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Biology and Biological Control of Leafy Surface

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Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

*Biological
Control*

BIOLOGY AND BIOLOGICAL CONTROL OF LEAFY SPURGE



**ROB BOURCHIER, RICH HANSEN, RODNEY LYM,
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The Forest Health Technology Enterprise Team (FHTET) was created in 1995 by the Deputy Chief for State and Private Forestry, USDA, Forest Service, to develop and deliver technologies to protect and improve the health of American forests. This book was published by FHTET as part of the technology transfer series.

<http://www.fs.fed.us/foresthealth/technology/>



Cover photo. a) Infestation of leafy spurge, *Euphorbia esula*, L. b) Leafy spurge hawk moth larva, *Hyles euphorbia*. c) Leafy spurge, *Euphorbia esula* L. d) Leafy spurge flea beetle, *Aphthona czwalinae*. e) Adult *Oberea erythrocephala*. USDA APHIS.

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INTRODUCTION

CAROL BELL RANDALL, DR. RODNEY LYM

1. Overview

Leafy spurge (*Euphorbia esula* L.) is an exotic, deep-rooted, perennial weed native to Europe and Asia (Fig. 1). It was first reported in the United States in

Newbury, Massachusetts, in 1827, where it likely established from contaminated soil left from ship ballasts. This invasive weed quickly spread westward across North America, accelerated by multiple reintroductions from contaminated crop seed including oat (*Avena fatua* L.), smooth brome (*Bromus inermis* Leyss.) and alfalfa (*Medicago sativa* L.) brought by European settlers. Leafy spurge is now abundant on the northern Great Plains of the United States and the prairie provinces of Canada, where it often forms stands dense enough to displace native plants and restrict cattle grazing (Fig. 2).



Figure 1. Leafy spurge infested landscape. Inset, leafy spurge flower. USDA-APHIS.

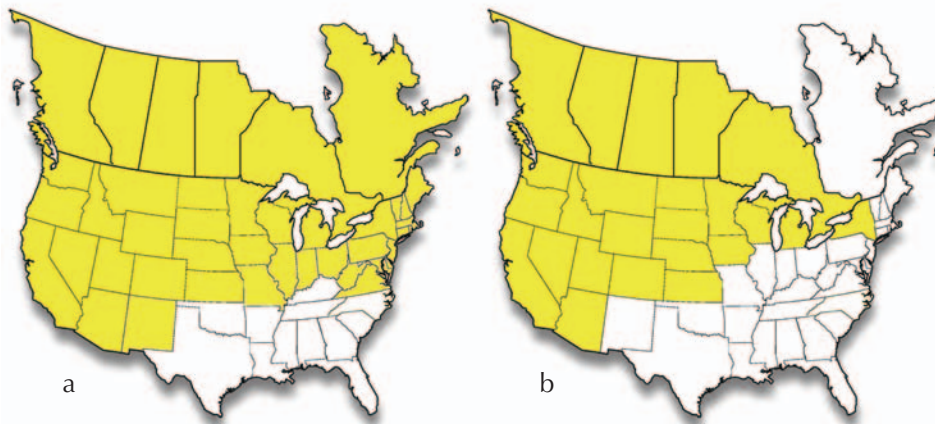


Figure 2. a) United States and Canadian provinces where leafy spurge occurs. b) States and Canadian provinces where leafy spurge is considered a noxious weed.

Leafy spurge will grow over a wide variety of terrains, from flood plains to river banks, grasslands, ridges, and mountain slopes. It is primarily found in untilled non-cropland habitats, such as pastures, rangeland, forest openings and edges, roadsides, and waste areas. The plant will grow in very diverse environments from dry to sub-humid and from



Figure 3. Leafy spurge plant, injured by *Oberea erythrocephala*, exuding characteristic latex. USDA-APHIS.



Figure 4. Savannah sparrow. Jack Bartholmai.

sub-tropic to subarctic regions. Leafy spurge is estimated to infest over 5 million acres in the Northern Great Plains and Rocky Mountain West of the United States and Canada. Once the weed has been introduced into a new area, topography does not seem to limit its spread.

When damaged, leafy spurge exudes a milky latex that discourages grazing by most wildlife and domestic cattle and horses (Fig. 3). The latex contains “ingenol,” a toxic compound that is highly inflammatory and emetic to most mammals, including humans. The latex causes scours and weakness in cattle and may result in death. Domesticated animals will eat dried leafy spurge in hay, but some livestock and most wildlife avoid eating growing plants. Economically, leafy spurge causes more than \$120 million in reduced business activity each year in the Northern Great Plains (Leistriz et al. 2004).

