Chapter 3: Understanding Federal Regulations as Guidelines for Classical Biological Control Programs

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INTRODUCTION

This chapter reviews the legislation and rules that provide the foundation for federal regulation of the introduction of natural enemies of insects as biological control agents. It also outlines the steps for complying with regulatory requirements, using biological control of *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA), as an example. The program to establish biological control agents for HWA in eastern North America dates from 1993 to present and involves importation from other continents, from other countries in the North American continent, as well as the interstate movement of biological control agents. Thus, biological control of HWA provides examples of regulation under old and new federal regulations and rules for foreign importation and interstate movement. With these regulations in mind, the several steps involved in putting a biological control program into practice will be reviewed—finding and importing agents, rearing and studying the agents in a containment facility (aka quarantine), acquiring data to show that the agent will be effective and safe, and release into a new environment. Monitoring the biological control agent's establishment and efficacy is also part of a biological control program, but this is not discussed here.

[1]Disclaimer: Views and statements are those of the author and should not be interpreted as official policy of any federal agency. Anyone considering releases of biological control organisms must follow official regulations and should not rely only on the information in this chapter.

In its broadest meaning, biological control is the action of an organism that maintains the population of another organism at a lower average density than would occur if it was absent. From an applied viewpoint, biological control is the use of natural enemies (predators, parasites, pathogens) to reduce a pest population and thus the damage it causes. Classical biological control is the introduction and permanent establishment of natural enemies in order to reduce populations of a non-indigenous pest. It is the movement (importation from a foreign country, release from containment, and interstate transport) of biological control organisms that is federally regulated.

The federal government recognizes that biological control is often a desirable, low-risk means to reduce pests of crops and other plants; however, the unregulated movement of certain biological control organisms may present an unacceptable risk. Thus, the government has a dual role to facilitate biological control and also to assess the risks and benefits of releasing specific organisms for biological control of pests. Regulations regarding the movement of entomophagous (insect-eating) biological control organisms have not been promulgated, although federal legislation was passed in 2000 authorizing regulation of all biological control organisms. Because regulatory policies now in place are not transparent and widely available, many practitioners may not be adequately informed. This review of the underlying legal framework and the steps in importing and releasing biological control organisms will hopefully fill some of the information gaps practitioners may have.

DEFINITIONS

The terminology associated with biological control varies with disciplines and can have uncertain and conflicting meanings. Even familiar terms when used by government agencies can have meanings that are narrower or broader than when used scientifically. Therefore, the following definitions are provided for terms used in this chapter.

Act means a public law passed by the United States Congress. These acts are listed sequentially by Congressional session (e.g., Public Law 106-224 is the Agricultural Risk Protection Act of 2000). These are organized by topic in the United States Code (USC).

Biological Control has various meanings, but the definition of DeBach (1964) is appropriate for this chapter—"the action of parasites, predators, or pathogens (disease-causing organisms) in maintaining another organism's population density at a lower average than would occur in its absence." DeBach recognized three approaches to achieve biological control: *classical biological control* is the purposeful introduction and permanent establishment of natural enemies to suppress populations of a pest; *augmentation* is the supplemental or inundative release of natural enemies in areas where they are missing, too scarce to provide adequate control, or arrive too late in the season to be effective; and *conservation of natural enemies* is management to enhance the survival and impact of established natural enemies.

Biological Control Organism, as defined by public law, means "any enemy, antagonist, or competitor used to control a plant pest or noxious weed" (7 USC 7702).

Indirect Damage is when an organism adversely affects another organism that is beneficial to plants, and those adverse effects cause losses in yields of crops or forage plants or a reduction in the viability or vigor of ornamental or native plants (*cf*. definition proposed but not adopted (USDA 2001)).

Movement, **Move**, and **Moving** includes release into the environment as well as the transport or facilitating the transport, by any means, into the country or between states.

Nonindigenous refers to a plant or animal that is not native to a place. Introduced, adventive, alien, exotic, nonnative, and nonindigenous (non-indigenous) species are used rather interchangeably to indicate a species living outside its native distributional range and that arrived there by human activity, either accidental or deliberate.

Regulations are general and permanent rules developed by executive branch agencies to administer and enforce the Acts passed by the U.S. Congress. These rules (also called administrative laws) are published in the Federal Register and codified under 50 titles in the Code of Federal Regulations (CFR), which is updated annually.

LEGAL FRAMEWORK

Legislation

In the United States, there are many acts that may pertain to biological control organisms, but three are especially important. The most significant is the Plant Protection Act (PPA) passed by Congress on June 20, 2000 (U.S. Congress 2000). This Act consolidated all or part of ten existing plant health laws into one law that gives the Secretary of the United States Department of Agriculture (USDA) broad authority to regulate movement of any plant, plant product, biological control organism, noxious weed, and plant pest. This authority has been delegated to the Department's Animal and Plant Health Inspection Service (APHIS) and the regulation of biological control organisms has been assigned to APHIS's Plant Protection and Quarantine (PPQ) program. Most of the provisions of the Federal Plant Pest Act (FPPA) of 1957, which regulated the importation and interstate movement of plant pests, were retained with the new act providing additional authority for regulation of noxious weeds and biological control organisms.

The PPA recognizes the need to prevent the dissemination of plant pests and to facilitate the use of biological control to protect plants from their pests, including noxious weeds. Excerpts from the Act (Table 1) illustrate how regulation of the movement of plant pests (Section 411) is simple and direct—movement of plant pests is prohibited without a permit; whereas Section 412, regulating the movement of biological control organisms, is much more complex. For example, Section 412 (a) states that any biological control organism would be regulated only if it is determined that this is necessary to prevent the introduction or dissemination of a plant pest; and Section 412 (l) indicates that certain biological control organisms may be listed as exempt from restrictions on movement in interstate commerce. The following section on regulations illustrates the difficulty of developing administrative rules to both promote and ensure the safety of biological control organisms.

