed; **2.** Species of the same (or closely related) genus; **3**. Species in the same family as the target weed (**3a**. Plants in same sub-tribe; **3b**. Plants of other sub-tribes; **3c**.Plants in same subfamily other tribes; and **3d.** Plants in other subfamilies); **4**. Threatened and endangered species in the same family; **5**. Species in other families in the same order having similar characteristics as target plant; **6** Species in other orders that have some physiological, morphological or biochemical similarities to the target weed including environmentally and economically important species; **7**. Any plant on which the biological control agent OR its close relatives have been found or recorded to feed and/or reproduce.

[B] Plant origin: introduced (I), native (N) to North America or (F) Foreign not in North America (Milbrath and Biazzo, 2007 or USDA Plants Database, 2011)

[C] Distribution from USDA Plants Database, 2011.

[D] Indicates the mean number of larvae that were successfully reared to pupation.

[E] One larva fed but died in the second instar

[F] One larva fed and survived to the final instar but died before pupation. Test was repeated and displayed no feeding.

*Indicates plant species on the approved TAG list (Milbrath and Biazzo 2007). All other plants on this list were either added to satisfy Category 7 requirements or were tested as additional representatives for each category.

b. Protocol for Releasing the Agents

1. Method to Ensure Pure Cultures and Correct Identification of the Agents

Our current cultures are pest free and have been reared in quarantine since 2008. If additional cultures of *Hypena opulenta* are needed, they will be obtained from original collection sites through CABI EU-CH. Voucher specimens of *H. opulenta* are kept at the URI Insect Quarantine Laboratory.

2. General Release Protocol to Ensure the Absence of Natural Enemies and Cryptic or Sibling Species

We expect to use the current *H. opulenta* colony at URI for all insects to be used in the proposed releases. As indicated, the current cultures are pest free. If there are unforeseen problems with the *H. opulenta* colony and additional material is required it will be collected from the same locality in the Ukraine as the tested populations. Once in containment the new material will be reared for at least one generation before any field releases to ensure that populations are pest free and no cryptic species are present.

3. Specific Location of Rearing Facility

Viable cultures of *Hypena opulenta* will continue to be reared at the University of Rhode Island Insect Quarantine Laboratory in Kingston, RI. As a backup for the current colony, a starter colony will be transferred from URI to the Insect Microbial Containment Facility AAFC Lethbridge in preparation for the proposed releases.

4. Intended Sites, Timing, Methods, and Number of Agents for Initial Release

United States: We intend to release *Hypena opulenta* 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 open field plots of *V. rossicum* and *V. nigrum*. We plan to release about 500 adults into each of these sites on the island. We will monitor release sites as described below.

Canada: We are planning to release on property owned by the University of Toronto: the Koffler Scientific Reserve at Jokers Hill in King Township 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 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.*

Fig. 4 Swallow-wort sites on Naushon Island, MA. Release sites include sun and shade plots. We'll also survey distant sites (Uncatena and Veckatimest and a stand near the south end of the island) for agent spread and establishment.

Fig. 5 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. 4) which we have monitored since 2008.

c. Post Release Monitoring

We have established sites on Naushon Island and Conanicut Island that have been monitored since 2008. Initially, we were using two $1m^2$ quadrats per site collecting data on number of stems per $0.5m^2$, number of seedlings per $0.1m^2$, 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 $0.5m^2$ 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 both sites once the agents are released.

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 not continuously, but in several years 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 agents 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 the agents have established and how far they have spread. These findings will allow us to make decisions on future release sites.

Fig. 6 Forested and open field sites of black swallow-wort on Naushon Island. Both sites (labeled Black in Fig. 4) are proposed for agent release.

d. Benefits and Risks

Hypena opulenta has displayed very minimal feeding and no complete development on any species outside of the genus *Vincetoxicum.* We conducted additional tests to determine if *H. opulenta* larvae would move to other plants in later instars if swallow-worts were defoliated. In these tests, larvae were reared on *V. nigrum* for two instars and then 10 larvae were transferred to each of the non-target test plants. Third instars did little or no feeding on *Cynanchum*

racemosum, *C. laeve*, *C. ascyrifolium*, *Asclepias verticillata*, *Matelea gonocarpos*, and *Gonolobus stephanotrichus*, and all died in less than 3 days without further development in trials where we had 100% survival of controls. Larvae transferred to *Urtica dioica* never fed, but survived a bit longer, lasting up to 5 days, and some larvae on *Asclepias syriaca* fed a little, and were able to live up to 8 days, but none progressed to the fourth instar.

Our host range testing indicates that *H. opulenta* presents no risk to non-target plants. Further, these agents have not displayed any behavior that would lead us to believe they would have any other negative environmental impacts or cause harm to native species.

In contrast, there would be numerous benefits if this agent was able to establish and have an impact on *Vincetoxicum* either immediately or over time. Effective biological control could lead to reduction in swallow-worts throughout North America with positive impact on native plant and animal species, in both managed and unmanaged ecosystems.

VI Potential Environmental Impacts

a. Human Impacts

A potential reduction in swallow-wort would relieve pressure on farmers, nursery owners and recreational managers because it would eliminate costs, efforts, and risks associated with applying herbicides or mechanical control. These agents would have no foreseeable negative impacts on humans.

b. Potential Economic Impacts

If these agents were able to limit swallow-wort populations, there could be considerable savings in time and money spent on controlling these invasive plants in managed ecosystems and enhanced recreational and wildlife benefits in natural ecosystems.

c. Plant Impacts

One consequence of pale swallow-wort invasion is an alteration of the local mycorrhizal community to one that decreases native plant species performance but enhances swallow-wort performance (Smith et al., 2008). In addition to the common native plants species affected by these changes to the soil community, there are several endangered plant species that are at risk from unmanaged populations of swallow-worts. This risk could be ameliorated, if swallow-wort populations are reduced, and natural areas with large *Vincetoxicum* infestations may see a return of native plant species.

d. Non-plant Impacts

Reducing swallow-wort infestations will increase native biodiversity in previously infested sites, helping to restore native ecological processes. It may also increase monarch butterfly populations by reducing the frequency of oviposition on swallow-wort instead of native milkweed populations.

e. Proposed Methods for Mitigation

The proposed biocontrol agent *H. opulenta* does not pose any risk to non-target plant species and therefore no undesireable effects are anticipated. For the US, releases on an island will provide some time for agent evaluation (and conceivably elimination if required) before natural or purposeful spread to the mainland US. In Canada *Vincetoxicum* patches at the release sites will be selected to minimize the initial dispersal potential of the insects such that their impact can be evaluated and populations potentially eliminated prior to adult dispersal if this is deemed necessary.

f. Abiotic and Edaphic Effects

This agent will not have any foreseeable negative impact on soil, air or water quality. In contrast, a reduction in swallow-wort may improve local soil quality in areas of infestations because swallow-worts contain toxic substances which may have allelopathic properties and change soil community composition (Christenson, 1998; Smith et al., 2008; Douglass et al., 2009).

g. 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.

VII Conclusion

Extensive research evaluating the host range of *H. opulenta* has shown that it can only develop on *Vincetoxicum* spp. and poses no risk to native North American plant species. This insect species causes extensive defoliation of *V. nigrum* and *V. rossicum*. Impact studies with *H. opulenta* demonstrate that it only takes one generation to significantly reduce aboveground biomass, flowering, seedpod production and number of seeds of *V. rossicum* in the following year. The multiple, overlapping generations of *H. opulenta* are expected to have a substantial impact on *Vincetoxicum* spp. under field conditions – particularly in the shade. Repeated defoliation over several years should facilitate interspecific competition in mixed plant communities and potentially significant reduction in *Vincetoxicum* populations. This biocontrol agent is a safe and effective candidate for field release in North America and given the severity of current swallow-wort infestations and lack of effective control measures, the timely release of *H. opulenta* is recommended.

References Cited

Arnett, R. H. 2000. American Insects: A Handbook of the Insects of America North of Mexico. CRC Press.

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

Bon M.C., F. Guermache, M. Rodier-Goud, F. Bakry, M.Bourge, M. Dolgovskaya, M. Volkovitsh, R. Sforza, S. Darbyshire and L. Milbrath 2011. Ploidy level and genome size of *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopow) Barbarich (Apocynaceae), two invasive vines in North America. Proceedings 13th International Symposium on Biological Control of Weeds. Kona Hawaii, USA, 11-16 September 2011.

Broughton, S. 2000. Review and evaluation of *Lantana* biocontrol programs. Biological Control Volume 17, Issue 3, March 2000, pp. 272-286.

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

Cappuccino, N., Mackay, R., and Esiner, C. 2002. Spread of the invasive alien vine *Vincetoxicum rossicum*: Tradeoffs between seed dispersability and seed quality. American Midland Naturalist 148: 263-270.

Casagrande, R. A. and J. Dacey, 2001. Monarch butterfly (*Danaus plexippus*) oviposition on black swallowwort (*Vincetoxicum nigrum*). Rhode Island Nat. Hist. Surv. 8: 2-3.

Casagrande, R.A. and J.E. Dacey. 2007. Monarch butterfly oviposition on swallow-worts (*Vincetoxicum* spp.). Environmental Entomology 36:631-636.

Christensen, T. 1998. Swallow-worts. Wildflower. Summer issue, pp. 21-15.

Centre De Données Sur Le Patrimoine Naturel Du Québec. 2008. *Les plantes vasculaires menacées ou vulnérables du Québec. 3e édition.* Gouvernement du Québec, ministère du Développement durable, de l'Environnement et des Parcs, Direction du patrimoine écologique et des parcs, Québec. 180 p.

COSEWIC. 2010. COSEWIC assessment and status report on the Four–leaved Milkweed *Asclepias quadrifolia* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. x + 40 pp.
http://www.sararegistry.gc.ca/virtual_sara/files/cosewic/sr_Four-leaved%20Milkweed_0810_e.pdf

Day, M.D., Wiley, C.J., Playford, J. & Zalucki, M.P. 2003a. Lantana: current management status and future prospects. ACIAR Monograph No. 102. Australian Centre for International Agricultural Research, Canberra, Australia.

DiTommaso, A. and J.E. Losey. 2003. Oviposition preference and larval performance of monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species. Entomologia Experimentalis et Applicata 108:205-209.

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

Douglass, C.H., Weston, L.A., and DiTommaso, A. 2009. Black and pale swallow-wort (*Vincetoxicum nigrum* and *V. rossicum*): the biology and ecology of two perennial, exotic and invasive vines. In: Management of Invasive Weeds. Ed. by Inderjit, Springer, the Netherlands, 261– 277.

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

Förare, J. 1995. The biology of the noctuid moth *Abrostola asclepiadis* Schiff. (Lepidoptera:Noctuidae) in Sweden. Entomologisk Tidskrift 116: 179-186.

Förare, J. and C. Solbreck. 1997. Population structure of a monophagous moth in a patchy landscape. Ecological Entomology 22: 256-263.

Gassmann, A,, R. Schwan, A. Mosyakin, N. Häfliger, R. Dejong, and I. Toševski. 2011. Annual Report 2010. Biological control of swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*. 2010 Annual Report of CABI Europe – Switzerland.

Guermache, F., Bon, M., Jones, W.A., and L.R. Milbrath. 2010. A look at ploidy level of *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopow) Barbar. (Apocynaceae) from the perspective of a study of their invasion success. 15th European Weed Research Society Symposium Proceedings, Kaspovar, Hungary, 12-15 July 2010.

Gleason, H. A. and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. 2nd ed. The New York Botanical Garden, Bronx, NY.

Goater, B., Ronkay, L., and Fibiger, M., 2003. Noctuidae Europaeae Vol. 10 Catocalinae & Plusiinae. Entomological Press, Sorø, 452 p., 16 color plates.

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

Grimble D.G., Beckwith, R.C., and P.C. Hammond. 1992. A survey of the Lepidoptera fauna from the Blue Mountains of eastern Oregon. Journal of Research on the Lepidoptera 31: 83-102.

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

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

Lauvanger, E.G. and L. Borgen. 1998. The identity of *Vincetoxicum* in Norway. Nord. J. Bot. 18: 353-364.

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

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

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

Liede, S. 1997. Subtribes and genera of the tribe Asclepiadeae (Apocynaccae, Asclepiadoideae) – a synopsis. Taxon 46: 233-247.

Lumer, C. and S. E. Yost. 1995. The reproductive biology of *Vincetoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York State. Bulletin of the Torrey Botanical Club 122: 15-23.

Manitoba Conservation Data Centre. 2009. http://www.gov.mb.ca/conservation/cdc/ecoregions.html

Markgraf, F. 1972. *Vincetoxicum* N. M. Wolf, pp. 71-73. *In:* T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb (eds.), Flora Europaea. Volume 3. Diapensiaceae to Myoporaceae. Cambridge University Press, Cambridge UK.

McCabe, T.L. and V. Vargas. 1998. The larva of *Hypena manalis* (Lepidoptera: Noctuidae: Hypeninae). Journal of the New York Entomological Society 106:109- 113.

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

Mikkola, K. and Honey, M.R. 1993. The Noctuoide (Lepidoptera) described by Linnaeus. Zoological Journal of the Linnean Society 108: 103-169.

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

Milbrath, L.R. 2010. Phytophagous Arthropods of Invasive Swallow-Wort Vines (*Vincetoxicum* spp.) in New York. Environ. Entomol. 39: 68-78.

Milbrath L.R. and Biazzo, J. 2007. Proposed host specificity test plant list for testing potential biological control agents of swallow-worts. Unpublished report submitted to USDA-APHIS-PPQ, 41 pp.

Miller G.C. and V. Kricfalusy 2008. Dog-strangling vine - *Cynanchum rossicum* (Kleopow) Borhidi: A review of distribution, ecology and control of this invasive exotic plant. Report Toronto Regional Conservation Authority (www.trca.on.ca) . 62pp http://www.rougepark.com/unique/reports/RP_DSV_Report_Dec07.pdf

Moore, R.J. 1959. The dog-strangling vine *Cynanchum medium*, its chromosome number and its occurrence in Canada. Can. Field-Nat. 73: 144-147.

NHIC 2011. Natural Heritage Information Centre. Ontario Ministry of NaturalResourceshttp://nhic.mnr.gov.on.ca/MNR/nhic/nhic.cfm

Oldham, M.J., and S.R. Brinker. 2009. Rare Vascular Plants of Ontario, Fourth Edition. Natural Heritage Information Centre, Ontario Ministry of Natural Resources. Peterborough, Ontario. 188 pp.Pedigo, L.P., J.D. Stone, and G.L. Lentz. 1973. Biological Synopsis of the green cloverworm in central Iowa. Journal of Economic Entomology 66: 665-673.

Pringle, J. S. 1973. The spread of *Vincetoxicum* species (Asclepiadaceae) in Ontario. The Canadian Field Naturalist 87: 27-33.

Roberts, P. M. and G. K. Douce, Coordinators. 1999. Weevils and Borers. A County Agent's Guide to Insects Important to Agriculture in Georgia. University of Georgia, Col. Ag. Env. Sci., Cooperative Extension Service, Tifton, GA, USA. Winter School Top Fifty Agricultural Insect Pests and Their Damage Sessions, Rock Eagle 4-H Ctr., Jan. 20, 1999.

Robinson, B.L. and M.L. Fernald. 1908. *Gray's New Manual of Botany*, 7th ed. American Book Company, New York.

SK Conservation Data Centre 2011. http://www.biodiversity.sk.ca/Docs/vasctrak.pdf

Sheeley, S. E. 1992. Life history and distribution of *Vincetoxicum rossicum* (Asclepiadaceae): an exotic plant in North America. M. S. thesis, Syracuse University of New York, College of Environmental Science and Forestry, Syracuse, New York.

Sheeley, S. E. and D. J. Raynal. 1996. The distribution and status of species of *Vincetoxicum* in eastern North America. *Bulletin of the Torrey Botanical Club* 123: 148-156.

Sipes, S.D., P.G. Wolf, V.J. Tepedino, and J. Boettinger. 1994. Population Genetics and Ecology of Jones Cycladenia. Bureau of Land Management Cost Share Program, Utah State Office. 27p. + append.

Smith, L.L, DiTommaso, A., Lehmann, J., and S. Greipsson. 2008. Effects of arbuscular mycorrhizal fungi on the exotic invasive vine pale swallow-wort (*Vincetoxicum rossicum*). Invasive Plant Science and Management 1: 142-152.

Solbreck, C. 2000. Ecology and biology of *Euphranta connexa* (Fabr.) (Diptera: Tephritidae)–a seed predator of *Vincetoxicum hirundinaria* Med. (Asclepiadaceae). Entomologisk Tidskrift 121: 23–30.

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

Tewksbury, L., R. Casagrande, and A. Gassmann. 2002. 16. Swallow-worts, pp. 209-216. *In:* R. Van Driesche, S. Lyon, B. Blossey, M. Hoddle, and R. Reardon (eds.), Biological Control of Invasive Plants in the Eastern United States. USDA Forest Service Publication FHTET-2002-04, Morgantown, WV.