Leafy spurge alters plant species composition and can negatively affect wildlife. Bird populations, including those of the grasshopper sparrow (*Ammodramus savaanarum*) and savannah sparrow (*Passerculus sandwichensis*), have been reduced in areas of high leafy spurge density (Fig. 4). Leafy spurge causes reduced habitat utilization by bison (*Bos bison*), deer (*Odocoileus* spp.) and elk (*Cervus elaphus*). Leafy spurge is a major threat to the endangered western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles), because the invasive weed has established and spread into much of the only remaining habitat suitable for orchid survival (Fig. 5). The orchid is a native plant of the tall grass prairie, and was placed on the federal threatened species list in 1989.

Traditionally, herbicides have been used to control leafy spurge and long-term herbicide programs have been relatively successful. However, herbicide use is not always acceptable due to its high cost, potential for groundwater contamination, and prohibition in environmentally sensitive areas. Consequently, non-chemical methods for control have been developed, including the introduction of multiple biological control agents.

2. Biological control of weeds

Most invasive plants in North America are not native; they arrived with immigrants and commerce from different parts of the world. Generally, these non-native plants are introduced without their natural enemies, the complex of organisms that feed on the plant in its native range. This lack of predation is one reason non-native plant species become major pests when introduced outside of their native range.

Biological control of weeds is the deliberate use of living organisms to limit the distribution and abundance of a target weed. In this manual, “biological control” refers to “classical biological control,” which uses host-specific natural enemies from the weed’s

native range. Natural enemies (or biological control agents, bioagents, and biological control organisms) can damage or destroy a weed’s flowers, seeds, roots, foliage, or stems. This damage may kill the plant outright, reduce weed vigor and reproductive capability, or help cause or promote secondary infection from pathogens—all of which reduce the weed’s ability to compete with other plants. The aim of biological control is to reunite host-specific natural enemies with the target invasive plant to reduce the weed’s impacts and restore at least a part of the ecological balance present in the invasive plant species’ native range.

There are both advantages and disadvantages to biological control of weeds (Table 1). Regarding advantages, biological control is selective against a specific weed or closely related group of weeds, can provide long-term control, and is thought to be less damaging to the ecosystem than some other weed management methods. Once established at a site, biological control agents are self-perpetuating and will continue to attack the target weed year after year. Most biological control agents are able to disperse to new target weed patches, even in difficult terrain. Over the long-term the ability of biological control agents to provide continuous weed suppression and to move to new infestations as they become established make them a cost-effective weed management tool.

Disadvantages of biological control of weeds include the uncertainty about whether the biological control agents will effectively suppress the target weed to desired levels, the long time that might elapse before impacts are observed, and the risk of adverse non-target impacts on unintended plant species. Biological control agents can not be removed from the ecosystem once they are established, so they are and must be selected carefully and studied extensively before they are introduced.



Figure 5. Western prairie fringed orchid. <http://www.fws.gov>

Table 1. Advantages and disadvantages of classical biological control as a weed management tool.

Advantages	Disadvantages
Target specificity	Protracted time until Impact is likely
Continuous action	Uncertainty over ultimate scale of impact
Long-term cost effective	Uncertain “non-target” effects in the ecosystem
Gradual in effect	Irreversible
Generally environmentally benign	Not all exotic weeds are appropriate targets
Self dispersing, even into difficult terrain	Will not work on every weed in every setting

Natural enemies used in classical biological control of weeds include insects, mites, nematodes and fungi. Beetles, flies, and moths are among the most commonly used insects. To be considered for release in the United States, biological control agents must feed and develop only on the target weed, and in some cases, on a few closely related plant species. Also, a potential biological control agent's life cycle should be closely matched, or synchronized, with that of the target weed. For example, if properly synchronized, foliage-feeding insects would be in the feeding stage when the weeds are actively growing. Properly synchronized root-feeding insects would be in the feeding stage when resources in below-ground tissues are maximized.

The most effective biological control agents tend to be those which damage the most vulnerable or most problematic/persistent part of the host plant. Root- and stem-feeding biological control agents are more effective against perennial plants that primarily spread by root compared to flower- and seed-feeding biological control insects which are more useful against annual or biennial species that only spread by seeds. In the case of leafy spurge, which has an extensive root system, the most effective biological control agents are those that feed on the roots.