The second important piece of legislation affecting biological control programs in the United States is the National Environmental Policy Act (NEPA). This law, effective January 1, 1970, applies to all federal activities, including projects receiving federal funds and permits issued by federal agencies. Non-federal applicants are not responsible for compliance; rather, the federal agency that proposes the action, provides the funds, manages the land where the activity will occur, or issues the permit is responsible for compliance. To fulfill NEPA provisions, the agency first determines which of three levels of analysis is required. The simplest level of analysis is for an activity that has been predetermined to be Categorically Excluded (CE). These are specific activities listed by the agency that it has determined have limited environmental effects. Examples of CE activities listed by APHIS include (a) interstate movement of nonindigenous species between containment facilities; (b) importation of nonindigenous species into containment facilities; and (c) releases into a State's environment of pure cultures of organisms that are either native or are established introductions (see 7 CFR Ch. III 372.5). If the activity is characterized

Table 1.—Excerpts from the Plant Protection Act.

Section 411. REGULATION OF MOVEMENT OF PLANT PESTS. (7 USC 7711)

(a) PROHIBITION OF UNAUTHORIZED MOVEMENT OF PLANT PESTS.—No person shall import, enter, export, or move in interstate commerce any plant pest, unless the importation, entry, exportation, or movement is authorized under general or specific permit.

Section 412. REGULATION OF MOVEMENT OF PLANTS, PLANT PRODUCTS, BIOLOGICAL CONTROL ORGANISMS, NOXIOUS WEEDS, ARTICLES, AND MEANS OF CONVEYANCE. (7 USC 7712)

(a) IN GENERAL—The Secretary may prohibit or restrict the importation, entry, exportation, or movement in interstate commerce of any plant, plant product, biological control organism, noxious weed, article, or means of conveyance, if the Secretary determines that the prohibition or restriction is necessary to prevent the introduction into the United States or the dissemination of a plant pest or noxious weed within the United States.

(b) POLICY.—The Secretary shall ensure that processes used in developing regulations under this section governing consideration of import requests are based on sound science and are transparent and accessible.

(c) REGULATIONS.—The Secretary may issue regulations to implement subsection (a), including regulations requiring that any plant, plant product, biological control organism, noxious weed, article, or means of conveyance imported, entered, to be exported, or moved in interstate commerce.

(e) STUDY AND REPORT ON SYSTEMS APPROACH [for plant pathogens]

(e) NOXIOUS WEEDS.—

(g) BIOLOGICAL CONTROL ORGANISMS.—

(1) REGULATIONS.—In the case of biological control organisms, the Secretary may publish, by regulation, a list of organisms whose movement in interstate commerce is not prohibited or restricted. Any listing may take into account distinctions between organisms such as indigenous, nonindigenous, newly introduced, or commercially raised.

(2) PETITION TO ADD OR REMOVE BIOLOGICAL CONTROL ORGANISMS FROM THE REGULATIONS.—Any person may petition the Secretary to add a biological control organism to, or remove a biological control organism from, the regulations issued by the Secretary under this subsection.

(3) DUTIES OF THE SECRETARY.—In the case of a petition submitted under paragraph (2), the Secretary shall act on the petition within a reasonable time and notify the petitioner of the final action the Secretary takes on the petition. The Secretary's determination on the petition shall be based on sound science.

as being limited in scope to specific sites, specific species, or activities that potentially would impact few environmental values or systems, then the agency would prepare an Environmental Assessment (EA). The purpose of the EA is to determine the significance of the environmental effects and to examine alternative means to achieve the objective. The EA includes a brief discussion of: (1) the need for the proposal, (2) alternative courses of action, (3) the environmental impacts of the proposed action and alternatives, and (4) a listing of agencies, institutions, and persons consulted. The applicant, the public, and other agencies may be involved in preparing or commenting on the draft EA. The process concludes with either a Finding of No Significant Impact (FONSI), or the application is denied. The third NEPA category, the Environmental Impact Statement (EIS), is used for changes in policy, such as changes in agency regulations, and for activities that do not qualify for a FONSI.

The third important piece of federal legislation is The Endangered Species Act of 1973. This act requires federal agencies, in consultation with the U.S. Fish and Wildlife Service (F&WS) or the NOAA Fisheries Service, to ensure that any action that a federal agency authorizes, funds, or carries out is unlikely to jeopardize the continued existence of a listed species, or result in the destruction or adverse modification of designated critical habitat of such species. Proposals to release a nonindigenous species into a new environment would be reviewed by the F&WS for potential impacts on endangered species. The federal agency, such as APHIS, conducting an EA usually asks the F&WS for its opinion regarding threats to federally-listed endangered or threatened species. The F&WS is also responsible for monitoring the movement of wildlife, which includes insects (50 CFR 14—Importation, Exportation and Transportation of Wildlife). The F&WS is particularly concerned about species that are listed according to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Import permits from F&WS are required for species that are on the CITES list. Currently there are no entomophagous insects on the list; however, shipments of all biological control organisms should be accompanied by a Declaration for Importation or Exportation of Fish or Wildlife (USFWS Form 3-177).

Regulations

Implementation of the Acts of Congress is done usually by agencies in the Executive Branch by a process of "rule-making." The proposed regulations are published in the Federal Register for public comment and as final regulations. These are then compiled in the Code of Federal Regulations (CFR), which is available on the internet.

Regulations for movement of biological control organisms are provided in 7 CFR Part 330–Federal Plant Pest Regulations; General; Plant Pests; Soil, Stone, and Quarry Products; Garbage. In response to passage of the PPA of 2000, Section 330.102 was revised in April, 2001 to include biological control organisms among the items that "the Secretary of Agriculture may prohibit or restrict the importation, entry, exportation, or movement in interstate commerce, . . . if the Secretary determines that the prohibition or restriction is necessary to prevent the introduction into or the dissemination within the United States of a plant pest or noxious weed." Although the CFR was updated to reflect USDA's current authority to regulate the importation and movement of entomophagous biological control organisms, new rules to exercise this authority have not been incorporated into the Regulations. Extensive revisions were proposed in the Federal Register of October 9, 2011, but public concerns about the proposed rules and reorganization of security following events of September 11, 2001 sidetracked their adoption. Nonetheless, these proposed rules (USDA 2001) provide insight into the complexity of developing detailed rules for the regulations for the many aspects of biological control of arthropods and noxious weeds.