Tutin, T. G. et al. Flora Italiana. 2006. Almanacco Distanze aeree Natura Italiana. 28 June 2011. *http://luirig.altervista.org/gifeurbig2/3649.gif*.

USDA, NRCS. June 2011. The PLANTS Database, Version 3.5 (http://plants.usda.gov). 2 June 2011. Data compiled from various sources by Mark W. Skinner. National Plant Center, Baton Rouge. LA 70874-4490 USA.

Wapshere, A.J. 1989. A testing sequence for reducing rejection of potential biological control agents for weeds. Ann. Appl. Biol. 114: 515-526.

Weed, A. S. 2010**.** Biology and ecology of European natural enemies of swallow-worts (*Vincetoxicum*) and the potential for biological control. Ph.D. dissertation, University of Rhode Island, Kingston, RI.

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

Weed, A.S., Gassmann, A. Casagrande, R.A. 2011a. Effects of leaf and root herbivory by potential insect biological control agents on the performance of invasive *Vincetoxicum* spp. Biological Control 56: 50-58.

Weed, A.S., Casagrande, R.A., A. Gassmann, and A. Leroux. 2011b. Performance of potential European biological control agents of *Vincetoxicum* spp. with notes on their distribution. Journal of Applied Entomology DOI: 10.1111/j.1439-0418.2010.01594.x

Weston, L., Barney, J. and A. DiTommaso. 2005. A Review of the Biology and Ecology of Three Invasive Perennials in New York State: Japanese Knotweed (*Polygonum cuspidatum*), Mugwort (*Artemisia vulgaris*) and Pale Swallow-wort (*Vincetoxicum rossicum*). Plant and Soil 277: 53-69.

Appendix 1

Timelines from discovery of candidate agent to release into nature in North America.

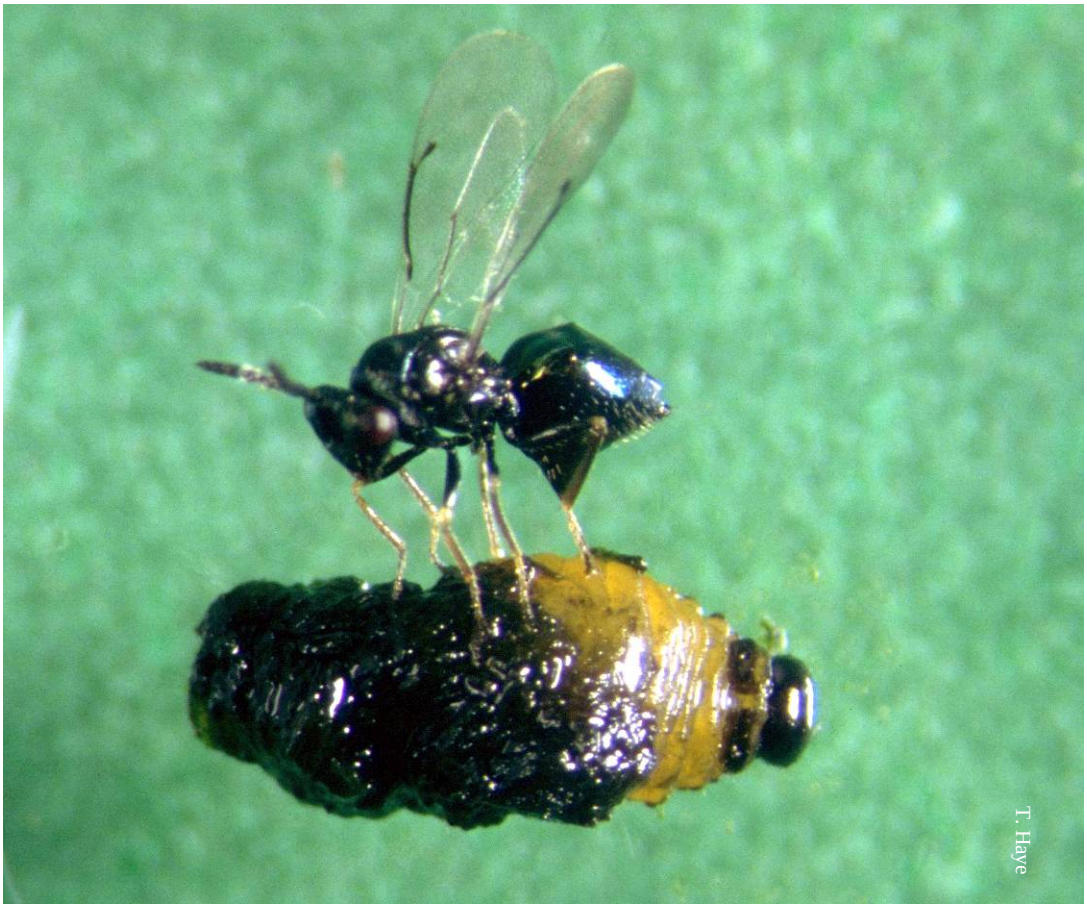
Year /Date	Action
2006	Identification and collection of *Hypena opulenta* as potential biocontrol agent bor *Vincetoxicum* from Donetsk, Ukraine. (Weed and Casagrande 2010)
2006 -2011	Host range testing at University of Rhode Island and CABI Delemont Switzerland to confirm host specificity and biology of *Hypena opulenta*. (Hazelhurst *et al.* 2012)
Nov 2011	Joint release petition for Canada and the United States submitted to USDA and CFIA for review . http://web.uri.edu/biocontrol/home/black-swallowwort/
June 2012	Following review by Canada Biological Control Committee and USDA-APHIS Technical Advisory Group, a formal response is issued by the regulators, requesting additional testing /replications for key plant species and testing of additional genetic stock of *Hypena opulenta* . This required additional field collections from the original site in Donetsk, Ukraine, which were made in summer 2012.
January 2013	Completion of the additional testing requested and submission of supplemental data package to regulators.
September 2013	Following second review of additional data package submitted in January the Canada Biological Control Commiteee and USDA-APHIS Technical Advisory Group on Biological Control of Weeds recommended release of *Hypena opulenta.*
September 2013	The Canadian Food Inspection Agency approved release of *Hypena opulenta* in Canada.

Hazlehurst, A.F., Weed, A.S., Tewksbury, L., Casagrande, R.A., 2012. Host specificity of *Hypena opulenta*: a potential biological control agent of *Vincetoxicum* in North America. Environ. Entomol. 41, 841–848.DOI: http://dx.doi.org/10.1603/EN12093

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

APPENDIX F
Model Petition for Release of an Entomophagous Species for Classical Biological Control of an Arthropod

Petition for cage- and open field release of *Tetrastichus setifer* (Hymenoptera: Eulophidae) for biological control of the Lily Leaf Beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae) in Canada



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***Disclaimer**: This sample petition is provided for information purposes and as a general template for required information, at the time of publication of this guide. Prospective petitioners should consult with the Canadian Food Inspection Service, at the time of petition preparation, for any revisions to the information needed and current petition requirements.*

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Abstract

Terastichus setifer Thomson (Hymenoptera: Eulophidae) is proposed as a classical biological control agent of lily leaf beetle, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae), an invasive alien pest of *Lilium* spp. in Canada since the 1940's. A univoltine gregarious European species, *T. setifer* attacks lily leaf beetle larvae causing death before development of the host is completed.

Host range tests were carried out in Rhode Island from 2001-2003. Of 7 non-target species tested, only a single larva of the 150 *L. trilineata* tested was attacked and found to contain parasitoids. It was concluded that this anomaly was likely an artifact (confinement) of the experimental setup, supported by no attacks of *L. trilineata* by *T. setifer* when a slightly modified experimental protocol was used.

The host specificity of *T. setifer*, its widespread European distribution and its capacity to produce up to 26 parasitoids (average of 7) per host is expected to result in substantial impact on lily leaf beetle in Canada.

1.0 INTRODUCTION

The lily leaf beetle *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae), a pest of cultivated and native lilies, was introduced into North America in Montreal in the 1940s (LeSage 1983). Over the past three decades it has expanded its range considerably. It is now a common pest throughout eastern Canada and the northeastern USA (LeSage and Elliott 2003; Majka and LeSage 2008), and it has been reported as far west as Alberta (Ken Fry, personal communication). The extensive native range—Northern Africa to Scandinavia and east to the Pacific—suggests that *L. lilii* is capable of becoming established throughout the North American continent (Kenis et al. 2003).

The lily leaf beetle feeds mainly on true lilies (genus *Lilium*) and several species in the closely related genus *Fritillaria*. Populations of the beetles reach high densities and complete defoliation is common. Because cultivated lilies are themselves introduced from Asia and *Fritillaria* does not occur outside of cultivation in Eastern North America, presently the lily leaf beetle it is mainly known as a horticultural pest. However, considering the enormous popularity of lilies in urban and suburban gardens (the lily trade is worth $65 million in the US (Gold 2003)), and the unsightly damage caused by lily beetle larvae, the importance of *L. lilii* as a pest cannot be underestimated.

Even more worrisome is the fact that the *L. lilii* has recently been reported infesting populations of Canada lily *Lilium canadense* L. in Quebec (Bouchard et al. 2008) and New Brunswick (Majka and LeSage 2008). Canada lily is a native species that is listed as threatened or vulnerable throughout its range (Gilbert 2005). Although the beetle has not yet been reported from populations of the native wood lily *Lilium philadelphicum* L., which is also threatened or endangered in parts of its range (Bouchard et al. 2008), the larvae perform well on the plant in the lab (Ernst et al. 2007). Moreover, as the range of the *L. lilii* expands westward, it will eventually come into contact with several other native lilies, of which roughly half are threatened or endangered (USDA PLANTS Database, http://plants.usda.gov). Thus, *L. lilii*, heretofore considered a garden pest, is on the verge of becoming a threat to native plant biodiversity as well.

Lilioceris lilii rarely attains pest status throughout continental Europe; especially in regions were lilies are native, such as Central and Eastern Europe, where it is controlled by a suite of native parasitoids (Gold et al. 2001; Haye and Kenis 2004). Populations found on native lilies are particularly heavily parasitized (Haye and Kenis, 2004). In North America, no arthropod natural enemies of *L. lilii* have been reported (Majka and LeSage 2008, Gold et al. 2001). In 2001, following host-specificity screening tests, classical biological control of *L. lilii* was begun in North America, with the release of over 3000 females of the larval parasitoid *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae) in eastern Massachusetts. *T. setifer* is now well established at several release sites throughout New England, and has spread over 7 miles from the original release sites (Casagrande and Tewksbury 2007). Parasitism rates up to 100% have been recorded and local beetle densities have declined (Tewksbury et al. 2005). Thus, *T. setifer* shows great promise for the control of lily leaf beetle in North America.

2.0 PROPOSED ACTION

2.1 Objectives of the release

We propose to release *T. setifer* as a classical biological control agent for lily leaf beetle at experimental plots on the Central Experimental Farm in Ottawa, Ontario. *T. setifer* adults reared from larvae collected at the Rhode Island release sites will be released in an experimental garden. The goal of these releases is to initiate a self-perpetuating population of *T. setifer*, which can subsequently be redistributed elsewhere in Canada where *L. lilii* is established.

2.2 Choice of biological control agent

Tetrastichus setifer is one of three agents already released in North America for the control of *L. lilii*. In the United States, *T. setifer* has been released in Massachusetts, Rhode Island, Maine and New Hampshire, and is successfully established in all four states. Documented parasitism ranges from 27-100% (Casagrande and Tewksbury 2006). The widespread distribution of *T. setifer* in its native range suggests that it is capable of surviving in a variety of habitats, including those in Canada where *L. lilii* has established and is likely to spread into.

2.3 Rearing/containment facility

Tetrastichus setifer will be reared at the National Arthropod Containment Facility (NACF) operated by Dr. Peter Mason and Ms. Andrea Brauner in Ottawa, ON. Material imported from the United States and Europe will be used to establish colonies for release. Pathogen- and parasite-free parasitoids will be used for release in agricultural sites infested with lily leaf beetle.

2.4 Disposal of unwanted hitchhikers

Shipments of *T. setifer* adults, pupae in host cocoons and/or parasitized larvae received at the NACF will be inspected for contaminants. Pupae in host cocoons and parasitized larvae will be reared and any pathogens, predators, parasitoids, and hyperparasitoids will be removed and destroyed by autoclaving. Autoclaved material will then be disposed of in the regular garbage.

2.5 Location of the release

The initial release will be done in an experimental garden at AAFC's Central Experimental Farm in Ottawa (45º 23′ N; 75º 43′ W). Subsequent releases will be done in both large lily gardens and natural populations of *L. canadense* that have been reported to support populations of *L. lilii* in Quebec (Bouchard et al. 2008) and Alberta.

2.6 Methods of release

Release of *T. setifer* will be made using the methods outlined in Tewksbury et al. (2005). Host cocoons containing overwintering parasitoid larvae will be maintained in the cold storage (2^{o}C) for 5 months after which they will be stored at 4^{o}C. When required, the cocoons will be warmed to 25^{o}C for adult emergence. Adult parasitoids will be released into plots containing lilies.

2.7 Agencies and or individuals involved in the release

Carleton University and Agriculture and Agri-Food Canada will be the lead agencies for the release and monitoring program. Dr. Naomi Cappuccino, Dr. Peter Mason, Andrea Brauner, and technical staff will be involved.

2.8 Current biological control of L. lilii in Canada

No biological control agents have been released in Canada for biological control of *L. lilii*. Moreover, no effective native natural enemies have been observed in Canada (Majka and LeSage 2008). In the northeastern United States three parasitoids have been released (Tewksbury 2006). *Tetrastichus setifer* has been released in MA, RI, ME and NH and is successfully established in all four states and documented parasitism ranges from 27-100%. *Lemophagus errabundus* Gravenhorst (Hymenoptera: Ichneumonidae) has been released in MA and RI only and not only established from releases in 2003 and 2004, but has spread a considerable distance. Although *Diaparsis jucunda* (Holmgren) (Hymenoptera: Ichneumonidae) has been released in RI, ME, and NH, it has not established in any of these states.

3.0 TARGET PEST INFORMATION

3.1 Taxonomy

Classification:

Order: Coleoptera
Family: Chrysomelidae
Subfamily: Criocerinae
Tribe: Criocerini
Genus: *Lilioceris*
Species: *lilii* (Scopoli)

Common names:

lily leaf beetle
scarlet lily beetle
criocère du lys

The lily leaf beetle is a distinctive, easily recognized chrysomelid. The elytra (wing covers) are shiny and bright red, contrasting with the beetle's black antennae, eyes, head, legs, and underside. The adult varies in length from about 6 to 8 mm, and has 11-

segmented antennae, notched eyes, and two visible indentations on the thorax. Adults produce chirping sounds by means of a stridulatory apparatus.

3.2 Economic impact of the target pest

The lily leaf beetle is considered to be a minor economic pest in Canada (OMAFRA 2009). However, it defoliates cultivated *Lilium* and *Fritillaria* species, as well as native lilies (Livingston 1996). A thick fecal shield covers the larvae, which feed on leaves and flowers, often defoliating plants before pupating in the soil. Young adults emerge in mid-summer and feed before overwintering in leaf litter. The impact of *L. lilii* on *Fritillaria* and *Lilium* can be quite severe (Figure 1). Majka and LeSage (2008) described the situation in the Halifax area: "Beetle populations can rapidly increase over the season, can cause severe defoliation, and affected plants eventually languish and die. C.G.M. interviewed one gardener in Waverly, NS who, in 1992 had over 50 species and cultivars of lilies growing in a large garden on his property. Although he took very active measures to curb the numbers of beetles, including hiring neighborhood children to pick them off affected plants, in 2006 only one species of lily survived on his property." Many home gardeners have considered replanting their lilies to another type of perennial to get away from the pesticide sprays needed to control this pest (Casagrande and Tewksbury 2006).

Figure 1. Lily damaged by lily leaf beetle, *Lilioceris lilii* (Scopoli), note that the leaves have been stripped from the plant.

Native species are also at risk from attack by lily leaf beetle. Bouchard et al. (2008) found *L. lilii* on the native Canada lily *Lilium canadense* throughout much of southern Quebec. Majka and LeSage (2008) reported that *L. lilii* had colonized Canada lily in the

Marysville area of New Brunswick. They also noted that *L. canadense* is present in both the Annapolis Valley of Nova Scotia, and the Fredericton area of New Brunswick, areas where *L. lilii* has been found. According to Majka and LeSage (2008), "Roland (1998, pp. 1203) stated that *L. canadense* is, 'now becoming rare in most parts of its range.'" *Lilium canadense* occurs from the Maritime Provinces south to Alabama and Georgia. It is ranked S1 (extremely rare) in Alabama, Kansas, North Carolina, Rhode Island, and Ontario; and S2 (very rare) in Delaware, Indiana, Tennessee, Georgia, and Nova Scotia (Dolan 2004). Given the present decline of *L. canadense* and the impact of *L. lilii* on other species of lilies, this transfer of *L. lilii* from a horticultural to a native plant poses a potentially significant threat to this already rare, native lily. Ernst et al. (2007) identified several endangered native lilies including *L. canadense, L. philadelphicum, L. michiganense* Farw., and *L. superbum* L. as being at risk of being colonized by *L. lilii* and recommended that populations of these lilies be monitored closely. Thus, lily leaf beetle has potential to cause damage to natural ecosystems.