Host specificity is the most important consideration for a natural enemy to be used as a biological control agent. Host specificity means that the biological control agent cannot survive on plants other than the target weed. Potential biological control agents often undergo more than five years of rigorous testing to ensure that host specificity requirements are met. These tests are necessary to ensure that the biological control agents are both effective and safe, and that they will damage only the target weed

The United States Department of Agriculture - Animal and Plant Health Inspection Service - Plant Protection and Quarantine (USDA-APHIS-PPQ) is the federal agency responsible for authorizing the importation of biological control agents into the United States. The Canadian Food Inspection Agency (CFIA) serves the same role in Canada. Federal laws and regulations are in place to minimize the risks to native plant and animal communities associated with introductions of exotic organisms to manage weeds. The Technical Advisory Group for Biological Control Agents of Weeds (TAG) is an expert committee with representatives from regulatory agencies, federal land management and environmental protection agencies from the United States, Canada and Mexico. TAG reviews all petitions to import new biological control agents into the United States and makes recommendations to USDA-APHIS about the safety and potential impact of prospective biological control agents. Weed biological control researchers work closely with USDA-APHIS-PPQ and TAG to accurately assess the environmental safety of potential weed biological control agents and programs. The Canadian counterpart to TAG is the Biological Control Review Committee (BCRC).

In addition, each state in the United States has its own approval process to permit field release of weed biological control agents.

3. Code of Best Practices for Biological Control of Weeds

Biological control practitioners have adopted a Code of Best Practices for Biological Control of Weeds (see page 6 and Appendix A). By following the code, practitioners reduce the potential for causing environmental damage through the use of biological control by voluntarily restricting biological control activities to those most likely to result in success.

The code of best practices was developed by delegates and participants to the X International Symposium for Biological Control of Weeds to reduce the potential for negative impacts from biological control of noxious weed activities.

Although weed biological control is an effective and important weed management tool, it does not work in all cases and will not eradicate, or completely remove, the target weed. Often, biological control can be integrated with other chemical, mechanical, or cultural methods of weed control.

4. Biological control of leafy spurge

Biological control of leafy spurge in the United States began in 1966 with the release of the leafy spurge hawkmoth (*Hyles euphorbiae* L.) in Gallatin County, Montana (Fig. 6).

Funding for biological control research was greatly enhanced following a regional leafy spurge symposium held in Bismarck, ND in 1979. Exploration for biological control agents was expanded in regions where leafy spurge was native. Once potential biological control insects were located they were screened and the most promising were brought into North America. To date, a total of twelve insect species native to Europe and Asia have been permitted for release in the United States and Canada as classical biological control agents of leafy spurge. (The biology of these agents is presented in Chapter 2.)



Figure 6. Leafy spurge hawk moth, *Hyles euphorbiae*. USDA-APHIS.

5. Management vs. control of leafy spurge

The management of noxious weeds involves a multi-year approach that incorporates a number of control activities in any given year.

Long-term management of leafy spurge is extremely difficult to achieve. The most cost-effective control method depends on the size and location of the infested area. Small patches of leafy spurge can be eliminated with a persistent herbicide program, but large areas will require additional control measures. No single method will control leafy spurge in all the environments in which it is found. A combination of treatments, such

Code of Best Practices for Biological Control of Weeds

1. Ensure target weed's potential impact justifies release of non-endemic agents.
2. Obtain multi-agency approval for target weed.
3. Select agents with potential to control target weed.
4. Release safe and approved agents.
5. Ensure only the intended agent is released.
6. Use appropriate protocols for release and documentation.
7. Monitor impact on target weed.
8. Stop releases of ineffective agents, or when control is achieved
9. Monitor impacts on potential non-target species.
10. Encourage assessment of changes in plant and animal communities.
11. Monitor interaction among agents.
12. Communicate results to public.

The Code of Best Practices was developed by delegates and participants to the X International Symposium for Biological Control of Weeds to reduce the potential for negative impacts from biological control of noxious weed activities.

as biological with cultural or chemical control practice, is necessary to control and stop the spread of leafy spurge, especially when the weed infests large acreages.

6. Is biological control of leafy spurge right for you?

The simultaneous use of multiple weed control methods is called Integrated Weed Management or IWM. A good weed management program relies on realistic management objectives, accurate weed identification and mapping, and post treatment monitoring to answer the most basic question: "Is it working?" Most successful weed management programs incorporate a number of appropriate weed control methods: chemical (herbicides), mechanical, cultural, and biological control.