In 2009, APHIS-PPQ asked for public comment to help it determine which alternative it should examine in preparing an Environmental Impact Statement for Movement of Plant Pests, Biological Control Organisms, and Associated Articles (USDA 2009). The alternatives proposed were:

(1) Take no action—leave current rules unchanged

(2) Revise requirements for movement of plant pests to cover biological control organisms consistent with the scope of the PPA (preferred alternative)

(3) Implement a comprehensive risk reduction program (more expansive regulations to address specific risk categories)

The statement "Establishment of clear, coherent, and streamlined regulations at the national level will be important to ensuring objective assessment of the risks and benefits of biological control in the U.S." (Mason et al. 2005) still applies today. Although new regulations of biological control organisms have not yet been issued, APHIS has new policies and procedures for regulation of entomophagous biological control organisms that are largely unpublished or available on web pages; thus, it is important to check with the agency regarding compliance if you wish to import, release, or move any biological control organism.

HISTORICAL REGULATION OF BIOLOGICAL CONTROLS FOR HWA

Regulation of the importation of entomophagous biological control agents from foreign countries to approved containment facilities has changed little in the last 50 years. What has changed is regulation of the first-time release of an entomophagous biological control agents into the environment. The hemlock woolly adelgid (HWA) provides a good example of these changes (Table 2), which fall into three groups:

Table 2.—Imported arthropods released from containment for biological control of the hemlock woolly adelgid and procedures used to assess potential risk of release.

Species, author, year described	Origin	Released from containment	Evaluation process used by APHIS	NEPA compliance[5]
Diapterobates humeralis (Hermann) 1804	Japan[1]	1992	OPRA[3], Limited Review	?
Sasajiscymnus tsugae (Sasaji & McClure) 1997	Japan	1995	OPRA, Limited Review	APHIS, FS, NPS
Scymnus sinuanodulus Yu & Yao 1997	China	1998	First-tier Risks (Not Regulated)	FS, NPS
Laricobius nigrinus Fender 1945	Canada[2]	2000	First-tier Risks (Not Regulated)	FS, NPS
Scymnus ningshanensis Yu & Yao 2000	China	2000	First-tier Risks (Not Regulated)	FS
Scymnus camptodromus Yu & Liu 1997	China	2000	First-tier Risks (Not Regulated)	—[6]
Laricobius osakensis Montgomery & Shiyake 2011	Japan	2010	NAPPO[4], Independent Review, Public Comment	APHIS

[1]Widespread, including North America
[2]Endemic to western North America; permits not issued for subsequent movement from western States to eastern States
[3]OPRA=Organism Permitting and Risk Analysis conducted by the Biological Assessment and Support Team (BATS) of APHIS-PPQ
[4]Standards to release entomological biological control agents adopted by the North American Plant Protection Organization
[5]Compliance with the National Environmental Policy Act by Federal Agencies, Animal & Plant Health Inspection Service, Forest Service or National Park Service
[6]Has not been released into the environment

(1) Following the passage of NEPA in 1970, APHIS prepared an EA for the first-time release of all biological control organisms into the environment. The process for obtaining a release permit was not much different from the process currently used, except that it was less rigorous in the information required and the scope of the review. The permit granted, however, was usually restricted to a single State and of limited duration.

(2) Sometime between 1995 and 1997, Federal lawyers interpreted that the FPPA of 1957 did not apply to the release of entomophagous insects, but only to their importation and holding in containment. When an application to release an entomophagous insect from containment was received, APHIS would decline jurisdiction if it determined that the organism met First-Tier Risk criteria (Table 3). Because a permit was not issued, the need for an EA was not triggered. After APHIS declined jurisdiction, biological control practitioners could move entomophagous organisms to the laboratory for further research or mass rearing; however, if they were federal employees, received federal funds for the project, or the organisms would be released on federal lands, then NEPA applied and an EA was needed prior to a release into the environment. Biological control agents for weeds (e.g., herbivorous insects) still required an EA for release from containment and into the environment. For these "weed-eaters", APHIS has had in place, for more than fifty-years, published guidelines and a Technical Advisory Group (TAG) to evaluate applications for release (http://www.aphis.usda.gov/plant_health/permits/tag/index.shtml).

(3) In early 2006, under the authority of the PPA of 2000, APHIS resumed issuing permits for the release of entomophagous organisms from containment and preparing EAs for their first-time release into the environment. The current procedure is similar to that used before 1995, except that the EA provides a more thorough analysis of risks and benefits, and public comment on the EA is solicited. Although it can take a year or more for final approval, the permit is usually comprehensive, with few restrictions, and is often a key to quickly obtaining subsequent approvals from States and other federal agencies, if needed.

Although APHIS's policies and procedures for the regulation of entomophagous biological control organisms are not formally established, my understanding of these is incorporated in the following procedures. It is strongly advised to first check with APHIS before attempting the first-time introduction of any biological control organism in any State, regardless of whether the source of the organism is domestic or foreign.

Table 3.—First-tier risk assessment of nonindigenous invertebrates and micro-organisms proposed as candidates for release from containment.

1)	This organism has been identified to species /strain /biotype by a recognized authority.
2)	All reasonable efforts have been made to exclude undesirable plant pests and other contaminants.
3)	This organism does not feed on or infect living plant tissues.
4)	This organism does not feed on, infect or contaminate plant products.
5)	This organism does not transmit plant pathogens.
6)	No life stage or sex of this organism develops as a parasite or pathogen of a primary parasite.
7)	Release of this organism is not expected to cause significant losses in yields of crop or forage plants by causing major, population-level damage to commercially important pollinator or important natural enemies of plant pests or weeds.
8)	This organism is not expected to feed upon, attack, infect or otherwise adversely impact endangered or threatened plants or animals in the United States.

THE PROCESS FROM EXPLORATION TO RELEASE

The process for introduction of biological control agents is often presented as a series of sequential steps; however, the path usually is neither linear nor uniform. In reality an outline of procedures is analogous to a roadmap that shows a fairly straight mountain road, which upon travelling is found to have ups and downs, switch-backs, and wrong turns, but also the excitement of new discoveries. Table 4 is an example of a "roadmap" intended to help with planning. Chapters in other books give a broader, more general explanation for each step of a program to introduce new natural enemies (Van Driesche and Bellows 1996, Bellows and Fisher 1999). The emphasis in this chapter is on compliance with federal regulations and aspects of the process that usually are not provided in generalized descriptions.