3.3 Life history of the target pest

Lilioceris lilii (Figure 2) is univoltine and overwinters as an adult in soil and leaf litter. Following a pre-oviposition period of 2 weeks (Haye and Kenis 2004), eggs are laid on the undersides of host plant leaves in linear clusters of 2-16 (Müller and Rosenberger 2006). They hatch in approximately one week (Haye and Kenis 2004). Larvae feed on the undersides of leaves, and later may attack buds and flowers (Fox-Wilson 1942). The larvae cover themselves with their own excrement. The function of this fecal shield is still unclear, but may serve to protect them from a variety of generalist predators (Emmel 1936, Eisner *et al*., 1967, Jolivet & Verma, 2002. Nolte (1939) assumed that the shield could also serve as insulation preventing dehydration of the larva. Schaffner and Müller (2001) determined that the fecal shield was attractive to the parasitoid *Lemophagous pulcher* Szepligeti (Hymenoptera: Ichneumonidae). The larvae pass through four instars and after 10-21 days leave the plant to pupate in the soil, in a loose cocoon made by gluing together soil particles. The pupal stage lasts about three weeks at 22º C (Haye and Kenis 2004). Emerging adults feed, but do not mate, and must undergo an obligate diapause before reproducing (Haye and Kenis 2004). Earlier accounts of a second generation (e.g., Lataste 1932) are probably due to the observation of longer-lived individuals ovipositing well into late summer (Haye and Kenis 2004).

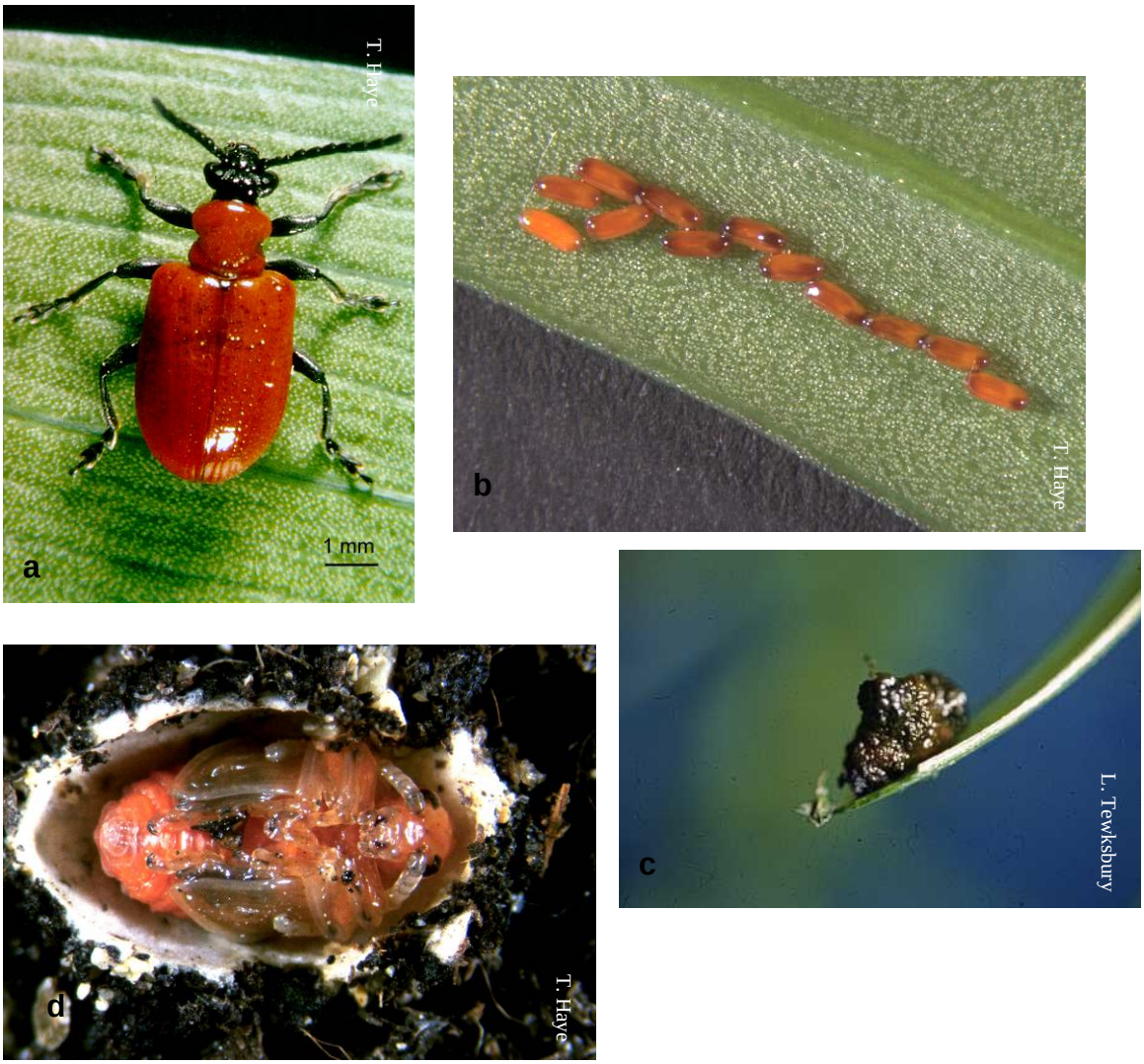

Figure 2. Lily leaf beetle, *Lilioceris lilii* (Scopoli): a) adult; b) eggs; c) larva with fecal shield; d) pupa in cocoon.

3.4 Distribution of the target pest

Lilioceris lilii has a large native distribution, ranging from Europe across Asia to China and extending from North Africa to Scandinavia (Figure 3). It is considered to be exotic in Great Britain (Fox-Wilson 1942) and possibly also in countries such as Sweden and the Netherlands, where no native lilies and fritillaries and where it was probably introduced together with cultivated lilies from central or eastern Europe (Rämert et al. 2009). In North America (Figures 3-4), it was first recorded in Montreal in 1943 (Brown 1946), although there was possibly an earlier introduction in which the beetle was wrongly identified as *Lema melanocephala* Say (Majka and LeSage 2008). The beetle spread to Ottawa in 1981 and was first recorded from the US in Cambridge

Massachusetts in 1992. Since then, its spread across the eastern half of North America has been rapid, and it now occurs from Nova Scotia to Manitoba, as well as in the six New England states and New York. Lily leaf beetle was first reported in Alberta in 2003, apparently the result of infested material being transported from Ontario (Anonymous 2009). It now occurs in at least 7 confirmed localities throughout Alberta (Ken Fry, personal communication). Based on its Eurasian distribution, Haye and Kenis (2000) predict that the beetle could spread throughout much of North America.

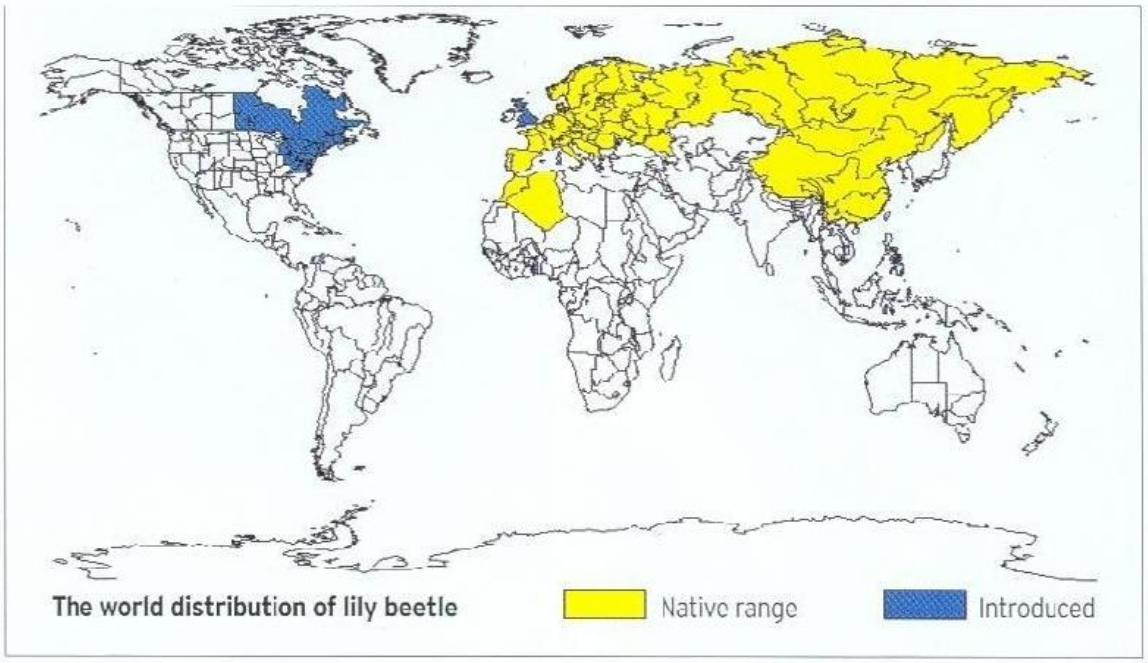

Figure 3. Global distribution of Lily leaf beetle, *Lilioceris lilii* (Scopoli) (from Salisbury 2008).

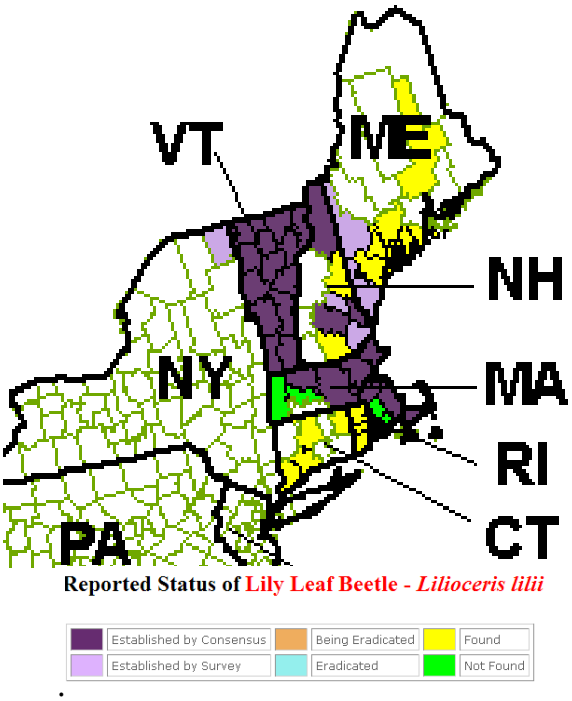

Figure 4. Distribution of Lily leaf beetle, *Lilioceris lilii* (Scopoli), in the northeastern U.S.A. to 2007: green indicates not present, all other colours indicate presence (NAPIS - **http://pest.ceris.purdue.edu/searchmap.php?selectName=INAMGRA&maptype=alltime**).

3.5 Economically and environmentally important species related to the target

There are no other species in the genus *Lilioceris* in North America (Integrated Taxonomic Information System. http://www.itis.gov/ accessed Aug 21, 2009). Two other species from the tribe Criocerini occur in North America: the common asparagus beetle *Crioceris asparagi* (L.) and the spotted asparagus beetle *C. duodecimpunctata* (L.). Both are important pests of asparagus that are native to Europe. The other tribe in the subfamily Criocerinae is the Lemini, represented by approximately 35 species in the genera *Lema*, *Neolema* and *Oulema*, most of which are native (Integrated Taxonomic Information System. http://www.itis.gov/ accessed Aug 21, 2009). Of these, only *Lema daturaphila* Kogan & Goeden (=*L. trilineata* White), *Oulema collaris* (Say) and *O. sayi* (Crotch) occur in Canada (Integrated Taxonomic Information System. http://www.itis.gov/ accessed Aug 21, 2009). *Lema daturaphila*, the three-lined potato beetle, feeds on a variety of plant families and is a pest on potatoes (Price 1997).

4.0 BIOLOGICAL CONTROL AGENT INFORMATION

4.1 Taxonomy

Classification:

Order: Hymenoptera
Family: Eulophidae
Subfamily: Tetrastichinae
Genus: *Tetrastichus*
Species: *setifer* Thomson

4.2 Identification of Biological Control agent and voucher specimens

The identity of *T. setifer* released in Canada will be confirmed by Dr. G. Gibson, Agriculture and Agri-Food Canada, Ottawa, Ontario who is a taxonomic authority on Eulophidae. Voucher specimens of *T. setifer* from the culture and any newly imported populations from the original culture source areas (Europe or United States) used for release will be deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes in Ottawa.

4.3 Natural geographic range, areas where introduced

Haye and Kenis (2004) reared *T. setifer* from *L.lilii* collected from gardens in Switzerland, Germany, Austria, Italy, France, Belgium and Netherlands. It has also been reported from England (Salisbury 2003), as well as from Sweden (Rämert et al. 2009), Slovakia, the Czech Republic and the former Yugoslavia (Graham 1991).

Following release in North America starting in 2001, *T. setifer* has become established in Massachusetts, Rhode Island, Maine and New Hampshire.

4.4 Source of the biological control agent

Tetrastichus setifer released in the U. S. were collected from various locations throughout Europe (Haye and Kenis 2000; Gold 2004). Populations initially released in Canada will be derived from the established U.S. populations. If additional material is warranted, collections will be made in the area of Europe where the *T. setifer* population established in the U.S. originated.

4.5 Host-agent interactions

Haye and Kenis (2004) surveyed three species of *Lilioceris—L. lilii, L. merdigera* (L.) and *L. tibialis* (Villa) —for parasitoids throughout Europe. They reported that *Tetrastichus setifer* parasitized 3 to 39 % of *L. lilii* larvae collected from cultivated lilies in gardens. *T. setifer* was also found in populations of *Lilioceris lilii* on *Lilium martagon* L. from natural sites. As well, the parasitoid attacked *Lilioceris merdigera* feeding on *Polygonatum* in wooded sites, where it caused 4% mortality, and *Lilioceris tibialis* on *Lilium martagon* at 1300 to 2000 m elevation in the Alps, where the parasitism rate was 42%. Thus, *T. setifer* appears to be somewhat of a habitat generalist. A recent survey in

Swedish gardens showed that it is the second most important parasitoid in this region, reaching 1-25% parasitism (Rämert et al., 2009).

4.6 Life history

Tetrastichus setifer (Figure 5) attacks all four larval instars of *L. lilii*, although it has more difficulty penetrating the thick fecal shield of older larvae (Haye & Kenis 2004). The parasitoid overwinters as a mature larva in *L. lilii* cocoons in the soil, emerging in the spring over a several-week period (Casagrande and Kenis 2004). Adults can live up to two months in the laboratory at 15° C (Haye and Kenis 2004). From 2 to 26 parasitoid individuals can emerge from a single host (mean of 7.0 ± 4.0 SD) (Casagrande and Kenis 2004).