When biological control is successful, biological control agents behave like a pest species of the target weed, meaning they increase in abundance until they suppress the target weed. As local weed populations decline, biological control agent populations decline with them, due to starvation and/or dispersal to new target weed patches

Some factors to be aware of before starting biological control activities include:

- The efficacy of biological control agents cannot be guaranteed.
- Biological control will not work every time in every situation.
- Biological control will not eradicate the weed.
- Biological control may not, by itself, provide the desired level of control.
- It might take years before you notice impacts.

For these reasons, we recommend you develop an integrated weed management program in which biological control is but one of many management tools. Here are some questions you should ask before you begin a biological control program.

- **What are my weed-management goals? (Eradicate vs. reduce weed abundance)**
Biological control does not eradicate target weeds, so it is not a good fit with an eradication goal. Depending on the target weed and biological control agent used biological control can be very effective at reducing the abundance of a target weed. If your goal is to reduce weed abundance, than biological control may help you achieve it.
- **How soon do I need results?** (Yesterday vs. one to two seasons vs. within five years.) Biological control takes time to work, so another weed control method may be a better choice if you need to show immediate results. Generally, it can take one to three years after release to confirm that biological control agents are established at a site, and even longer for agents to cause significant impact to the target weed. Because of the long, drawn-out time it takes for impacts to occur, biological control may not be your best choice if you are looking for results within one to two seasons. In certain weed infestations, even five years may not be long enough for biological control to reach its weed-management potential.
- **What resources can I devote to my weed problem?** If you have a small weed problem (small infested area), weed control tools such as herbicides or hand pulling, followed up with annual monitoring for re-growth, may be the most cost-effective, because you may achieve rapid control which will prevent the weed from infesting more area. However, if an invasive weed is well established, biological control will likely be the most cost-effective and successful strategy.

The ideal biological control response:

- Is based on an understanding of current weed conditions.
- Is part of a broader integrated weed management program.
- Has considered all forms of weed control and determined that biological control is the best alternative for the given area.
- Has realistic goals and timetables and ensures appropriate monitoring.

7. About this manual

This manual provides background information on leafy spurge and each of its biological control insects. It also provides guidelines to establish and manage biological control agents as part of a leafy spurge management program.

Chapter 1 provides a detailed description of leafy spurge and some closely related species, including scientific name; description of the leaves, stems, flowers, seeds, and habitat; and occurrence in the United States and Canada. Photographs and drawings are provided.

Chapter 2 describes the leafy spurge biological control agents. Included is information on biological control agent's native range, original source of North American releases, part of plant attacked, life cycle, description, destructive stages, host specificity, known

non-target effects, habitat preferences, and availability. This chapter is particularly useful for identifying biological control agents in the field.

Chapter 3 includes detailed information and guidelines on how to plan, implement, monitor, and evaluate an effective leafy spurge biological control program. Included are guidelines and methods for:

- Planning a leafy spurge biological control program as part of an integrated spurge management plan.
- Selecting and preparing release sites.
- Collecting, handling, transporting, shipping, and releasing leafy spurge biological control agents.
- Monitoring biological control agents and vegetation.

Chapter 4 discusses the role of leafy spurge biological control in the context of an integrated leafy spurge management plan.

The **Glossary** defines technical terms frequently used by those involved in leafy spurge biological control.

The **References** lists only the publications cited directly in this manual.

The **Appendices** are as follows:

Appendix A: Code of Best Practices for Biological Control of Weeds

Appendix B: Troubleshooting Guide; When Things Go Wrong

Appendix C: Leafy Spurge Biological Control Release Form

Appendix D: Leafy Spurge Monitoring Plan Questionnaire

Appendix E: Leafy Spurge Biological Control Insect Monitoring Form

Appendix F: Leafy Spurge and Cypress Spurge Biological Control Qualitative Monitoring Form

Appendix G: Leafy Spurge Biological Control Vegetation Monitoring Form

Appendix H: Build Your Own *Apthona* Accelerator

Appendix I: PPQ Form 526 Permit Application to Transport Biological Control Agents

Appendix J: Recovery and Sampling Report Form

Appendix K: Leafy Spurge Vegetation Sampling; Daubenmire Quadrats

CHAPTER 1: GETTING TO KNOW LEAFY SPURGE

DR. MARK SCHWARZLÄNDER, DR. LUKE SKINNER

1. Introduction

Leafy spurge, family Euphorbiaceae, is in the genus *Euphorbia*, which, with more than 1,600 species, is one of the world's largest, most complex, and variable classifications of flowering plants (Gassmann et al. 1991).