Initial Surveys

The literature on the target pest and its natural enemies should be compiled for both where the pest is indigenous and where the natural enemies will be introduced. This literature survey should also include relatives of the target pest and their natural enemies. Besides taxonomic information, this compilation should include the distribution and host records of the natural enemies, if available. This information not only forms the basis to define suitable areas to explore and what groups of natural enemies to search for in these areas, but also what taxonomic expertise may be needed. The ability to identify natural enemies is critical for biological control programs as the potential candidates often are undescribed species—five of the seven natural enemies imported for control of HWA (Table 2) were species previously unknown to science.

Table 4.—Synopsis of steps and activities for obtaining and introducing biological control organisms.

Step	Partial List of Activities
Survey potential release areas	For potential release areas, define the climate and existing natural enemies attacking the target pest; start monitoring target population and potential non-target hosts.
Initial planning/preparation (Where, What, When)	Define search area and targets; review rules for export from search area; establish support team and funding; obtain use of approved containment facility; obtain import permit (PPQ-526)
Exploration/collection	Have suitable equipment for collecting, studying and keeping agents alive; do preliminary host range study; make arrangements for additional collections and study of natural enemies in collection area(s)
Shipment	Have proper forms for export (if needed) and import (PPQ-526); have suitable shipping materials; alert APHIS and your quarantine officer of shipment
Rearing, and evaluation in containment	Free agents of contaminants, obtain positive identification of candidate agents, develop rearing methods, study biology and potential host range in target release area(s)
Biology in indigenous habitat	Obtain information on biology and feeding range of selected natural enemies in indigenous area
Release from containment	Prepare release petition using NAPPO guidelines; apply for release permit (PPQ-526); APHIS-PPQ prepares an EA and solicits comments
Field release/establishment	Mass rear, as needed; decide where, when, how many/location for releases; obtain State and local permission for release; initial assessment of establishment and efficacy

A field survey for natural enemies of the target pest, and its relatives, in the areas where it has become established should be made prior to making plans to collect and import natural enemies of the target pest. What native natural enemies may interact with the prospective natural enemy (e.g., its parasites and competitors) should also be identified. This background information about existing fauna in potential release areas helps to define the missing components (e.g., natural enemies) and to identify potential nontarget or alternative hosts of the prospective natural enemy. Potential nontarget or alternate hosts and possible interactions with native natural enemies are very important considerations in risk analysis.

Another purpose of the pre-introduction surveys is to establish a reference collection of positively identified natural enemies in the prospective introduction areas. The biological control program for the balsam woolly adelgid, *Adelges piceae* (Ratzeburg), failed to recognize native congeners of some of the introduced species resulting in false reports of the establishment of introduced species (Montgomery et al. 2011). Pre-introduction surveys of HWA natural enemies made in Connecticut and North Carolina (Montgomery and Lyon 1995, Wallace and Hain 2000) provided some background information for the HWA biological control program.

Where, What, When

Although the literature on the distribution of the target pest may indicate where to search for its natural enemies, its biology and natural enemies may not have been reported, since introduced insects are often not pests in their indigenous regions. For HWA, we know where it originated—Japan (Havill et al. 2006)—and that HWA is also indigenous in western North America and in China, but these populations differ genetically from the population in the eastern United States (Havill et al. 2007). Other areas where HWA is indigenous include Taiwan, Nepal, Vietnam, and India, but these areas have climates that are less similar to target release areas in the eastern United States than areas already explored. Because of concerns about climate matching, the populations of *Laricobius nigrinus*, from moderate, coastal climates that were released for biological control of HWA in the eastern U.S. were supplemented with populations from cold, mountainous areas (Mausel et al. 2011).

Unlike most insects, adelgids have no parasites and no specific pathogens; thus, the search for natural enemies is limited to predators. Past introductions of biological controls for HWA have been limited to predatory beetles—lady beetles and derodontids (Cheah et al. 2004). Surveys in western North America have identified *Leucopis* flies (Diptera: Chamaemyiidae) that are part of the predator complex that feeds on HWA (Kohler et al. 2008), but their introduction is hampered by their abundant parasites, difficulty in rearing them, and taxonomic problems.

Ideally, what to import would be based on the knowledge that the natural enemy actually regulates indigenous HWA populations. Life table analysis is a robust method, introduced by Varley et al. (1973), for describing the sources of and the quantifying mortality of a population in order to provide insight into the regulation of insect populations. Unfortunately, life tables are difficult to construct for field populations, especially when predators, rather than parasites, are the source of mortality. Additional information on construction of a life table can be found in Morris (1957), Royama (1981), Buonaccorsi and Elkinton (1990), and Bellows and Van Driesche (1999). A study of white fly mortality is a good example of application of a life table (Naranjo and Ellsworth 2005). McClure's (1995, 1997) analysis of HWA mortality in Japan is one of the few efforts to collect mortality caused by prospective biological controls in their indigenous environment. He concluded that both density-dependent negative feedback (host resistance) and natural enemies played important roles in keeping populations of HWA low in Japan (McClure 1997), but it is unclear what natural enemies were responsible. For example, one article points to an orbatid mite, which dislodged HWA eggs (McClure 1995), another to four insect predators (McClure 1997), and another to a lady beetle (Sasaji and

McClure 1997). He also noted that HWA mortality was high (>99%) in forests, where HWA density was low, but that mortality from predators was very low (<9%) on cultivated hemlocks with high HWA populations. He concluded that "the best biological control agents of introduced pests may not be those that help maintain pest populations at non-outbreak levels in natural habitats, but rather those that are most responsive to pest outbreaks in cultivated and disturbed habitats" (McClure 1997).

In considering if natural enemies of relatives of the target species should be introduced, the life history and habitat requirements of the relatives and targets should be considered. The natural enemies released to control balsam woolly adelgid in North America were not successful, partly because they were collected from other adelgids and were not adapted to the climate (Schooley et al. 1984). It seems unlikely that reintroduction of these species for biological control of HWA would be successful.