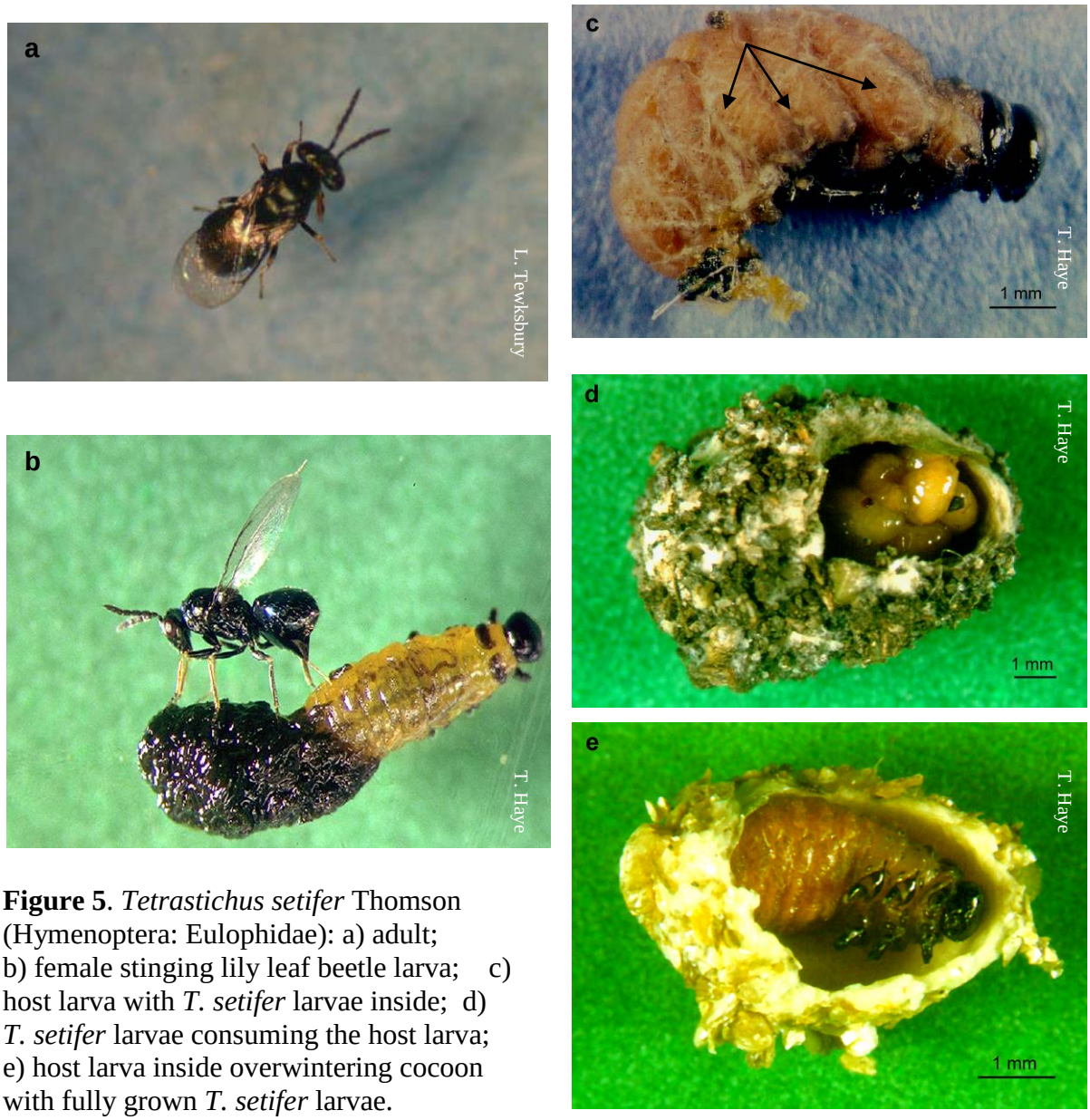

Figure 5. *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae): a) adult; b) female stinging lily leaf beetle larva; c) host larva with *T. setifer* larvae inside; d) *T. setifer* larvae consuming the host larva; e) host larva inside overwintering cocoon with fully grown *T. setifer* larvae.

4.7 Known host range

Tetrastichus setifer has been reared from naturally occurring populations of *Lilioceris lilii*, *L. tibialis*, and, more rarely, *L. merdigera* (Haye and Kenis 2004). It has never been found in Criocerinae outside the genus *Lilioceris*, despite extensive rearing programs aimed at finding European biological control agents for the two asparagus pests, *Crioceris asparagi* and *C. duodecimpunctata*, and the cereal leaf beetle, *Oulema melanopus* (Casagrande and Kenis 2004).

4.8 History of past use of control agent

Tetrastichus setifer has become established in Massachusetts, Rhode Island, New Hampshire, and Maine following releases beginning in 2001. The agent has spread from the original release sites and is causing parasitism rates of up to 100% at some sites (Casagrande and Tewksbury 2006). Current data collected in 2009 (Tewksbury, personal communication) indicates that results are similar to 2006. In Rhode Island *T. setifer* was found in 13 of 16 homeowner properties sampled. Those properties that were within a mile of the original release site had 90-100 % parasitism in every sample. Parasitism was found up to five miles from the original release site, although the parasitism rate at five miles away was 33%.

4.9 Elimination of hitchhikers from culture

Thus far, no contaminants (pathogens, parasites or hyperparasitoids) have been observed or identified from *T. setifer*. If recovered, these will be destroyed as outlined in section 2.4.

4.10 SOP for handling in quarantine

As mentioned in sections 2.3 and 2.4, *T. setifer* adults, pupae in host cocoons and/or parasitized larvae will be shipped to quarantine in Petri dishes packed in vermiculite and no host plant material will be included in the shipment. Any contaminants will be removed and destroyed. At the NACF, the *L. lilii* pupae will be maintained until emergence of *T. setifer* adults. Any hyperparasitoids will be removed and destroyed. A voucher series of any hyperparasitoids will be retained for identification and incorporation into the CNC.

4.11 Closely related species in North America

Tetrastichus setifer is in the eulophid sub-family Tetrastichinae, one of the largest and most ecologically diverse groups of Hymenoptera. Early taxonomic treatments of the group (e.g., Burks 1979) placed the majority of species in the genus *Tetrastichus* (LaSalle 1993). Graham (1987, 1991) revised the European Tetrastichinae, dividing *Tetrastichus* into several genera. LaSalle (1993) reviewed the North American species in light of Graham's classification. According to LaSalle's reclassification, there are 21 species in the genus *Tetrastichus* in North America. Of those, six are recorded from coleopteran hosts (LaSalle 1993).

Several species of *Tetrastichus* have been introduced as biological control agents of dipteran, lepidopteran, coleopteran and homopteran pests in North America, but only

two—*T. coeruleus* (Nees) (= *T. asparagi*) and *T. julis* Walker —have become established in their new range (LaSalle 1993). *T. coeruleus* provides partial control of its host, the common asparagus beetle *Crioceris asparagi* (L.) (Capinera and Lily 1975). *T. julis* is even more successful, providing substantial control of the cereal leaf beetle *Oulema melanopus* (Harcourt et al 1977, Ellis et al 1979).

5.0 HOST-SPECIFICITY TESTING

Host specificity testing studies were conducted by Dr. M. Kenis and graduate students (T. Haye, C. Scarborough) of CABI Europe Switzerland (European studies) and Dr. R. Casagrande, L. Tewksbury, H. Faubert and graduate student M. Gold of the University of Rhode Island (North American studies).

5.1 Selection of test insects

The host specificity assessment of *T. setifer* followed best practice procedures being developed at the time (see Van Driesche and Reardon 2004). An initial non-target test list was constructed using multiple criteria for the selection of appropriate species (Kenis et al., 2003, Casagrande and Kenis 2004). Non-target species were included in the initial list if they satisfied one (or preferably more) of the following selection criteria: (1) phylogenetic affinity to target (shared species, genus, family or superfamily), (2) ecological similarity to target (shared food plant or feeding niche), (3) known hosts of other *Tetrastichus* species and (4) outgroup species (different family). The original test list consisted of approximately 11 non-target species, but this was pared down to 10 species based on availability of non-target host material. The final test list shown in Table 1 includes all non-target species tested with *T. setifer* from 2002 to 2004.

Table 1. Non-target test list for *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae).

Insect Species (Family: Subfamily)	Native Range	Host Plant [Family]	Selection Criteria
Target			
Chrysomelidae: Criocerinae *Lilioceris lilii* (Scopoli)	Europe	*Lilium martagon* L., *Lilium* spp., *Fritillaria* spp., *Cardiocrinum* spp. [Liliaceae]	
Non-target			
Lilioceris tibialis (Villla)	Europe	*Lilium martagon* L., *L. bulbiferum* L. [Liliaceae]	Congener, similar biology & ecology
Lilioceris merdigera L.	Europe	*Polygonatum multiflorum* L., *P. verticillatum* (L.), *Convallaria majalis* L. [Ruscaceae] *Allium ursinum* L., *A. schoenoprasum* L. [Lilliaceae]	Congener, similar biology & ecology
Crioceris asparagi (L.)	Europe	*Asparagus officinalis* L. [Liliaceae]	Same subfamily
Lema daturaphila Kogen & Goeden (=*L. trilineata* White)	NA	*Solanum tuberosum* L., *Nicotiana tabacum* L. [Solanaceae]	Same subfamily
Chrysomelidae: Chrysomelinae *Leptinotarsa decemlineata* (Say)	NA	*Solanum melongena* L, *Solanum tuberosum* L. *Lycopersicon esculentum* Mill. [Solanaceae]	Different subfamily, ease of rearing
Chrysomelidae: Galerucinae *Galerucella calmeriensis* (L.), *Galerucella pusilla (*Duftschmidt)	Europe	*Lythrum salicaria* L. [Lythraceae]	Different subfamily, availability, ease of rearing
Coccinellidae: Epilachninae *Epilachna varivestis* Mulsant	NA	*Phaseolus vulgaris* L., *Glycine max* L., *Vigna unguiculata* (L.) Walp., *Trifolium pratense* L., *T. repens* L., *Medicago sativa* L., *Pueraria lobata* (Willd.) Ohwi, and *Bidens* spp. *Desmodium* spp. [(Fabaceae]	Unrelated, availability, ease of rearing
Epilachna borealis Mulsant	NA	*Cucumis melo* L., Cucumis sativa L., *Cucurbita* spp. [Cucurbitacae]	Unrelated, availability, ease of rearing

5.1 Tests on species in the genus Lilioceris

5.1.1 Methods - These tests were conducted in Europe by CABI Europe Switzerland (Haye, 2000; Kenis et al., 2001, 2002; Scarborough, 2002, Haye and Kenis, 2004) and were summarized by Casagrande and Kenis (2004). The work consisted of: 1) evaluation of sympatric populations in the field and 2) laboratory host specificity screening.

Sympatric populations - Third and fourth instar larvae of various *Lilioceris* species were collected between May and July, 2002, from four natural sites in the Jura region of Switzerland and artificial site in the same region (Scarborough, 2002). All five sites had sympatric populations (separated by less than 500 m) of the beetle *L. lilii* feeding on

Lilium martagon and cultivated *Lilium* spp. and the beetle *L. merdigera* feeding on *P. multiflorum* and *P. verticillatum*. At a sixth site, situated in the Alps, sympatric populations of the beetles *L. lilii* and *L. tibialis* were found feeding on the lily *L. martagon*.

Larvae from all sites were reared on excised host plants in 1.3 liter plastic containers with a bottom layer of wet fine vermiculite and allowed to pupate. After emergence of adult beetles and some non-diapausing parasitoids, the containers were sifted and the parasitoids that had emerged were identified based on cocoon features and adult emergence.

Host specificity screening - These tests were carried out by Haye (2000), Kenis et al. (2001, 2002), and Scarborough (2002). Laboratory rearing of the three species was set up in cages using adults or eggs collected from field populations in Switzerland. Larvae were fed with cultivated lily (for *L. lilii* and *L. tibialis*) and cultivated onion (for *L. merdigera*)

Parasitoids used in these experiments (*L. pulcher, L. errabundus, D. jucunda,* and *T. setifer*) were reared from cocoons collected in previous years and held over winter at 2^{o}C. The cocoons, held in Petri dishes in polystyrene boxes lined with damp cellulose paper, were moved to room temperature (20-24^{o}C) and monitored daily for adult emergence. For the ichneumonid species, males of a single species were held together in 1.3 liter containers in groups of four or five and provided with moist cotton wool dipped in honey. Females were placed in cages with males for approximately 24 hours for mating and then held separately for another 24 hours before use in experiments. Between tests, parasitoids were kept in incubators at 11-17^{o}C, 16:8 L:D photoperiod and ambient humidity, with access to moist cotton wool and honey.

In choice tests, three larvae of *L. lilii* and three larvae of either *L. merdigera* or *L. tibialis* were placed in a 9.4 cm diameter Petri dish and one parasitoid was introduced for ten minutes, during which time ovipositions on individual larvae were directly observed. Because the eulophid *T. setifer* oviposits for up to 30 minutes compared to a few seconds for the three ichneumonids, experiments with *T. setifer* were run for 3 hours. Following each test, larvae were reared on their proper host plants and held over wet fine vermiculite in 0.15 liter containers until they were dissected to determine parasitism.

In no-choice tests, a single female was introduced into a dish of its dominant host (typically *L. lilii*) and observed for 10 minutes to count ovipositions. She was removed and allowed 10 minutes before a second exposure to three larvae of the alternate host. Again, ovipositions were recorded during this second exposure, after which the female was provided a second 10-minute rest. A third 10-minute exposure to the initial test species was conducted to confirm her ability (or willingness) to oviposit. Exposed beetle larvae were reared over wet, fine vermiculite before dissection to determine parasitism.

5.1.2 Results and discussion

Sympatric populations – Parasitism of *Lilioceris* spp. varied between geographic locations and host species (Table 2). The parasitoid complex consisted of *Diaparsis jucunda*, *Lemophagus errabundus*, *L. pulcher*, and *Tetrastichus setifer*.

Table 2. Parasitism (%) among the sympatric populations of *Lilioceris lilli* (Scopoli), *L. merdigera* L., and *L. tibialis* (Villa) (Coleoptera: Chrysomelidae) in two regions in Switzerland. Numbers in parenthesis indicate the number of parasitized beetles (after Scarborough 2002).

Location	Host species	% Parasitism (n=)			
		Diaparsis jucunda	***Lemophagus errabundus* + *L. pulcher***	***Tetrastichus setifer***	**Total**
Jura	*Lilioceris lilli* (Scopoli)	48.6 (142)	25.7 (75)	2.1 (6)	76.4 (223)
	Lilioceris merdigera (L.)	5.6 (10)	74.6 (132)	0.6 (1)	80.8 (143)
	Lilioceris tibialis (Villa)	--	--	--	--
Western Alps	*Lilioceris lilli* (Scopoli)	79.2 (38)	4.2 (2)	10.1 (5)	93.8 (45)
	Lilioceris merdigera (L.)	--	--	--	--
	Lilioceris tibialis (Villa)	0.8 (3)	20.3 (77)	8.1 (31)	29.3 (111)

Host specificity screening - *Tetrastichus setifer* was reared from all three test species. Individuals of *T. setifer* reared from *L. lilii* spent significantly more time on *L. lilii* than on *L. tibialis* in paired choice tests ($P = 0.009$) and showed a significant preference for *L. lilii* over *L. merdigera* ($P = 0.0002$). However *T. setifer* reared from *L. tibialis* showed no preference between that host and *L. lilii* (Table 3).

Table 3. Summary of *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae) choice tests with European *Lilioceris* spp. (Coleoptera: Chrysomelidae), 2000 (after Scarborough 2002, Casagrande and Kenis 2004).

Parasitoid Origin	Test Species	Mean Contact Duration (secs) ±SD		Mann Whitney *U*
Lilioceris lilli (Scopoli)	*L. lilii* – v – *L. tibialis*	*L. lilii*	874.3 ± 607.6	W = 398.0, p = 0.009
		L. tibialis	306.0 ± 234.0	
Lilioceris tibialis (Villa)	*L. tibialis* – v – *L. lilii*	*L. tibialis*	1,416.0 ± 1,062.2	W = 272.5, p = 0.5971
		L. lilii	1,596.7 ± 609.5	
Lilioceris lilli (Scopoli)	*L. lilii* – v – *L. merdigera*	*L. lilii*	1,029.2 ± 640.6	W = 478.5, p = 0.0002
		L. merdigera	385.4 ± 387.1	

These results are consistent with those observed by Haye and Kenis (2004) in non-sympatric populations at natural sites on wild plants in Switzerland. Although all four major parasitoids occasionally attack all three congeneric hosts in natural settings, strong host preferences are shown. *L. lilii* is mainly attacked by *D. jucunda*, which is found in

very low numbers on the two other hosts and only in the vicinity *of L. lilii* populations. *L. pulcher* is by far the main parasitoid of *L. merdigera*. *T. setifer* has been observed in high numbers attacking populations of *L. tibialis*, and *L. errabundus* is found occasionally on all the three hosts. These strong host preferences shown in natural habitats in Switzerland do not necessarily reflect their potential as biological control agents, given that all four parasitoids have been found as dominant parasitoids of *L. lilii* in gardens in different regions of Europe (Haye and Kenis, 2004; Rämert et al., 2009). In Europe, *Tetrastichus setifer* is only occasionally the dominant parasitoid of *L. lilii*, but it is the only species found to be abundant in all European regions investigated (Haye and Kenis 2004; Rämert et al, 2009).

5.1.3 Conclusions

When congeneric *Lilioceris* hosts were available in natural settings in Europe, *Tetrastichus setifer* attacked *L. lilii* and *L. tibialis*, but was only rarely reared from *L. merdigera* (Scarborough 2002). Both *L. tibialis* and *L. merdigera* were suitable hosts that supported *T. setifer* development in the lab (Casagrande and Kenis 2004). In choice tests, *T. setifer* raised from *L. lilii* prefered *L. lilii*; however, females raised from *L. tibialis* showed no preference (Casagrande and Kenis 2004). The fecal shield appears to play an important role in host selection. *L. lilii* larvae were preferred over *L. merdigera*; however, switching the larvae's fecal shields caused female *T. setifer* to prefer *L. merdigera* (Scarborough 2002).

5.2 Tests on species outside the genus Lilioceris

5.2.1 Methods

Source and rearing of parasitoids - Parasitoids used in these experiments were reared from *L. lilii* larvae collected in Europe. In 1998, these were collected in northwestern France (Gold et al. 2001), and in subsequent years, they were collected throughout Europe (Haye and Kenis, 2000; Gold, 2004). Field-collected larvae were held in 1.4 l plastic containers under laboratory conditions (ca 25ºC) and fed lily leaves until cocoon formation. Resultant cocoons were then held under similar conditions until all adult *L. lilii* emerged. Parasitized cocoons were then held at 4ºC in a growth chamber for a minimum of two months before shipment in chilled containers to the University of Rhode Island (URI) Biological Control Laboratory. In the quarantine laboratory, parasitoids were held at 4ºC until needed for experiments and then warmed to 25ºC for adult emergence. From 1999-2003, 12,978 parasitized *L. lilii* cocoons were shipped to URI, including 4,352 *T. setifer*, 4,895 *D. jucunda* and 3,731 *Lemophagus* spp. Parasitoids that emerged were used in research. The remaining cocoons were dissected and information on species was provided to CABI for parasitoid distribution surveys. Only field-collected parasitoids were used in the host specificity studies.

The pest beetle *L. lilii* was maintained in quarantine at the URI Biological Laboratory in a colony that was started (and periodically refreshed) with adults collected near Boston, Massachusetts. Beetles were reared on potted Asiatic and Oriental lilies grown from organically produced bulbs in a greenhouse under ambient temperature conditions and a minimum of 16h daylight, supplemented by 400 watt sodium vapor or 1000 watt mercury

vapor lights on timers. In the laboratory, beetles were reared in screen cages (45 cm on a side) under fluorescent lights with a 16:8 (L:D) photoperiod. Newly emerged adult beetles were fed for a minimum of one week and then stored in plastic freezer cartons with paper towels in a refrigerator at 7^{o}C for three months, after which they were removed and used in rearing (Gold, 2004).

Host range tests - Newly emerged adult parasitoids were held in 1.8 liter plastic jars in growth chambers under fluorescent lights with a 16:8 (L:D) photoperiod and a day:night temperature cycle of 20:15^{o}C. The jars were removed from the growth chambers for 4h during host specificity tests at ambient room temperature (25^{o}C). These tests were conducted on a table next to a window with supplemental fluorescent lighting. Potential hosts included the common asparagus beetle, *Crioceris asparagi* (L.), which is in the same tribe, Criocerini, as *L. lilii,* as well as two Criocerinae from the tribe Lemini, the cereal leaf beetle *Oulema melanopus* (L.) and the three-lined potato beetle *Lema daturaphila*. Four other potential hosts were more distantly related to *L. lilii*: three non-Criocerinae chrysomelids—the willow leaf beetle *Plagiodera versicolor* Laicharting, loosestrife beetles *Galerucella* sp. (*Galerucella calmariensis* (L.) and *G. pusilla* Duftschmidt) and a phytophagous coccinellid, *Epilachna varivestis* Mulsant. (Table 1).

Test larvae were placed on stems of their host plant for a minimum of 2h before exposing them to parasitoids in all experiments because Schaffner and Müller (2001) showed that some species of *L. lilii* parasitoids are attracted to plants damaged by *L. lilii* larvae. For these feeding periods and subsequent parasitoid exposures, 10-12 second or third instar larvae were placed on an excised stem of a host plant, and that stem was placed in a floral water tube filled with tap water. In the tests with ichneumonid species, one to five female wasps (generally three, rarely one) and one male wasp were placed in a jar with the test larvae for 2 hours. In the tests with eulophid species, ten females and at least one male *T. setifer* were placed in a jar for 2 hours. Wasps were provided water and honey with either a damp wick in a floral water tube and a streak of honey or honey water on a wick. Immediately after exposure to the test larvae, the same parasitoid adults were given a second exposure to 10-12 second or third instar *L. lilii* larvae on a lily stem using the same protocol as above. When parasitism was found in a test larva, as well as in the subsequent test with lily leaf beetle larvae, the results were analyzed using a Chi-square test.

After parasitoid exposure, larvae were reared in 240 ml plastic containers with a bottom layer of 50 cc of damp vermiculite and fed leaves of the host plant for approximately ten days before they were dissected to determine parasitism. In all experiments, the first exposure of a female parasitoid was to a nontarget test species (other than *L. lilii*), and these exposure data were used only if parasitoids successfully attacked *L. lilii* larvae after that first exposure. Depending upon the parasitoid species, between 35% and 71% of the tests were rejected because of lack of attack on *L. lilii*, involving well over 1,500 test larvae and an equivalent number of *L. lilii*. Among the possible 32 tests (8 test larvae x 4 parasitoid species) we obtained useful results (with positive results in controls) in 27 combinations with an average of 35.6 test larvae per test. The *L. lilii* controls in these tests averaged 27.3% parasitism.

5.2.2 Results and discussion

None of the putative hosts exposed to *T. setifer* were attacked except a single larva of *L. trilineata*, which was found to contain *T. setifer* larvae (Table 4). The parasitoid ratio (1/73) was significantly different (Chi-square test, $P = 0.001$) from the parasitism of the *L. lilii* control in this test (15/63), indicating a distinct preference of *L. lilii* as a host by this species. Gold (2004) also conducted preliminary tests in which *T. setifer* was exposed to *L. trilineata* using a slightly different protocol, and in those tests 0 of 79 larvae were parasitized. We consider the parasitism of a single *L. trilineata* larva out of 150 tested to be an anomaly, perhaps due to confinement in too small a container.

Table 4. Summary of *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae) non-target host range testing, 1999-2003 (after Gold 2004, Casagrande and Kenis 2004). N - number of replicates; *total over all tests.

Insect species	**N**	**Test Species**		***Liloceris lilii* control**	
		# Killed By Parasitoid	**Parasitoid-Induced Mortality (%)**	**# Killed / N**	**Parasitoid-Induced Mortality (%)**
Chrysomelidae					
Criocerinae					
Criocerini					
Liloceris lilii (Scopoli)	333*	118*	23.5-42.5	-	-
Crioceris asparagi (L.)	46	0	0	45 / 106	42.5
Lemini					
Lema daturaphila Kogen & Goeden	73	1	1.4	15 / 63	23.8
Chrysomelinae					
Leptinotarsa decemlineata (Say)	41	0	0	16 / 41	39.0
Galerucinae					
Galerucella calmariensis (L.) + *Galerucella pusilla* Duftschmidt	39	0	0	8 / 34	23.5
Coccinellidae					
Epilachna varivestis Mulsant	40	0	0	21 / 53	39.6
Epilachna borealis Mulsant	39	0		13 / 36	36.1

5.2.3 Conclusions

In the host-range trials that included a variety of potential hosts outside the genus *Lilioceris*, the only non-host that was attacked was a single larva of *L. trilineata* (out of 73 tested) (Casagrande and Kenis 2004). In subsequent tests with *L. trilineata*, no larvae (of 79) were parasitized (Gold 2004). Taken together, these tests indicate that *T. setifer* is a genus-specific parasitoid that is unlikely to expand its host range to include native chrysomelids or other beetles.

6.0 Environmental and economic impacts of the proposed release

6.1 Impacts on vertebrates

Release of *T. setifer* is not expected to have any direct impacts on humans or other vertebrates. Reduction of populations of the target species will result in fewer applications of insecticide, reducing input costs for producers and improving yields.

6.2 Implications of not releasing the biological control agent

The lily leaf beetle is an unsightly garden pest that is threatening to become an important environmental pest as it incorporates native lilies into its diet. As it expands its range westward, it will come into contact with an increasing number of potential hosts in the genus *Lilium*, of which roughly half are threatened or endangered (USDA PLANTS Database, http://plants.usda.gov). Given the lily beetle's ability to completely defoliate and eventually kill its host plant, the potential for the extirpation of local lily populations is substantial.

6.3 Direct impacts on target and non-target species

Impact on the target species, *L. lilii*, at U.S. release sites has already proven to be substantial, as *T. setifer* has caused up to 100% larval mortality (Tewksbury et al. 2005). The impact on non-target species is unlikely. *T. setifer* is a genus specialist, attacking larvae of the genus *Lilioceris*, in which there are no other species reported from North America. As *Lilioceris* is a genus of moderate-sized, flamboyantly coloured beetles, it is unlikely that any species remain to be discovered in North America in general or Canada in particular.

There remains a small possibility that *T. setifer* could attack other species in the sub-family Criocerinae, since one individual of *Lema trilineata* was attacked in laboratory host-range tests (Casagrande and Kenis 2004). *Lema daturaphila* (=*L. trilineata*) oviposits on plants containing tropane alkaloids in the genera *Datura, Physalis, Atropa*, and others but larvae feed on many plant families (Price 1997). *Lilioceris lilii* occasionally feeds on the solanaceous plant *Solanum nigrum*, when it occurs in proximity to lilies (N.C. personal observation). Thus, it is possible that *T. setifer* will come into contact with *L. trilineata* on its solanaceous hosts. If the parasitoid does eventually incorporate *L. trilineata* into its diet, this collateral damage will not constitute an environmental emergency, as *L. trilineata* is a pest as well, on potatoes. Two other Lemini, *Oulema collaris* and *O. sayi*, occur in Canada (Integrated Taxonomic Information System. http://www.itis.gov/ accessed Aug 21, 2009). Since *T. setifer* did not attack *Oulema melanopus* no-choice tests, it is unlikely to incorporate *O. collaris* or *O. sayi* into its diet.

The two Criocerinae in Canada that are most closely related to *L. lilii* (same tribe, Criocerini) are the two asparagus pests *Crioceris asparagi* L. and *C. duodecimpunctata*, both of which are native to Europe. *T. setifer* did not attack these either of species in no-

choice tests. In Europe, where the asparagus beetles and the lily beetle co-occur, *T. setifer* has never been reported from *Crioceris* sp., despite extensive rearing of these beetles in the context of their own biological control programs.

6.4 Effects on physical environment

There are no effects on the physical environment.

6.5 Indirect effects

As there are no known predators or parasitoids of *L. lilii* in North America, indirect effects through competition for hosts are negligible.

6.6 Possible direct or indirect effects on threatened and endangered species

The only indirect effect on threatened and endangered species is the positive effect on threatened lily populations of removing a potentially devastating herbivore.

7.0 Post-release monitoring

7.1 Biological control agent establishment and spread

Field release sites will be monitored for *T. setifer* establishment and dispersal. Surveys will be conducted in mid to late June and late July for a 5 year period after release. Thereafter, surveys will be recommended every 5 years to monitor dispersal.

Initial releases will be made in a lily leaf beetle-infested lily garden (100+ plants) established at the AAFC Central Experimental Farm in Ottawa. This site will be 'farmed' to encourage establishment and population increase of *T. setifer*. In addition, releases will be made at 1-3 sites with histories of lily leaf beetle infestation using the methods developed by Tewksbury et al (2005). Releases will consist of at least 50 females and a similar number of males. Development of the beetle populations will be monitored and when 4th instar larvae appear they will be collected just before they enter the soil to pupate. These larvae will be brought to the laboratory where they will be allowed to pupate in Petri dishes containing vermiculite to obtain estimates of parasitism. Measuring the background mortality is necessary for accurately judging the impact of the released parasitoids. To estimate background mortality, 25 sentinel plants with lily leaf beetle eggs and L1-L3 larvae will be placed in the experimental plots one week before the parasitoid mass-releases. These plants will then be removed immediately prior to making the parasitoid releases. Percent mortality from larvae on these plants will be measured based on the number of L4 larvae that develop.

7.2 Biological control agent and target densities over time

Field release sites will be monitored annually for 5 years to assess levels of attack by *T. setifer* and densities of lily leaf beetle, *L. lilii*. At specified locations, the density of lily leaf beetle on 25 or more randomly sampled plants will be determined during June, July and August. Biweekly, from each plant 4th instar lily leaf beetle larvae will be collected

(50-100 larvae in total), dissected in the laboratory and the number of *T. setifer* larvae will be documented.

7.3 Host-specificity and attack rates on the target species and non-target species

Existing lily populations will be monitored for presence of *L. lilii* and 4th instar larvae collected. As well, 2nd instar larvae of *L. lilii* will be set out on sentinel plants in close proximity to and at various distances away from the release location. These sentinel individuals will be retrieved when larvae reach 4th instar. All larvae will be dissected and/or reared in the laboratory. Data generated will allow us to determine how far from the target habitat *T. setifer* has moved in a year and how parasitism levels change as the parasitoid becomes established and disperses.

7.4 Changes in the target pest and in the growth, survival and reproduction of selected non-target species populations

Populations of lily leaf beetle will be monitored by measuring occurrence of larvae using counts on plants. Numbers of larvae collected will be documented at 5 locations at 1 km distances from the release point. Collections of lily leaf beetle larvae will be made at the same locations, in habitats where they can be found. These data will be compared with pre-release data to assess population changes.

7.5 Changes in species diversity and community structure

At the field release locations identified in section 7.2, the density of native beetle species will be determined annually by sweep net sampling and/or pan traps and/or plant inspections. These densities will be related to declines in lily leaf beetle densities to assess species diversity and community structure.

8.0 References

Anonymous. 2009. Lily leaf beetle. http://plantlilies.com/growlilies/68-beetle.html (last visited September 13, 2009).

Brown, W.J. 1946. Some new Chrysomelidae, with notes on other species (Coleoptera). *Canadian Entomologist* 28:47-54.

Casagrande, R.A. and M. Kenis. 2004. Evaluation of lily leaf beetle parasitoids for North American introductions. Pp. 121-137, *In* Van Dreische, R.G. and R.Reardon (editors). *Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice*. United States Department of Agriculture, Forest Service, Morgantown, WV, FHTET-2004-03, 243p.

Casagrande, R and L. Tewksbury. Jan. 2007. Lily Leaf Beetle Biological Control Research Report to the North American Lily Society.

Casagrande, R. and L Tewksbury. 2006. Lily Leaf Beetle Biological Control Update. http://www.umext.maine.edu/piscataquis/gardening/2006/vol4iss5/beetle.htm (last visited May 28, 2009).

Capinera, J.L. and J.H. Lily. 1975. Bionomics and biotic control of the asparagus beetle, *Crioceris asparagi*, in western Massachusetts. *Environmental Entomology* 4: 93-96.

Ellis, C.R., D.G. Harcourt, and D. Dubois-Martin. 1979. The current status in Ontario of *Tetrastichus julis* (Hymenoptera: Eulophidae), a parasite of the cereal leaf beetle *Proceedings of the Entomological Society of Ontario*109: 23-26.

Eisner, T., Tassel, E.V. and J.E. Carrel (1967). Defensive use of a 'fecal shield' by a beetle larva. Science, 158, 1471-1473.

Emmel, L. 1936. Aus dem Leben des Lilienhähnchens (*Crioceris lilii*). *Natur und Volk* 66: 424-428.

Ernst, C., N. Cappuccino and J.T. Arnason. 2007. Potential novel hosts for the lily leaf beetle *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae) in eastern North America. *Ecological Entomology* 32:45-52.

Fox Wilson, G. 1942. The lily beetle, *Crioceris lilii*. *Journal of the Royal Horticultural Society* 67:165-168.

Gold, M.S., R.A. Casagrande, L.A. Tewksbury, S.B. Livingston and M. Kenis. 2001. European parasitoids of *Lilioceris lilii* (Coleoptera: Chrysomelidae). *Canadian Entomologist* 133:671-674.

Gold, M.S. 2004. Biological control of the lily leaf beetle, *Lilioceris lilii*, in North America. Ph.D. Dissertation. University of Rhode Island, Kingston, Rhode Island, USA.

Harcourt, D.G., J.C. Guppy, and C.R. Ellis. 1977. Establishment and spread of the *Tetrastichus julis* (Hymenoptera: Eulophidae), a parasite of the cereal leaf beetle in Ontario. *Canadian Entomologist* 109:473-476.

Haye, T. and M. Kenis. 2000. Lily leaf beetle (*Lilioceris lilii* Scopoli). Annual Report 1999. CABI Bioscience, Delémont, Switzerland.

Haye, T., and M. Kenis. 2004. Biology of *Lilioceris* spp. (Coleoptera: Chrysomelidae) and their parasitoids in Europe. *Biological Control* 29:399-408.

Kenis, M., T. Haye, R.A. Casagrande, M.S. Gold, and L.A. Tewksbury. 2003. Selection and importation of European parasitoids for the biological control of the lily leaf beetle in North America, and prospects for control, in Europe. Pp. 416-419, *In* R.G. Van Driesche (editor) *Proceedings of the International Symposium on Biological Control of Arthropods. Honolulu, Hawaii, 14-18 January 2002*, United States Department of Agriculture, Forest Service, Morgantown, WV, FHTET-2003-05, 573 p.

Jolivet, P. and K.K. Verma. 2002. *Biology of Leaf Beetles.* Intercept, Andover, U.K.