The best time to observe natural enemies in their indigenous habitat may not be the best time to collect them to establish colonies in containment. The greatest diversity and abundance of natural enemies of HWA seems to be in the spring, when the eggs of the overwintering and spring generations of HWA are present. Most discovery and first-time importations of natural enemies of HWA were made in the spring, but successful establishment of breeding colonies was done with fall collections. This is because most HWA predators are univoltine and lay eggs in the spring; thus spring imports may have already produced most of their eggs. Predators that feed on HWA during the fall can be collected then and stored in the containment facility until spring, enabling oviposition by the predator to be synchronized with the life history of HWA in the target region.

The Containment Facility

Organisms imported for classical biological control are usually brought first into a containment facility (aka quarantine). Thus, well before a permit is requested, the applicant must have access to a containment facility that has been inspected and approved by APHIS-PPQ. The process of certifying a facility for containment of arthropods takes 1-4 months and APHIS should be consulted prior to its construction. APHIS consults with state officials about the construction of the containment facility and before issuing permits to import or release organisms into the state. It is good protocol to inform the state official about your program in advance. An approved containment facility must have a Standard Operating Procedure (SOP) and this should be reviewed in advance of importations to make sure that your activity fits within the SOP. The Quarantine Officer should also be provided with an outline of your proposed activities and a copy of the approved permit so that he or she can ensure all protocols and restrictions are followed. Additional information regarding containment can be found at http://www.aphis.usda.gov/plant_health/permits/organism/containment_facility_inspections.shtml.

Import Permits

The form PPQ-526, titled "Application for Permit Move Live Plant Pests or Noxious Weeds," is used to obtain a permit for importing biological control agents. (As the title suggests, this form is also used for movement of plant pests and noxious weeds.) It has existed for more than 25 years, although it has been modified several times and adapted for electronic filing. On the form, the word "pest" means the organism(s) for which you are seeking a permit, thus organisms intended for use as biological control agents are to be listed on the form as "pests to be moved." Use scientific names, but species group names—genus, family, order—may be acceptable since APHIS recognizes that little may be known about the natural enemy complex of the target organism in its indigenous habitat. If hosts of the natural enemy or foliage will be included in shipments, the scientific names of these are listed in a separate category. The countries where the collections will be made must be listed. Methods of containment and final disposition are required; thus, an approved containment facility and standard operating procedure usually is a prerequisite for importing biological control candidates. Application for permits should be made by the

research scientist or other leader of the project rather than the quarantine officer. A minimum of eight weeks should be allowed to receive the permit.

The application (PPQ-526) can be filed electronically or using a paper form (APHIS recommends the former). Electronic filing requires that the applicant receive a USDA eAuthentication Account with Level 2 Access. Obtaining an eAuthentication account involves filling out a simple form online and then going to the nearest USDA Service Center to show a driver's license or other government-issued photo ID. There are several advantages in using eAuthentication to apply for permits: (1) there is helpful guidance in filling out the permit, such as pull down menus for countries and organism names, (2) processing is much faster—initial review takes one week whereas a paper application takes one month, (3) progress of the approval can be tracked, (4) your template is saved for renewal or application for another permit, (5) tasks, such as ordering shipping labels and filing annual reports, can be done by email, and (6) you will receive advance notice of permit expiration.

Shipping

After receiving the permit, the permit holder will need to request the PPQ Form 599 Red/White labels to enable foreign shipments to enter the United States (the labels are not issued or used for domestic, interstate shipments). Each of these distinctive labels has an individual number and an address of a USDA Plant Inspection Station (PIS). The red and white labels are not reusable or transferable and records of each use are tracked electronically using a barcode on each label. When shipping natural enemies, a red and white label is affixed to the outside of the package and supplemental information is placed inside (minimum is the permittee name, permit number and label number). Legally, the package should include the USFWS Form 3-177 on the outside (see http://www.fws.gov/le/ImpExp/faqs.htm). To expedite the shipment, I make an invoice (Table 5) with all the information that may be needed to clear the package and place this invoice in a clear pouch on the outside of the container addressed to Inspectors with a copy between the inner and outer layer of the shipment packaging.

The permit includes detailed information on packaging the shipment, conditions regarding what may be shipped, as well as detailed step by step instructions regarding clearance for U.S. Customs and Border Protection (CBP) and APHIS PPQ Agriculture Plant Inspection Station (PIS). Shipments brought to the U.S. via commercial bonded-carriers or hand carried go first to CBP for clearance, with PIS and F&WS helping as needed. After clearance, the shipment may be transported to the containment facility by the same bonded carrier or it may be reshipped to the containment facility by APHIS-PPQ using the permittee's designated carrier, billed to the permittee's account. My experience is that the inspectors from these three Departments work closely, shipments are cleared very quickly, and delays can be traced to necessary information not being provided with the shipment, or to the carrier.

Table 5.—Outline of invoice letter.

1) Permit number and label number, permittee name.
2) The species (genus or family, if species unknown), both plants and animals, in the shipment
3) Statement that the shipment does not include CITES species
4) Statement that no venomous animals are in the shipment.
5) Statement that the contents have no commercial value
6) Address to forward the shipment to its final destination
7) Your carrier and billing account number for reshipment (APHIS will not pay shipping costs)
8) Name and phone number of a contact (quarantine officer) at the final destination.

Although hand-carrying live natural enemies from a foreign country directly to the containment facility can be arranged, this may not be safer or more expedient than shipping by bonded carrier. This privilege must be requested when applying for the permit and can be done only by the permittee or others designated on the permit. At least 20 days before the entry, each hand-carry event must be submitted and pre-authorized by the PPQ Permit Compliance Officer, who will notify CBP and provide you with a red and white label specifically prepared for the hand-carry event. The request should include details about who, when and where (i.e., the person who will carry, specific date, flight, and scheduled arrival), as well as details about what the package will contain, including foliage or other host material. Any deviations from what was pre-authorized, or changes in the airline or the travel date, will create the risk that the CPB officer will seize the package and send it to the nearest PIS or have it destroyed. Flight delays typical of airline travel should not create problems. After the package is released by CBP, it must be taken directly to the containment facility, and the quarantine officer (not the person who carried the package) must notify the PPQ Compliance Officer of the organisms received within 24 hours of their arrival.

Biology and Host Specificity Research

Information on the agent's biology and host specificity should be conducted in its indigenous habitat as well as in the containment facility. This is listed as two steps in Table 4, but one does not necessarily precede the other and they may occur simultaneously. Frequently, it is not until the species is imported and in the containment facility that its identity and potential for biological control is recognized. It often is necessary to return to where the species was collected to examine its biology and host range more thoroughly.

The biological information for a petition to release an entomophagous biological control agent not only includes its identity but also methods to distinguish it from its relatives. Additional information should be provided about closely related species so that potential interactions with these can be assessed. For example, *Laricobius nigrinus* Fender, which was introduced in the eastern United States, can hybridize in nature with the indigenous *L. rubidus* LeConte (Davis et al. 2011). The likelihood that the agent may compete for food resources with native predators and be attacked by predators, parasites, and pathogens currently established in the proposed release area should be discussed. Information should also be provided on the agent's dispersal capability and potential to thrive in the climate of the proposed release area.

Predictions of host range should be based on observations in the putative agent's indigenous environment as well as host specificity testing conducted in the laboratory. When a potential biological control is first discovered, the collectors should also search for relatives of the target pest and determine if the putative agent also attacks it. This can be done using simple tests—for parasitoids, potential hosts can be recovered and reared to see if the putative agent emerges, and with predators simple feeding tests in small dishes may be done overnight. Notes should be made of the flora and fauna in the putative agent's indigenous environment. Once the identity of the putative agent is confirmed, then literature searches may reveal other potential hosts.

Laboratory evaluation of host specificity begins with compiling a list of potential non-target species for testing that includes species with phylogenetic and ecological similarities, and species that may be endangered or of special ecological significance (Kuhlmann et al. 2005). Often a hierarchical framework is used that starts with no-choice tests done in small arenas followed by choice tests, to determine prey that are attacked and prey preferences, and then rearing trials to determine prey suitability for development (van Lenteren et al. 2006). A good example of hierarchical testing is the evaluation of *L. nigrinus* (Zilahi-Balogh 2005a,b), although Simberloff (2011) has questioned the adequacy of this. Natural enemies in the laboratory may utilize hosts that they would not utilize in nature; thus it is important to validate host specificity testing with knowledge

of the host range in the candidate's native areas. Anomalies in host specificity and biology in the laboratory can often be clarified with information about the natural enemy in its native range.

Movement from Containment and Environmental Release

In terms of federal regulation, the removal of entomophagous agents from containment facilities is regarded as a release into the environment. The same extensive information and thorough review as done for a "full" release into the environment would be needed for a "partial" release to rearing laboratory or for caged field studies. The process to remove a biological control agent from a containment facility begins with the same form used to import the organism into containment—Form PPQ-526. However, this time the permit application should include a separate report with the information (Table 6) requested in the North American Plant Protection Organization (NAPPO) "Guidelines for Petition for First Release of Non-indigenous Entomophagous Biological Control Agents" (NAPPO, 2008). The United States does not have a committee to review these petitions, as it has for biological controls for weeds; therefore, APHIS asks the Biological Control Review Committee, which has members from Mexico, the United States, and Canada, and is coordinated by Agriculture & Agri-Food Canada, to review petitions for release of entomophagous agents.

Preparing a draft environmental assessment (EA) is the next step if the above review is favorable and APHIS concurs. This draft is prepared by APHIS based on information supplied by the applicant in the petition, and other resources the agency may have. Native Tribes and states in affected areas are contacted for comments on the draft. Then, a notice is published in the Federal Register of the availability of the draft EA and that anyone can comment on it for a 30-day period. Public involvement is required by NEPA and APHIS implementing regulations (7 CFR 372.5). If warranted, a finding of no significant impacts to the environment (FONSI) is issued along with a final EA. Only then may the permit to release the organism from containment be issued. Although not up to date, Hunt et al. (2008) provides a review of the procedure in the United States and other countries.

The EA for the field release of *L. osakensis* in the continental United States is an example of this aspect of the regulatory process (APHIS 2009). The petition for its release was submitted October 30, 2008 and on April 9, 2009, the Biological

Table 6.—NAAPO guidelines for petitions for first release of nonindigenous entomophagous agents.*

(1) **Proposed action**, with the purpose, need, and reasons for the release as well as specific location, timing, and method for the initial release.

(2) **Target pest information**, including its taxonomy, economic impact, life history, and distribution as well as knowledge of other natural enemies (native and introduced) that attack the pest, and potential non-target species related phylogenetically or ecologically to the target pest.

(3) **Biological control agent information**, consisting of its taxonomy and recognition characters; depository of voucher specimens (some must be deposited in the U. S. National Collection); other closely related species or genera in North America; its current and potential geographic, habitat, and climatic range; source of the agent; its life history; its known host range; and its natural enemies and that it will be free of these when released.

(4) **Environmental and economic impacts of the proposed release**, based on known impact on vertebrates, direct impact on target and non-target species, indirect effects including competition with resident natural enemies, and any potential effects on threatened and endangered species.

(5) **Post-release monitoring**, including its establishment and spread and affect on target population densities, and, when sufficient data are available, the economic and environmental impact of the program.

*Abbreviated from North American Plant Protection Organization, Regional Standards for Phytosanitary Measures, No. 12 (NAPPO 2008)

Control Review Committee issued a favorable evaluation. The notice of the availability of the EA was published a year later on 20 May in Federal Register (USDA 2010), and the FONSI was issued June 22, 2010. The process from receipt of the petition to issue of the permit took 18 months, which is within published timelines.