Lataste, F. 1932. Addition à ma note du 10 mars 1931 Sur le criocère du Lis. Zooéthique. *Bulletin de la Société zoologique de la France* 57 :36-39.

LeSage, L. 1983. Note sur la distribution présente et future du criocère du lys, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae) dans l'est du Canada. *Le Naturaliste canadien* 110:95-97.

LeSage, L. and B. Elliott. 2003. Major range extension of the lily leaf beetle (Coleoptera: Chrysomelidae), a pest of wild and cultivated Liliaceae. *Canadian Entomologist* 135:587-588.

Majka, C.G. and L. LeSage. 2008. Introduced leaf beetles of the maritime provinces, 5: the lily leaf beetle, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae) *Proceedings of the Entomological Society of Washington* 110:186-195.

Müller, C. and C. Rosenberger. 2006. Different oviposition behaviour in Chrysomelid beetles: Characterisation of the interface between oviposition secretion and the plant surface. *Arthropod Structure and Development* 35:197-205.

Nolte, H. B. 1939. Zur Biologie des Lilienhähnchens. *Die Kranke Pflanze* 16: 7-10.

Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA). 2009. The Lily Leaf Beetle (Coleoptera: Chrysomelidae) http://www.uoguelph.ca/pdc/Factsheets/Insect/LilyLeafBeetle.htm (last visited May 28, 2009).

Price, P.W. 1997. *Insect Ecology*. John Wiley & Sons, New York, NY, U.S.A., 890p.

Rämert, B. **,** Kenis, M. **,** Kroon, H. and U. Nilsson. 2009. Larval parasitoids of *Lilioceris lilii* (Coleoptera: Chrysomelidae) in Sweden and potential for biological control. *Biocontrol Science and Technology* 19:335-339.

Salisbury, A. 2008. Lily beetles and prospects for control. *The Plantsman* 7:230-234.

Scarborough, C. 2002. An investigation of the parasitoids of the lily leaf beetle, Lilioceris lilii (Scopoli) (Coleoptera: Chrysomelidae): A case study for biological control in North America. M.Sc. Thesis. University of London, Imperial College, London, UK.

Schaffner, U. and C. Müller. 2001. Exploitation of the fecal shield of the lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), by the specialist parasitoid *Lemophagus pulcher* (Hymenoptera: Ichneumonidae). *Journal of Insect Behaviour* 14:739-757.

Stehr, F.W. 1970. Establishment in the United States of *Tetrastichus julis* (Hymenoptera: Eulophidae), a larval parasite of the cereal leaf beetle. *Journal of Economic Entomology* 63: 1968-1969.

Tewksbury, L.A., M.A. Gold, R. Casagrande and M. Kenis. 2005. Establishment in North America of *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), a parasitoid of *Lilioceris lilii* (Coleoptera: Chrysomelidae), pp. 142-143. *In* M.S. Hoddle (ed.), *International Symposium Biological Control of Arthropods*. USDA Forest Service, Davos, Switzerland.

Van Driesche, R.G. and R. Reardon (editors). 2004. *Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice*. United States Department of Agriculture, Forest Service, Morgantown, WV, FHTET-2004-03, 243p.

APPENDIX G
Model Petition for Release of an Entomophagous Species for Inundative/Augmentative Biological Control of an Arthropod

A petition for first release of the parasitoid wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) for biological control of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in greenhouse crops

Submitted by:
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***Disclaimer**: This sample petition is provided for information purposes and as a general template for required information, at the time of publication of this guide. Prospective petitioners should consult with the Canadian Food Inspection Service, at the time of petition preparation, for any revisions to the information needed and current petition requirements.*

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Abstract

We request permission to import into Canada, and release the parasitoid *Encarsia formosa* into greenhouse crops of tomato and other hothouse crops for biological control of greenhouse whitefly, *Trialeurodes vaporariorum*. This action is required in order to manage populations of greenhouse whitefly below economic injury thresholds. Economic injury through contamination of greenhouse produce with honeydew, and through direct growth and production losses due to phloem removal are causing significant economic losses in the industry. Current biological control agents are insufficient to reliably maintain populations of the greenhouse whitefly below economic injury levels, and the development of widespread insecticide resistance renders most insecticides ineffective. Moreover, the application of insecticides interferes with biological control agents for other pests and damages pollinator populations in greenhouses, causing further economic loss.

The candidate agent, *Encarsia formosa* has no cold-hardiness or overwintering (e.g. diapause) traits, and is unlikely to overwinter in Canada except in the most mild winters in the extreme west, on southern Vancouver Island, British Columbia. Host records and non-target host range testing demonstrate that the host range is restricted to nymphs of Aleyrodidae. Because the agent does not orient to odours of host plant/non-target whitefly combinations in y-tube assays, we predict that *E. formosa* adults will not specifically seek out populations of the native species. Thus the agent may attack native whiteflies in an opportunistic fashion, but this attack is unlikely to have any lasting effects on population dynamics of any of the species.

There are only a few (less than 10) species of whitefly in Canada, and the majority are economic pests. None of the whitefly species in Canada is of any conservation concern or economic benefit. We therefore request authorization to release *E. formosa* into Canada, and to develop colonies for commercial production and sales.

1. Proposed Action

1.1 Purpose of the release

The purpose of the release is to adapt and field test methods for biological control of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), using the parasitoid wasp, *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) on greenhouse tomato, and to develop the ongoing use of *E. formosa* for inundative and augmentative biological control of greenhouse whitefly in commercial tomato greenhouses in Canada

1.2 Need for the release (explains why the agent is being introduced)

The greenhouse whitefly is the key pest of greenhouse grown tomato crops in Canada and elsewhere. As described in section 2, insecticide resistance and subsequent loss of control of this species has led to routine crop failures and devastating economic losses in the greenhouse industry. Developing a new approach to managing this pest is essential. As described in section 3, the parasitoid *Encarsia formosa* is used successfully in the greenhouse industry in the Netherlands for biological control of greenhouse whitefly.

1.3 Reasons for choice of the entomophagous biological control agent

Encarsia formosa is widely and successfully used throughout Europe for biological control of the target pest on greenhouse tomato crops. The conditions inside greenhouses in Canada are sufficiently similar to conditions in Europe to allow us to predict that the wasp will perform in exactly the same manner. The host range is restricted to species in the family Aleyrodidae (whiteflies) and literature and evidence presented herein suggests a degree of specialization within the host family.

1.4 Specific location of rearing/containment facility and name(s) of qualified personnel operating the facility

Initial containment of the received specimens and maintenance of a small laboratory population was under a research importation permit (Permit Number) and took place in the PPC 1 containment facility at the Agassiz Research and Development Centre, which are certified by the Canadian Food Inspection Agency. The facility is managed by Dr. Dave Gillespie and his technician, Peggy Clarke. This facility was used for the conduct of all non-target testing described in this document, and a small colony continues to be maintained in this facility.

As a commercial product, *Encarsia formosa* will be mass produced in a warehouse type operation located in Somewhere, BC. The facility is secure, having alarm systems in place for equipment failures and break and enter, and card-lock access to component operations (e.g., rearing, packaging/shipping, office areas). The operations are run by general manager John Smith who oversees a staff of 10 employees. Mr. Smith has a M.Sc. in insect physiology and 15 years of experience in the commercial insect rearing industry.

1.5 Timing of the release (approximate date of release), as well as factors that affect the timing of release (e.g. life stage of target pest or of biological agent to be released, season, agricultural practices, weather)

Releases are planned around the life cycle and pest density of the greenhouse whitefly, and the affected crop grown. Therefore, multiple releases will be made by clients during the calendar year. *Encarsia formosa* oviposits in 3rd and 4th instar whitefly hosts, thus releases will be made when host populations are immature and releases will continue (several weeks) until immature stages are no longer present. Releases will be made in glasshouse and atrium environments which are controlled for temperature. A small-scale mass rearing program will be developed, and once numbers are sufficiently large, releases will be made, likely in March or April of 2017.

1.6 Location of planned first release (e.g., province/state and region)

A small-scale mass rearing program will be developed, and once numbers are sufficiently large, releases will be made, likely in March or April of 2017. The first release will be made in a small-scale production greenhouse at the Agassiz Research and Development Centre, Agassiz, British Columbia. Subsequent releases will be in commercial greenhouse tomato facilities in southwestern British Columbia

1.7 Methods to be used after agent importation (e.g., rearing, multiplication, release)

An initial population of approximately 20,000 individuals per week will be produced in the containment facility, and these will be used for releases in greenhouses, which do not have containment. Once efficacy has been demonstrated, the rearing program will be transferred to Mr. Recneps Nairb, A Biological Control Company, Somewhere, Canada, who will increase production and sell this natural enemy to commercial greenhouse growers across Canada.

As described in section 3, a mass rearing system will be developed that will ultimately allow sales for ongoing augmentative release of *E. formosa* in commercial greenhouses across Canada.

1.8 Methods to be used for disposing of any host material, pathogens, parasites, parasitoids, and hyperparasitoids accompanying an import

Because the present colony was developed under a previous importation permit, contaminants have been removed from the colony. However, if additional individuals need to be imported, similar procedures will be followed as previously. *Encarsia formosa* will be shipped as parasitized whitefly scales. These are black, in contrast to unparasitized scales, and emerge roughly 7 days after the whitefly adults. Therefore, we will hold the received material in small, sealed dishes in containment until whitefly adults have emerged, and destroy these in alcohol. As they emerge, *E. formosa* adults will be individually removed, inspected to verify species, and transferred to rearing cages with whitefly nymphs of a suitable stage. There is a small chance that other aphelinid parasitoid species would also have parasitized the whitefly scales. These scales are not sclerotized and darkened to the same degree as those parasitized by E. formosa, and can be recognized and destroyed fairly easily by removal of the individual scales into alcohol. Adults of these other species are also quite distinct from *E. formosa* and, if present, will

also be removed when adults are transferred to rearing cages. Males of *E. formosa* and of many other species of Aphelinidae that parasitize whiteflies develop as hyperparasitoids of female parasitoids in whiteflies. These are very rare in the case of *E. formosa*, so presence of large numbers of males will be an indication that other species are presents and lead to the destruction of the shipment in alcohol.

1.9 Agencies or individuals that will be involved in the release and monitoring

Dr. Dave Gillespie and Peggy Clarke, AAFC, Agassiz Research and Development Centre will be involved in the initial releases and monitoring, in Centre greenhouses and in commercial greenhouses in the lower Fraser Valley.

Mr. Recneps Nairb, A Biocontrol Company, the commercial supplier will coordinate ongoing monitoring by growers. Growers will be involved in release and monitoring in commercial greenhouses in the Lower Fraser Valley.

2. Target Pest Information

2.1 Taxonomy: scientific name, full classification, synonymy, common names (if any), and sufficient characterization to allow unambiguous recognition

Class Insecta
Order Hemiptera
Suborder Sternorrhyncha
Family Aleyrodidae
Genus *Trialeurodes*
Species *vaporariorum* (Westwood, 1856)

Common Names
English: greenhouse whitefly,
French: aleurode des serres,
Spanish: mosca blanca de las hortalizas

Synonyms
Trialeurodes vaporariorum (Westwood, 1856) - Valid name
Aleurodes vaporariorum Westwood, 1856
Asterochiton lecanoides Maskell, 1879
Aleurodes papillifer Maskell, 1890
Aleurodes nicotianae Maskell, 1895
Aleyrodes sonchi Kotinsky, 1907
Trialeurodes mossopi Corbett, 1935
Trialeurodes sesbaniae Corbett 1936
Trialeurodes natalensis Corbett, 1936

NOTE: Synonyms and common names are easily obtained on the internet. The Encyclopedia of Life is an excellent general source for the taxonomy and synonyms of most species (http://eol.org/).

2.2 Economic impact and benefits (if any) of the target pest

Trialeurodes vaporariorum is a key pest of greenhouse tomatoes, and of many other greenhouse vegetable and ornamental crops in Canada (Raworth *et al*. 2002). Losses are from a number of causes. Honeydew from adults and nymphs may contaminate fruit surfaces, necessitating either washing of fruit, or in the case of products that cannot be washed, disposal of product. If numbers are sufficiently high, large accumulations of honeydew promote the growth of black sooty mold (*Cladosporium or Alternaria* spp.) that obscure leaf surfaces and reduce photsynthesis. Direct damage (loss of growth, reduction of fruit production) results from the presence of moderate to high numbers (Hoddle 2004, Avilla *et al*. 2004). Greenhouse whitefly can transmit criniviruses, which can cause major losses in tomato crops (Orfanidou *et al*. 2014). This species is also a pest of many tropical and subtropical crops (e.g. Vazquez *et al*. 1995).

2.3 Biology and reproductive potential of the target pest

Adults of *Trialeurodes vaporariorum* (Figure 1) lay eggs on expanding leaves and fresh growth near the growing points of plants. The number of eggs produced varies with plant species and plant nutrition. On tomato at 22°C, females produce an average of 153 eggs, which require 29 days to read the adult stage; and adults live for 36 days (van Lenteren and Martin 1999).

Figure 1. *Trialeurodes vaporariorum*: Left, whitefly adult and eggs, Right, whitefly adults.

Stages are egg, first instar (crawler), second, third, fourth instar nymphs, puparium and adult. Eggs are attached to leaves by a pedicel, which is embedded into the leaf and are mostly laid on the underside of leaves. If females are not disturbed, eggs are typically aggregated, and can be laid in characteristic semicircles. Eggs hatch after 8 days and produce a first-instar crawler which can move on the leaf and selects a suitable site to settle (Figure 2). This stage completes development in about 6 days, and subsequent instars require 2, 3, and 4 days. The puparium completes development in about 5 days, after which the adults emerge. There is no overwintering stage, and no stage diapauses in this species (Byrne and Bellows 1991).

Figure 2. Tomato fruit contaminated with honeydew and sooty mold; Whitefly nymphs

Trialeurodes vaporariorum is haplo-diploid and reproduces by arrhenotoky. Unmated females produce only males, and mated females produce both males and females (Byrne and Bellows 1991).

2.4 Global distribution of the target pest

Trialeurodes vaporariorum has cosmopolitan distribution and occurs on every continent except Antarctica (http://www.cabi.org/isc/datasheet/54660). In northern regions, it is restricted to protected environments such as greenhouses, conservatories and indoor plantscapes. It can be found out of doors in Southwestern British Columbia (Maw *et al*. 2000).

2.5 Economically, ecologically important (e.g., keystone, endangered) species in North America (introduced and native) that are phylogenetically related or occur in the same habitat as the target pest

In greenhouses, *Bemesia argentifolii* Bellows & Perring and *Bemesia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) can occur as damaging pests. In Canada, outside of greenhouses, there are no whitefly species of economic concern. The ash whitefly, *Siphoninus phillyreae* (Haliday) (Hemiptera: Aleyrodidae) is a polyphagous species that is invasive in the United States and spreading northwards. Populations are present in Oregon State. It may eventually be present in Canada. A great many warm-temperate and subtropical species of Aleyrodidae are pests of economic and environmental concern in the United States.

Canadian whitefly species are all in the Aleyrodinae which is the largest subfamily (Table 1). As of March, 2016, none of these species is on the Canadian endangered species list (http://www.registrelep-sararegistry.gc.ca/species/schedules_e.cfm?id=1). No Aleyrodidae species are included on the endangered species list in the United States nor are any currently under consideration (http://ecos.fws.gov/tess_public/pub/SpeciesReport.do?groups=I&listingType=L&mapstatus=1).

Table 1. Whitefly species present in Canada ('[]' denotes species found only in greenhouses, conservatories or other confined situations; G denotes that the species is a pest in greenhouses) based on Maw *et al*. (2000).

	Distribution	**Pest Status**
Aleuroplatus berbericolus Quantance & Baker	BC	
Aleuroplatus epigaeae Russel	NB, PE, NS	
Aleuroplatus plumosus (Quaintance)	NF	
Aleyrodes asumaris Shimer	ON, QC?	
Aleyrodes spiraeoides Quaintance	BC	
Bemisia argentifolii Bellows & Perring	[ON], [NS]	G
Bemisia tabaci (Gennadius)	[ON], [NS]	G
Dialeurodes chitendeni Laing	BC	
Trialeurodes merlini (Bemis)	BC	
Trialeurodes packardi (Morrill)	ON, QC	
Trialeurodes vaporariorum (Westwood)	BC, [AB], [SK], [MB], [ON], [QC], [NB], [NS]	G

2.6 Regulatory or pest status of the target pest in state, provincial or federal law

Trialeurodes vaporariorum has no special regulatory status in Canada, and its importation and presence on imported plants is governed by the Plant Protection Act. As far as we are aware, this species does not have official pest status requiring any special recognition or regulation in Canada or its provinces.