Movement within the Continental United States

The PPA uses the level of a State in defining the area where an organism is considered to be established or native. Although this definition may not have scientific basis, it reflects the importance of States in federal laws. The procedure to obtain a federal permit to move an entomophagous biological control organism from a State where it is native to another State where it is not established is the same as for the release of an organism imported from a foreign country, except that a petition following NAPPO guidelines is not prepared since NAPPO addresses only species not established in the North American continent. The policy of regulating movement of entomophagous insects between States began sometime after 2006—a new edition of PPQ Form 526 (Dec 2011) has deleted the statement on its reverse side that it does not apply to interstate shipment of entomophagous insects (http://www.aphis.usda.gov/plant_health/permits/downloads/forms/ppqform526.pdf).

Many States also regulate the importation of biological control organisms into their State from other States. These states generally "piggy-back" on the federal permit and the purpose of the State permit is usually to provide a notification of specific release information, such as release date, place, and number to be released. Since new federal regulations regarding interstate movement of entomophagous biological control organisms have not been issued, it is best to consult with APHIS-PPQ and affected states prior to interstate movement of any biological control organism. It is anticipated that the pending proposal of new regulations will nominate more than 150 phytophagous and entomophagous biological control organisms for interstate movement without a permit and have procedures for nominating additional species. This will provide for dissemination of biological controls still expanding their range and not established in all states where the target pest occurs.

IMPLICATIONS FOR BIOLOGICAL CONTROL

Federal regulations are often viewed as an inconvenience or obstacle to biological control programs; however, understanding the regulations can not only facilitate compliance but also guide the development of biological control programs. The information required for a permit aligns with the information a conscientious scientist would obtain prior to releasing a new biological control organism. The regulations reflect the need to assure the safety of biological controls and to facilitate their dissemination. The NAPPO guidelines not only provide a framework for scientifically based risk assessment by regulatory agencies, but also can serve as guidelines in planning a biological control program.

The hemlock woolly adelgid illustrates the need to regulate the movement of biological controls between ecological regions, whether the biological controls are native or imported from another country. This adelgid has regional populations in the United States, one that is indigenous to western North America, and another that is nonindigenous to eastern U.S., which originated in Japan (Havill et al. 2006, Havill et al. 2007). The permit for the release of *Laricobius osakensis*, a HWA predator from Japan, is valid for the continental United States. While it seems unlikely that someone would deliberately introduce *L. osakensis* to the western U.S., the consequences of its establishment there were not considered by the EA. *Laricobius nigrinus*, from the western U.S. and western Canada, was released in the eastern U.S. at a time when APHIS did not regulate the environmental release of entomophagous insects. It has since been found that *L. nigrinus* hybridizes with *Laricobius rubidus*, a related beetle native to eastern U.S. (Havill et al. 2010). It is only through the use of molecular

genetics that this problem was identified, and this issue highlights the need for both classical morphological identification, and information on the phylogeny of pest populations and their natural enemies in their native and introduced habitats.

In summary, under existing regulatory authority and current policies, APHIS requires a permit for all importations and for any movement that crosses the "border" of the containment facility, or the border of a state. Federal regulatory authority is necessarily grounded in political (State) boundaries, and often fails to incorporate concepts such as ecological zones. While the example of biological control of HWA illustrates the need to regulate the movement of entomophagous insects, it remains unclear how to do this in a manner that facilitates biological control programs while protecting the environment from adverse impacts. Public response to new, proposed regulations will likely reflect the complex interactions of ecological and political boundaries, and variation between the intended use and the behavior of an organism.

LITERATURE CITED

APHIS. 2009. *Proposed Field Release of Laricobius osakensis (Coleoptera: Derodontidae), a Predatory Beetle for Biological Control of Hemlock Woolly Adelgid (Adelges tsugae), in the Continental United States—Environmental Assessment, December 2009.* APHIS, PPQ, Riverdale, MD: 37 p. http://www.aphis.usda.gov/plant_health/ea/downloads/Laricobius-osakensis2009.pdf (Last accessed 2 Jan 2012).

Bellows, T.S.; Fisher, T.W., eds. 1999. *Handbook of Biological Control.* London: Academic Press: 1046 p.

Bellows, T.S.; Van Driesche, R.G. 1999. Life table construction and analysis for evaluating biological control agents. In: Bellows, T.S.; Fisher, T.W., eds. *Handbook of Biological Control.* London: Academic Press: 199-224.

Buonaccorsi, J.P.; Elkinton, J.S. 1990. Estimation of contemporaneous mortality factors. *Researches on Population Ecology* 32: 151-171.

Cheah, C.A.S.-J.; Montgomery, M.E.; Salom, S.M.; Parker, B.L.; Costa, S.; Skinner, M. 2004. Biological Control of Hemlock Woolly Adelgid. Reardon, R.; Onken, B., tech. coords. FHTET-2004-04. Morgantown, WV: U.S. Department of Agriculture, Forest Service: 22 p.

Davis, G.A.; Havill, N.P.; Adelman, Z.N.; Caccone, A.; Kok, L.T.; Salom, S.M. 2011. DNA barcodes and molecular diagnostics to distinguish an introduced and native *Laricobius* (Coleoptera: Derodontidae) species in eastern North America. *Biological Control* 58: 53-59.

DeBach, P. 1964. The scope of biological control. In: DeBach, P., ed. *Biological Control of Insect Pests and Weeds.* New York: Reinhold: 3-20.

Havill, N.P.; Foottit, R.G.; Von Dohlen, C.D. 2007. Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. *Molecular Phylogenetics and Evolution* 44: 357-370.

Havill, N.P.; Montgomery, M.E.; Yu, G.; Shiyake, S.; Caccone, A. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America* 99: 195-203.

Havill, N.P.; Davis, G.A.; Klein, J.; Caccone, A.; Salom, S.M. 2010. Hemlock woolly adelgid biological control: molecular methods to distinguish *Laricobius nigrinus*, *L. rubidus*, and their hybrids. In: McManus, K.A.; Gottschalk, K.W., eds. *Proceedings, 21st U.S. Department of Agriculture Interagency Research Forum on Invasive Species 2010*; 2010 January 12-15; Annapolis, MD. Gen. Tech. Rep. NRS-P-75. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 25-28.

Hunt, E.J.; Kuhlmann, U.; Sheppard, A.; Qin, T.K.; et al. 2008. Review of invertebrate biological control agent regulation in Australia, New Zealand, Canada and the USA: Recommendations for a harmonized European system. *Journal of Applied Entomology* 132: 89-123.