2.7 Knowledge of status of other biological control agents (indigenous and introduced) that attack the target pest

A very large number of parasitoids, pathogens and predators are known to attack *T. vaporariorum*. For example, the CABI Invasive Species Compendium (http://www.cabi.org/isc/datasheet/54660) lists 56 different species. Not all of these are present in Canada, and none of the species that are present invade Canadian greenhouses in sufficient numbers to have any impact on populations of *T. vaporariorum*. The predator *Dicyphus hesperus* Knight (Hemiptera: Miridae) has been developed and tested as a predator of whitefly on tomato (McGregor *et al*. 1999) and is commercially reared. However, it is not able to consistently maintain numbers below economic injury levels. A coccinellid predator *Delphastis catalinae* (Horn) (Coleoptera: Coccinellidae) is available, but is not adapted to searching on tomato and becomes trapped on sticky hairs on stems. The pathogen *Beauvaria bassiana* (Balsamo) Vuillemin (Hyphomycetes) is registered for use on greenhouse crops, but, although helpful for reducing numbers in the short-term, applications do not maintain populations of *T. vaporariorum* below economic thresholds (Avilla *et al*. 2004).

2.8 Life stage(s) of target pest that are vulnerable to the biological control agent
Encarsia formosa can oviposit in all four nymphal stages and the early pupa of *T. vaporariorum*, although the 3rd and 4th instar are preferred (Stenseth 1987).

3. Biological Control Agent Information

3.1 Taxonomy: scientific name (order, family, genus, species, scientific authority), synonymy, common names and name of the taxonomic specialist confirming the identification of the biological control agent
Class Insecta
Order Hymenoptera
Family Aphelinidae
Genus *Encarsia*
Species *formosa* Gahan 1924
Common Names – None

Synonyms *Trichaporus formosus* (Gahan, 1924)

3.2 Methods used to identify the biological control agent (e.g., morphological, molecular)
With experience, suitable keys and reference specimens, diagnosis of this species using traditional morphological methods is relatively simple. Schmidt *et al*. (2001) give good diagnostic characters for Australian fauna, and these characters work very well for Canadian fauna as well.

Diagnosis – **Female**. Head and mesosoma and base of metasoma brown, contrasting with pale remainder of body. Forewing without bare area. Tarsus of middle leg 4-segemented. **Male**. Body predominately brown, legs lighter. Lower half of head, vertex partly and ocellar area brown.

Molecular primers have been developed (bar codes for CO1) and these are useful for routine confirmation of identity. Dr. John Huber (AAFC, Canadian National Collection of Arthropods) is a specialist in Aphelinidae and has confirmed the identity of the individuals comprising our research colony. Since parasitism by *E. formosa* turns the host pupa black, diagnosis in the immature stages is also possible based on morphological approaches.

3.3 Location of reference specimens (national collection)
Reference specimens have been deposited in the Canadian National Collection (confirmation letter from Dr. Owen Lonsdale, Collections Manager, attached, Appendix 1).

3.4 Natural geographic range, other areas where introduced, and expected attainable range in North America (also habitat preference and climatic requirements of the biological control agent)

There are no reports of diapause or other overwintering adaptations in *E. formosa*, and it is unlikely that this parasitoid will overwinter in any but the most temperate of climates in Canada (southern Vancouver Island, and Point Pelee, Ontario, for example). The species is virtually cosmopolitan (Hoddle *et al*. 1998), and can be expected to be present as a whitefly parasitoid in greenhouses, and in warmer climates around the world.

3.5 Source of the biological control agent (laboratory/rearing facility/containment facility, original collection locality, name of collector, and name of identifier)

The agent is currently in rearing in a containment facility operated by D. Gillespie and P. Clarke, AAFC, ARDC, and this will be the source of the biological control agent for first release. This material was originally sourced from a laboratory colony maintained at the Wageningen Agricultural University by Dr. A. Prof., which was initiated from specimens collected in a greenhouse tomato crop. The identity of this material was established from specimens provided to Dr. John Huber when the initial importation was made.

3.6 Host/biological control agent interactions (e.g., predator, parasitoid, pathogen, parasite, competitor, and antagonist)

Encarsia formosa is a solitary, primary endo-parasitoid of *T. vaporariorum*. It also kills and host feeds on some individuals to obtain nutrients required for egg production.

3.7 Biology and reproductive potential (including dispersal capability and damage inflicted on target pest)

Figure 3. *Encarsia formosa* – clockwise from top left: adult, parasitized whitefly pupae, parasitized pupae on a release card, parasitized (black) and unparasitized (white) whitefly pupae.

3.7.1 Reproductive biology and development
The biology of *Encarsia formosa* (Figure 3) was reviewed by Hoddle *et al*. (1998) (attached, Appendix 2). Females deposit single eggs in all immature stages except the egg, first instar and mature pupa. There is a preference for 3rd and 4th instar and pre-pupal stages of *T. vaporariorum* (Nechols and Tauber 1977). Eggs hatch, but larvae do not moult to the second instar until the host has reached the fourth instar, which results in emergence of adult parasitoids being relatively well synchronized with the presence of susceptible hosts in the next generation. The host puparium becomes thickened and black (melanised) when the parasitoid larva eventually kills the host. At 22°C, development from egg to adult takes from 16 to 23 days, depending on the host stage attacked, and the host plant (Hoddle *et al*. 1998) and females live for 8 to 12 days.

At typical greenhouse temperatures, females deposit from 8 to 12 eggs per day over an 8 to 20 day lifespan, depending on temperature (Stensenth 1985, Hoddle *et al*. 1998). The population in our lab is thelytokous (parthenogenic, females only), and this is maintained by the presence of a Wolbachia endosymbiont. Haplolid males are rarely produced as hyperparasitoids of female parasitoid larvae.

In Europe, the wasp is typically released at rates of 0.1 to 2 females per m^2, every 7 to 14 days, depending on the abundance of *T. vaporariorum.* Releases are made by placing parasitized whitefly nymphs among the crop. In most applications, nymphs are previously removed from leaves and glued to small cards. The abundance of *T. vaporariorum* may be monitored to track populations, either by direct counting on tomato leaves or by yellow sticky traps.

There is no record of overwintering adaptations such as freezing tolerance and/or diapause in *Encarsia formosa.*

3.7.2. Flight and dispersal

Host location and dispersal is mediated by a combination of visual stimulus (yellow spectrum), and semiochemicals from both the plant and the host (Birkett *et al*. 2003; Guerrieri *et al*. 1997). Exposure to cold temperatures (7°C) reduces flight capacity in adults (Luczynski *et al*. 2007). Little is known about long-distance dispersal in this species. As the adult is very small, dispersal on wind currents may be a factor.). Flight occurs at temperatures above 13° and adults can disperse up to 5 metres in 90 minutes C (van der Laan *et al*. 1982).

3.7.3. Abiotic tolerances

The effects of abiotic environment (Temperature, Relative humidity) were reviewed by Hoddle *et al*. (1998). The development threshold is in the range of 10 to 13°C, and estimates of degree-day requirements range from 189 to 207 DD. the upper lethal temperature is 38.8 °C. Wasps tolerate temperatures exceeding 35°C for a few hours in the day, and are effective even when greenhouse temperatures are less than 10°C at night. In general, *E. formosa* successfully controls *T. vaporariorum* across a wide range of greenhouse temperatures.

3.8 Known host range based on published scientific literature, host data from museum specimens, and unpublished records

The host range of *Encarsia formosa* was determined from records in Noyes, J. Universal Chalcidoidea database (http://www.nhm.ac.uk/our-science/data/chalcidoids/database/). Whitefly names have been updated to conform with Evans (2007, http://keys.lucidcentral.org/keys/v3/whitefly/PDF_PwP%20ETC/world-whitefly-catalog-Evans.pdf). All recorded hosts are in the subfamily Aleyrodinae. Host records in Evans provide no records for parasitism of hosts in the Aleurodicinae by *E. formosa*.

Table 2. Whitefly species reported as hosts of *Encarsia formosa* (from Evans 2007).

Aleuroglandulus subtilis Bondar
Aleurothrixus floccosus (Maskell)
Aleurotrachelus trachoides (Back)
Aleyrodes lonicerae Alker
Aleyrodes proletella (Linnaeus)
Aleyrodes singularis Danzig
Aleyrodes spiraeoides Quaintance*
Aleyrodes sp.
Bemisia argentifolii Bellows and Perring
Bemisia sp.
Bemisia tabaci Quaintence & Baker*
Massilieurodes chittendeni (Laing)*
Dialeurodes citri (Ashmead)*
Lipaleyrodes atriplex (Froggatt)
Lipaleyrodes euphorbiae David & Subramaniam
Tetraleurodes mori (Quaintance)
Tetraleurodes sp.
Trialeurodes abutiloneus (Haldeman)
Trialeurodes ricini (Misra)
Trialeurodes vaporariorum (Westwood)*
Trialeurodes variablis (Quaintance)
Trialeurodes sp.
Aleyrodidae unspecified

*species with an asterisk occur in Canada.

3.9 History of past use of the biological control agent.

Encarsia formosa has been widely and successfully used for biological control of *Trialeurodes vaporariorum* in greenhouses and protected culture throughout the world (Hoddle *et al*. 1998, van Lenteren and Martin 1999; Hoddle 2004; Avilla *et al*. 2004).

3.10 Pathogens, parasites, parasitoids, and hyperparasitoids (order, family, genus, species, scientific authority) of the agent and how they will be eliminated from the imported culture of the agent.

Various species of *Encarsia* and *Eretmocerus* (Aphelinidae) produce males as heteronomous hyperparasitoids of other *Encarsia* species including *E. formosa*. These are not anticipated to be a problem as stated above. Females of contaminant species are easily recognized and can be eliminated by observation, and contaminant males emerging from *E. formosa* present no risk. An unidentified male *Syrphophagus* sp. (Hymenoptera: Encyrtidae) has been reported emerging from *E. formosa* as a hyperparasitoid (Ajita 2000). *Syrphophagus* spp. are hyperparasitoids of aphid parasitoids (Braconidae and Aphelinidae) and may occur in greenhouses (Acheampong *et al*. 2013). Contamination by male *Syrphophagus* is not a risk because aphids and aphid parasitoids will not be present in the culture system.

There are no specific entomopathogens or pathogens of risk to crops, livestock or humans (fungi, bacteria, viruses and similar organisms) that are associated with *E. formosa*. The bacterial endosymbiont *Wolbachia* sp. is an integral part of the reproductive biology and ecology of *E. formosa* in nature and will not be eliminated from the imported culture.

3.11 Procedures stating how the biological control agent will be handled in containment (e.g., scaling up for release of a pure culture of the agent).

Numbers of *E. formosa* will be increased by standard rearing procedures for this species.

- Tobacco plants (*Nicotiana tabaci* L.) will be produced to a suitable size in a screened and sealed greenhouse. Entrance to this greenhouse will be limited, and restricted to personnel associated with the project. Clean lab coats will be required in the greenhouse and will be available in an entry foyer. All entries and exits from the greenhouse will be logged.
- Four sealed growth rooms will be maintained with suitable environmental conditions.
- In the first room, tobacco plants will be exposed to whitefly adults for 24 hours. Plants will be fogged with pyrethrum, which paralyzes the adults and by shaking they can be removed from the plants.
- Plants will be moved into the second room and held for 12 days, when the whitefly scales will be at a suitable stage for parasitism.
- Plants and nymphs will be exposed to *E. formosa* adults in the third room for 48 hours. Parasitoid adults will be removed by shaking and blowing on the plants, and the plants transferred to the fourth room.
- Fourteen days after exposure to parasitoids, some of the parasitized scales will be either allowed to emerge in room 3, to maintain parasitoid adult numbers. Material for release will be removed from the leaves by a washing and sorting process (protected information, A Biocontrol Company), and scales will be glued onto cards for distribution into release greenhouses.

3.12 Closely related genera, sibling species, cryptic species and ecologically similar species of the biological control agent in North America, when they occur.

Schauff *et al*. (1996) list and describe 27 species of North American *Encarsia* and these are all parasitoids of Coccid and Aleyrodid scales. They also note that there are likely many undescribed species in this genus in North America. There is no current information on the presence of sibling or cryptic species in the genus *Encarsia*, and the genus is badly in need of review and revision. The genus *Eretmocerus* (Aphelinidae) also parasitizes whitefly scales. The number of described species in North America is likely in the range of 24 species (estimated from Noyes). Parasitoid wasps in the genus *Amitus* (Hymenoptera: Platygastriidae) also parasitize whitefly scales.

4. Host-Specificity Testing

4.1 Selection of non-target test arthropods:

The multiple criteria method proposed by Kuhlmann *et al*. (2006) was used to select non-target species for host range testing (Table 3).

Table 3. Species included in host range testing of *Encarsia formosa*.

Species	Criteria	Host plant	Source
Aleyrodidae:			
Trialeurodes merlini	Same genus, native	*Arbutus menzii*	Field collected nymphs on leaves
Bemesia tabaci	Same family, sympatric; occurs in greenhouses on tomato.	*Nicotiana glauca*	Laboratory colony
Aleyrodes spiraeoides	Same family, Sympatric; can occur in greenhouses	*Nicotiana glauca*,	Laboratory colony
Aleuroplatus berbericolis	Same family, native	*Berberis aquifolium*	Field collected nymphs on leaves
Psyllidae:			
Bactericera cockerelli potato psyllid	Same order, Sympatric; occurs in greenhouses on tomato	Tomato	Laboratory colony
Coccidae:			
Coccus hesperidum brown soft scale	Same order, sympatric; can occur in greenhouses, but not known from tomato.	Pepper	Laboratory colony

4.2 Laboratory tests (replicated no-choice and choice feeding tests, oviposition tests, development tests), including information on offspring survival, sex ratio, and fecundity. Positive controls must be included.

We present results from laboratory tests performed in containment facilities by D. Gillespie and P. Clarke. Our objectives were to evaluate the acceptance of alternative hosts by *E. formosa*, using the species in the test list presented in section 4.1. We used a no-choice experimental design to evaluate feeding, oviposition and development of *E. formosa* on these species. We did not conduct any choice tests evaluating these traits because *E formosa* is highly unlikely to encounter hosts in a context where choice is relevant. We did conduct choice-tests in a Y-tube apparatus to evaluate orientation of *E. formosa* adults to host-host plant semiochemicals, because host location by *E. formosa* is highly dependent on these signals. If *E. formosa* adults cannot follow non-target semiochemical trails, they are unlikely to encounter or establish populations on these hosts in nature.

4.2.1 Insect sources

Female adult *E. formosa* were obtained from our rearing colony by holding black (parasitized) *T. vaporariourum* scales singly, in small containers until emergence. When they emerged, individual females were provided with 20 2nd instar scales on a piece of tomato leaflet, and a honey solution on a cotton wick. These resources ensured females had suitable resources to develop eggs. Females were used in experiments when 3 days old.

Third-instar scales of *T. vaporariorum* and *B. tabaci* were obtained by rearing. Female whitefly were placed in a small cage with tomato plants (30 cm tall) for 24 h, and then removed and cleaned. Plants were held until scales were at the third instar, when they were used for experiments. Third-instar scales of *A. spiraeoides* were obtained by rearing on pepper plants using similar techniques.

Second instar scales of *B. cockerelli* and *C. hesperidium* were produced by moving crawlers onto leaves of tomato, *Solanum esculentum* Dunal; and allowing them to establish.

Neither *T. merlini* nor *A. berbericolis* could be reared in the laboratory or established on tomato leaves. Mr. Nairb obtained leaves bearing aggregations of scales from field sites. These were held in our laboratory at 4°C for a maximum of 14 days before being used in experiments. In both cases, these came from single populations of each species, which introduced an unavoidable level of pseudoreplication.

In all cases, 2.5 cm disks were cut from leaves and the numbers of insects reduced to 10, 3rd instar scales in the case of whiteflies, and 10 2nd instar scales in the case of the other species.

4.2.2. Experimental protocols

The whitefly scales were removed from *E. formosa* holding containers after 2 days, and females were held without access to hosts, but with water and carbohydrates for 24 hours. This ensured a maximum tendency to oviposit. Females were then placed in test arenas for 24 h. These were Petri dishes containing 1, 2.5 cm leaf disk containing 10 test hosts of one species, on a moist cotton disk. Females were transferred after 24 h to a leaf disk containing 10 *T. vaporariorum* scales for a further 24 hours. This provided confirmation that the females were capable of laying eggs, and any female that did not lay eggs in the test dish and also failed to lay eggs subsequently, was not included in analysis. Dishes were held at 22°C until wasp adults emerged or until it was evident that this was not going to occur.

Positive controls consisted of wasps tested on target hosts followed by target hosts. Because we could not obtain all of the non-target hosts at the same time, we conducted tests with each, independently, and have used a Bonferroni correction on all tests in order to correct for the number of multiple comparisons.

4.2.3 Data collection

1. Females were observed for the first 60 minutes to observe attack on hosts. These data were analyzed as a contingency table (attack observed, not observed) for each test species.

2. Host feeding was evaluated by examining hosts after 24 hours for signs of feeding punctures and injury. These scales are characteristically collapsed and have visible puncture marks. These data were analyzed as a binomially distributed variable (number host fed, number not host fed) using a generalized linear model, and are presented as proportional data.

3. Development was evaluated by successful emergence of wasps from host scales. In the case of *T. merlini* and *A. berbericolis* we held in groups of 10 scales that were not attacked by *E. formosa* to determine the incidence of other parasitoid species. The proportion parasitized was corrected in each cohort of scales, because *E. formosa* is known to discriminate previously parasitized hosts. These data were analyzed as a binomially distributed variable (number parasitized, number not parasitized) using a generalized linear model, and are presented as proportional data.

4. We also determined development time (days to develop to adults) and size of females (length of body from vertex to tip). These were averaged for each dish (female) and were analyzed as using R. Days to develop were analyzed as a Cox Regression, and the size of females by ANOVA.

4.2.4 Results

Disclaimer: Providing fabricated data and tests would risk misinterpretation and eventual entry of the results of this sham petition into the scientific record. Therefore we provide summary tables without data or tests, provide a best-guess scenario of the results of testing, and then use these results to provide an example interpretation.

Table 4. Results of host range testing with *Encarsia formosa.* Mean ± S.E. numbers of *E. formosa* emerging from cohorts of ten non-target hosts exposed to the parasitoid. N = 30 cohorts of 10 scales each. The positive controls in all cases were cohorts of ten *T. vaporariorum.*

Species	**Number emerging from non-target**	**Number emerging from *T. vaporariorum***	**Number of adults non-targets emerging**	**Number of *T. vaporariorum* emerging**
T. merlini				
B.tabaci				
A.spiraeoides				
A. berbericolis				
B. cockerelli				
C. hesperidum				

Table 5. Results of y-tube orientation tests with *E. formosa* responding to either non-target or target host. Data are numbers of individuals responding to each potential stimulus.

Species	**Number responding to non-target**	**Number responding to *T. vaporariorum***	**Non-responders**
T. merlini			
B.tabaci			
A.spiraeoides			
A.berbericolis			
B. cockerelli			
C. hesperidum			

The assumed results on which further interpretation and discussion are based are

1. That E. formosa *attacks and emerges from* T. merlini, A. spiraeoides and B. tabaci *is not significantly different from that on* T. vaporariorum

2. That E. formosa *attacks and emerges from* Aleuroplatus berbericolis *at a lower rate that on* T. vaporariorum

3. *That* E. formosa *neither attacks nor emerges from either* B. cockerelli *or* C. hesperidium

4. *That* E. formosa *adults orient to* B.tabaci on tomato and A. spiraeoides on tobacco at similar rates to *T. vaporariorum*, but do not respond to the non-target host/host plant combinations of the other test species.

4.3 Information on the biological control agent from the area of origin based on field surveys or experimental field manipulation as feasible

Very little seems to be known about the impact of *E. formosa* in the area of origin. This species is likely Neotropical in origin. It is known to parasitize a wide range of hosts in the subfamily Aleyrodinae (Shauff *et al*. 1996; Noyes 2016). However, there seems to be no information pertaining to the effect of this species on host populations in natural settings or in crops outside of greenhouses. A study in Europe, where it is exotic, noted little evidence for non-target impact (Loomans and van Lenteren 1999).

5. Environmental and Economic Impacts of the Proposed Release

5.1 Known impact of the biological control agent on humans and other vertebrates.

We found no evidence in the scientific literature, or via an internet search that *Encarsia formosa* has any effects on humans or other invertebrates.

5.2 Expected benefits of releasing this biological control agent

Use of this agent as an augmentative and inundative control has provided biological control of *T. vaporariorum* on a wide range of crops, including tomato (Hoddle *et al*. 1999). This has provided a viable alternative to the use of insecticides, particularly broad-spectrum insecticides, and has allowed the industry to develop IPM programs based on reduced-risk insecticides, which may have somewhat lower efficacy. The crop losses resulting from resistance of *T. vaporariorum* to insecticides and damaging populations should be alleviated. Re-entry times have been eliminated, which allows scheduling of critical greenhouse operations and reduces the additional losses caused by delays in re-entry to the crop. Experience in Europe has been that the long-term costs of whitefly control with *E. formosa* are competitive with costs of pesticide applications. The elimination of pesticide use will make the use of *Bombus* spp. pollinators possible, reducing labour costs for that service. The elimination of pesticides; together with a more stable supply of tomatoes should allow better access to export markets in the USA.

5.3 Direct impact of the biological control agent on target pest and non-target species

Encarsia formosa has controlled populations of *Trialeurodes vaporariorum* when used in an augmentative or inundative release strategy in greenhouse tomato crops at rates from 0.5 to 2.0 per m^2 (Hoddle *et al*. 1999, van Lenteren and Martin 1999). Eggs are laid in immature nymphs. Mature *T. vaporariorum* nymphs are killed by developing parasitoid larvae, which pupate within the host exuviae. A proportion of available whitefly hosts are killed by host feeding; the parasitoid adult stings the host without laying an egg, and consumes the haemolymph that exudes from the wound. This behaviour provides female parasitoids with protein for development of eggs.

Encarsia formosa will kill nymphs of other whitefly species in the subfamily Aleyrodinae in exactly the same way as *T. vaporariorum*: by host feeding and by parasitism. There is no evidence that it will attack or feed on whiteflies in the subfamily Aleurodicinae, but neither is there evidence that it will not. The hypothesis that *E. formosa* will attack hosts in the Aleurodicinae could not be tested because species in this subfamily were not available and are not native to Canada. Despite long term and widespread release in greenhouses in both northern and southern Europe, there is no evidence that *E. formosa* has any capacity to reduce numbers of non-target whitefly species outside of greenhouses (Loomans and van Lenteren 1999). This is not unexpected, since *E. formosa* lacks overwintering strategies, and has a relatively high minimum development threshold. Either populations will not survive winter, or will be unable to develop sufficiently rapidly from a base temperature of 10 to 13°C that it can impact host

numbers in a single growing season. Finally, as shown in our tests, *E. formosa* adults were unable to locate non-target whitefly hosts associated with their normal host plants

Because *E. formosa* is thelytokous there is no risk that releases of this species will directly impact native parasitoids of whiteflies. Unlike many species, *E. formosa* does not produce males as hyperparasitoids of other species. Moreover, most native species have development thresholds that are considerably lower than *E. formosa* (Vet *et al*. 1980), so impacts through competition also seem unlikely.

5.4 Indirect impact (e.g., potential effects on organisms that depend on the target pest and non-target species, including potential competition with resident biological control agents and other natural enemies)
Trialeurodes vaporariorum occurs in an artificial system (i.e. greenhouse) whereby community dynamics are simplified (i.e. pest & host plant). Therefore indirect impacts are possible only with other introduced biological control agents. These impacts would be complementary as a biologically based pest management strategy is designed to utilize agents in different feeding niches for maximum impact.

5.5 Possible direct or indirect impact on threatened and endangered species in North America
At present (March 2016), there are no species of whiteflies or whitefly parasitoids on the threatened and endangered lists in Canada, or the United States. As noted in section 5.4, one species of whitefly *Trialeurodes merlini* was susceptible in our non-target tests, although *E. formosa* did not orient to the host/host plant odours and therefore may not be able to locate this species in nature. The reason we mention this is that the host plant of this whitefly, *Arbutus menziesii*, is a common tree in the maritime meadows habitat that is often adjacent to the endangered Garry Oak meadows habitat on Vancouver Island, British Columbia. It is virtually certain that *E. formosa* will not have any negative impacts on species in this habitat. The only species that it could attack is *Trialeurodes merlini*, and as a herbivore, reduction of populations should benefit arbutus health. Although some insect species use honeydew deposits from Hemiptera, including Aleyrodidae, as a carbohydrate source, we are not aware of any species that exclusively uses Aleyrodidae honeydew; indeed species with this habit take honeydew from any species of Hemiptera, and from various other sources including nectar from flowers. Thus, even drastic reduction of numbers of *T. merlini* would not impact the foodweb. Nonetheless, our post-release monitoring plan will consider this possibility.

5.6 Impact of the biological control agent on physical environment (e.g. water, soil and air)
Encarsia formosa has no negative impacts on the physical environment. Positive impacts are possible, and would flow from the reduction of insecticide use in greenhouses, which would reduce insecticide contamination of waste irrigation water, which could have positive benefits.

5.7 Proposed contingency plan to mitigate undesired environmental impacts

Because *E. formosa* will not establish permanent populations in Canada, environmental impacts would arise from escape of adults from greenhouses in the growing season. If such impacts are found and they are deemed serious enough to consider mitigation, then two possible courses are available. In the event of widespread impacts, the industry could simply stop releasing the parasitoid, thus preventing further impacts. If impacts are regional, for example, associated with species in the Garry Oak Meadows ecosystem, then releases could be restricted in Canada to places where this ecosystem does not exist (see also Section 6).

6. Post-Release Monitoring

The key risks in this proposed release are that *E. formosa* will escape from greenhouses following release, and secondly, that escaped *E. formosa* adults will attack *T. merlini* on Arbutus, resulting in the decline of the species on that host plant. For this reason, initial releases will be done at the Agassiz Research and Development Centre, which is well separated from the ecosystem of concern. Initial tests will place *T. vaprorariorum* populations on greenhouse tomato plants at 10m, 50 m, 100 m, 500 m and 1000 m distance from our greenhouse. These will be maintained and sampled monthly throughout the season to determine if *E. formosa* is present (easily detected by the blackened appearance of parasitized scales). These will be held in cages over the winter to demonstrate that *E. formosa* does not survive the winter. If *E. formosa* occur on *T. vaporariorum* on tomato plants outside of greenhouses then a subsequent trial will be conducted with *T. merlini* populations maintained on *Arbutus menziesii* trees in pots, and populations will be tracked over time to demonstrate lack of parasitism and lack of impact on population dynamics of the non-target species. Note that funding has been secured for this study through AAFC.

7. Pre-Release Compliance

7.1 Reference specimens

Reference specimens have been deposited in the Canadian National Collection of Insects, Arachnids and Nematodes. Approximately 250 specimens were supplied, in 95% alcohol, and receipt of these, in good condition suitable for DNA extraction, has been acknowledged by Dr. John Huber (Letter attached).

7.2 Information on the planned location and timing of the first release(s)

The first releases will be made at the Agassiz Research and Development Centre, Agassiz, British Columbia, Canada, (49° 14.587'N, 121° 45.724'W) starting on 15 February 2017, and continuing weekly through the growing season.

References

Acheampong S, Gilllespie D and Quiring D (2012) Survey of parasitoids and hyperparasitoids (Hymenoptera) of the green peach aphid *Myzus persicae* and the foxglove aphid, *Aulacorthum solani (*Hemiptera: Aphididae) in British Columbia. *Journal of the Entomological Society of British Columbia* **109**: 12 – 22.

Ajita H (2000) Geographical distribution and species composition of parasitoids (Hymenoptera: Chalcidoidea) of *Trialeurodes vaporariorum* and *Bemisia tabaci*-complex (Homoptera: Aleyrodidae) in Japan. *Applied Entomology and Zoology* **35**: 155-162.

Avilla J, Albajes R, Alomar O., Castañé C and Gabarra R (2004) Biological control of whiteflies on vegetable crops. Pp. 171–184 *In* Heinz K, Van Driesche R, and Parrella M (eds) *Biocontrol in protected culture. Ball Publishing, Batavia, IL*.

Birkett MA, Chamberlaln K, Guerrieri E, Pickett JA, Wadhams LJ, Yasuda T (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *Journal of Chemical Ecology* **29**: 1589-1600. doi:10.1023/A:1024218729423.

Byrne DN, Bellows TS (1991) Whitefly biology. *Annual Review of Entomology* **36**: 431–457.

Evans GA (2007) The whiteflies (Hemiptera: Aleyrodidae) of the world and their host plants and natural enemies. (http://keys.lucidcentral.org/keys/v3/whitefly/PDF_PwP%20ETC/world-whitefly-catalog-Evans.pdf).

Guerrieri E (1997) Flight behaviour of *Encarsia formosa* in response to plant and host stimuli. *Entomologia Experimentalis Et Applicata* **82**: 129-133. doi:10.1023/A:1002905428258.

Hoddle MS (2004) Analysis of fauna in the receiving area for the purpose of identifying native species that exotic natural enemies may potentially attack. Pp. 24–39 *In* Van Driesche RG, Reardon R (eds) *Assessing Host Ranges of Parasitoids and Predators Used for Classical Biological Control: a guide to best practice*. United States Department of Agriculture, Forest Health Technology Enterprise Team, Morgantown, West Virginia, United States, FHTET-2004-03.

Hoddle MS, Van Driesche RG, Sanderson JP (1998) Biology and Use of the Whitefly Parasitoid *Encarsia formosa*. *Annual Review of Entomology* 43: 645-669.

Kuhlmann U, Schaffner U, Mason PG (2006) Selection of non-target species for host specificity tests. Chapter 2, Pp. 15-37. In: Bigler, F., D. Babendreier, and U. Kuhlmann (eds) *Biological Control of Arthropods Using Invertebrates: Methods for Environmental Risk Assessment*. CABI Publishers, Wallingford, UK.

van der Laan EM, Burggraaf-van Nierop YD, van Lenteren JC (1982) Oviposition frequency, fecundity, and life-span of *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) and migration capacity of *E. formosa* at low greenhouse temperatures. *Med. Fac. Landbouww. Rijksuniv. Gent* 47:511–521.

van Lenteren JC, Martin NA (1999) Biological control of whitefly. Pp. 202-216 *In* Albajes R, Gullino ML, van Lenteren JC, Elad Y (eds) *Integrated Pest and Disease Management in Greenhouse Crops*. Kluwer Publishers, Dordrecht, The Netherlands.

Loomans AJM, van Lenteren JC (1999) Evaluating environmental effects of *Encarsia* species (Hymenoptera: Aphelinidae) introduced for whitefly control into Europe. *Bulletin OILB SROP (France)* (1999) **22**: 153-156.

Luczynski A, Nyrop JP, Shi A (2007) Influence of cold storage on pupal development and mortality during storage and on post-storage performance of *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae). *Biological Control* **40**: 107-117. doi:10.1016/j.biocontrol.2006.08.006.

Maw HEL, Foottit RG, Hamilton KGA, Scudder GGE (2000) Checklist of the Hemiptera of Canada and Alaska. National Research Council Press, Ottawa, ON, 220p.

McGregor RR, Gillespie DR, Quiring DMJ, Foisy M R J (1999) Potential use of *Dicyphus Hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes. *Biological Control* **16**: 104–110.

Nechols JR, Tauber MJ (1977) Age specific interaction between the greenhouse whitefly and *Encarsia formosa*: influence of the host on the parasite's oviposition and development. *Environmental Entomology* **6**: 143–149.

Orfanidou CG, Dimitriou C, Papayiannis LC, Maliogka VI, Katis NI (2014) Epidemiology and genetic diversity of criniviruses associated with tomato yellows disease in Greece. *Virus Research* **186**: 120-129. doi:10.1016/j.virusres.2013.12.013.

Raworth DA, Gillespie DR, Shipp JL (2002) *Trialeurodes vaporariorum* (Westwood), Greenhouse Whitefly, and *Bemisia tabaci* (Gennadius), Sweetpotato Whitefly (Hemiptera: Aleyrodidae). Pp. 264-269 *In* Mason PG, Huber JT (eds) *Biological Control Programmes in Canada 1981-2000*. CABI Publishing, Wallingford, UK, 583p.

Schauff ME, Evans GA, Heraty JM (1996) A pictorial guide to the species of *Encarsia* (Hymenoptera: Aphelinidae) parasitic on whiteflies (Homoptera: Aleyrodidae) in North America. *Proceedings of the Entomological Society of Washington* **98**: 1 – 35.

Schmidt S, Naumann ID, De Barro PJ (2001) *Encarsia* species (Hymenoptera: Aphelinidae) of Australia and the Pacific Islands attacking *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) - a pictorial key and descriptions of four new species. *Bulletin of Entomological Research* **91**: 369 – 387.

Stenseth NC (1987) Evolutionary processes and insect outbreaks. Pp. 553-563 *In* Barbosa P, Schultz JC (eds) *Insect Outbreaks*. Academic Press, New York, NY, United States.

Vazquez LL, Iglesia MDL, Lopez D, Jimenez R, Mateo A, Vera ER (1995). Whiteflies (Homoptera: Aleyrodidae) found on the principal agricultural crops of Cuba. [Spanish]. *Manejo Integrado de Plagas* **36**: 18 – 21.

Vet LEM, van Lenteren JC, Woets J (1980) The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). IX. A review of the biological control of the greenhouse whitefly with suggestions for future research. *Zeitschrift fur Angewandte Entomologie* [*Journal of Applied Entomology*] **90**: 26 – 51.