Kohler, G.R.; Stiefel, V.L.; Wallin, K.F.; Ross, D.W. 2008. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology* 37: 494-504.

Kuhlmann, U.; Schaffner, U.; Mason, P.G. 2005. Selection of non-target species for host specificity testing of entomophagous biological control agents. In: Hoddle, M.S., comp. *Second International Symposium on Biological Control of Arthropods*; 2005 September 12-16; Davos, Switzerland. FHTET-2005-08. Morgantown, WV: U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team: 566-583.

Mason, P.G.; Flanders, R.G.; Arrendondo-Bernal, H.A. 2005. How can legislation facilitate the use of biological control of arthropods in North America? In: Hoddle, M.S., comp. *Second International Symposium on Biological Control of Arthropods*; 2005 September 12-16; Davos, Switzerland. FHTET-2005-08. Morgantown, WV: U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team: 702-714.

Mausel, D.L.; Van Driesche, R.G.; Elkinton, J.S. 2011. Comparative cold tolerance and climate matching of coastal and inland *Laricobius nigrinus* (Coleoptera: Derodontidae), a biological control agent of hemlock woolly adelgid. *Biological Control* 58: 96-102.

McClure, M.S. 1995. *Diapterobates humeralis* (Oribatida:Ceratozetidae): An effective control agent of hemlock woolly adelgid (Homoptera: Adelgidae) in Japan. *Environmental Entomology* 24: 1207-1215.

McClure, M.S. 1997. Biological control in native and introduced habitats: Lessons learned from the sap-feeding guilds on hemlock and pine. In: Andow, D.A.; Ragsdale, D.W.; Nyvall, R.F., eds. *Ecological Interactions and Biological Control.* Boulder: Westview Press: 31-52.

Montgomery, M.E.; Lyon, S.M. 1995. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae). In: Salom, S.; Tigner, T.; Reardon, R., eds. *Proceedings: The First Hemlock Woolly Adelgid Review*; 1995 October 12; Charlottesville, VA. FHTET-96-10. Morgantown, WV: U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team: 89-102.

Montgomery, M.E.; Shiyake, S.; Havill, N.P.; Leschen, R.A.B. 2011. A new species of *Laricobius* (Coleoptera: Derodontidae) from Japan with phylogeny and a key for native and introduced congeners in North America. *Annals of the Entomological Society of America* 104: 389-401.

Morris, R.F. 1957. The interpretation of mortality data in studies on population dynamics. *Canadian Entomologist* 89: 49-69.

NAPPO. 2008. *RSPM No 12, Guidelines for Petition for First Release of Non-indigenous Entomophagous Biological Control Agents.* Ottowa, Ontario: Secretariat of the North American Plant Protection Organization. http://www.nappo.org/Standards/Standards(all)/RSPM12-Rev20-10-08-e.pdf. (Last accessed 23 March 2011).

Naranjo, S.E.; Ellsworth, P.C. 2005. Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomologia Experimentalis et Applicata* 116: 93-108.

Royama T. 1981. Evaluation of mortality factors in insect life table analysis. *Ecological Monographs* 51: 495-505.

Sasaji, H.; McClure, M.S. 1997. Description and distribution of *Pseudoscymnus tsugae* sp. nov. (Coleoptera: Coccinellidae), an important predator of hemlock woolly adelgid in Japan. *Annals of the Entomological Society of America* 90: 563-568.

Schooley, H.O.; Harris, J.W.E.; Pendrel, B. 1984. *Adelges piceae* (Ratz.), balsam woolly adelgid (Homoptera: Adelgidae). In: Kelleher, J.S.; Hulme, M.A., eds. *Biological Control Programmes against Insects and Weeds in Canada 1969-1980.* Farnham Royal, Slough, England: Commonwealth Agricultural Bureax: 229-234.

Simberloff, D. 2011. Risks of biological control for conservation purposes. *Biocontrol.* (Online version published 24 July 2011).

U. S. Congress. 2000. Title IV—*Plant Protection Act*, 114 Stat. 438, Public Law 106-224—Agricultural Risk Protection Act, June 20, 2000. http://www.aphis.usda.gov/brs/pdf/PlantProtAct2000.pdf. (Last accessed 2 Jan 2012).

USDA. 2001. Plant pest regulations; update of current provisions. Proposed rules. *Federal Register* 66(195): 51340-51358.

USDA. 2009. Environmental impact statement: Movement of plant pests, biological control organisms, and associated articles. Proposed rules. *Federal Register* 74(201): 53673-53674.

USDA. 2010. Availability of an environmental assessment for a biological control agent for hemlock woolly adelgid. *Federal Register* 75(97): 28232-28233.

Van Driesche, R.; Bellows, T. 1996. *Biological Control.* New York: Chapman and Hall. 447 p.

van Lenteren, J.C.; Bale, J.; Bigler, F.; Hokkanen, H.M.T.; Loomans, A.J.M. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology* 51: 609-634.

Varley, G.C.; Gradwell, G.R.; Hassell, M.P. 1973. *Insect Population Ecology: An Analytical Approach.* London: Blackwell Scientific Publications.

Wallace, M.S.; Hain, F.P. 2000. Field surveys and evaluation of native predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in southeastern United States. *Environmental Entomology* 29: 638-644.

Zilahi-Balogh, G.M.G.; Kok, L.T.; Salom, S.M. 2005a. A predator case history: *Laricobius nigrinus*, a derodontid beetle introduced against the hemlock woolly adelgid. In: Hoddle, M.S., comp. *Second International Symposium on Biological Control of Arthropods*; 2005 September 12-16; Davos, Switzerland. FHTET-2005-08. Morgantown, WV: U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team: 651-664.

Zilahi-Balogh, G.M.G.; Kok, L.T.; Salom, S.M.; Broeckling, C.D. 2005b. Comparison between a native and exotic adelgid as hosts for *Laricobius rubidus* (Coleoptera: Derodontidae). *Biocontrol Science and Technology* 15: 165.

Implementation and Status of Biological Control of the Hemlock Woolly Adelgid

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