Members of this genus have a highly specialized flowering branch, the cyathium (Fig. 7), that consists of a

central female flower surrounded by five groups of male flowers. The female flower develops before the male flowers, and all flowers are enclosed within a group of petal-like leaves, called "bracts." There are four marginal glands in the bracts. These produce nectar that attracts animals, which pollinate the plant.

All parts of plants in the genus *Euphorbia* emit milky, toxic sap when injured. (Fig. 8).



Figure 7. Leafy spurge plant and detail of cyathium. USDA-APHIS.



Figure 8. Leafy spurge plant exuding latex. USDA-APHIS.

2. Native North American *Euphorbia*

In North America leafy spurge joined a number of native related plants in the genus *Euphorbia* such as Horned spurge (Fig. 9), Flowering Spurge (Fig. 10) and Fire on the Mountain (Fig. 11). There are 53 native North American *Euphorbia* species listed in the PLANTS database (see "PLANTS Database," page 22). These species fall into four subgenera: *Agaloma*, *Chamaesyce*, *Esula*, and *Poinsettia* (Pemberton 1984).



Figure 9. Horned spurge, John L. Baker, Wyoming.



Figure 11. Fire on the mountain. ©Patrick Alexander, PLANTS database.



Figure 10. Flowering spurge, George N. Rysgaard, Minnesota.

In general, host-specificity testing for leafy spurge biological control agents has focused on native species in the subgenus, *Esula*. Test plants were identified based on a classification by Pemberton (1985) which identified 21 North American species of *Euphorbia* in the subgenus *Esula*. The most recent revisions of the genus *Euphorbia* reduced the number of native North American *Euphorbia* in the subgenus *Esula* from Pemberton's 21 species to 17 (Table 2).

3. Non-native spurges (genus *Euphorbia*)

Additional non-native *Euphorbia* species from distant lands have arrived in North America. The PLANTS database identifies nine non-native *Euphorbia* species in North America (Table 3). One of them, Cypress spurge, which is in the same subgenus as leafy spurge, has been the target of classical biological control studies using the same biological control agents identified for leafy spurge.

Table 2. North American native *Euphorbia* species identified in the PLANTS database.

Scientific name	Common name	Life strategy	Plant type	Distribution (United States)
<i>Euphorbia aaron-rossii</i>	Marble canyon spurge	Perennial	Forb/herb	AZ
<i>Euphorbia antisiphilitica</i>	Candelilla	Perennial	Subshrub/ forb/herb	NM, TX
<i>Euphorbia bicolor</i>	Snow on the prairie	Annual	Forb/herb	AR, LA, NM, OK, TX
<i>Euphorbia bifurcata</i>	Forked spurge	Annual	Forb/herb	NM, TX
<i>Euphorbia bilobata</i>	Blackseed spurge	Annual	Forb/herb	AZ, NM, TX
<i>Euphorbia brachycera*</i>	Horned spurge	Perennial	Forb/herb	AZ, CO, ID, MN, MT, ND, NE, NM, NV, SD, TX, UT, WY
<i>Euphorbia chamaesula*</i>	Mountain spurge	Perennial	Forb/herb	AZ, NM
<i>Euphorbia chapmanii</i>	Chapman's spurge	Perennial	Forb/herb	FL
<i>Euphorbia commutata*</i>	Tinted woodland spurge	Annual	Forb/herb	AL, AR, DC, FL, GA, IA, IL, IN, KY, LA, MD, MI, MN, MO, MS, NC, OH, OK, PA, SC, TN, TX, VA, WI, WV
<i>Euphorbia corollata</i>	Flowering spurge	Perennial	Forb/herb	AL, AR, CT, DC, DE, FL, GA, IA, IN, IL, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, NE, NH, NJ, NY, OH, OK, PA, RI, SC, SD, TN, TX, VA, VT, WI, WV
<i>Euphorbia crenulata*</i>	Chinese Caps	Annual/biennial	Forb/herb	AZ, CA, CO, NV, OR
<i>Euphorbia cuphosperma</i>	Hairy-fruit spurge	Annual	Forb/herb	AZ, NM
<i>Euphorbia curtisii</i>	Curtis' spurge	Perennial	Shrub	AL, FL, GA, NC, SC
<i>Euphorbia cyathophora</i>	Fire on the mounain	Annual/perennial	Forb/herb	AL, AR, AZ, CA, FL, GA, HI, LA, KY, IA, IL, IN, KS, MD, MN, MO, MS, NC, NE, NM, OH, PR, SC, SD, TN, TX, UT, VA, VI, WI
<i>Euphorbia dentata</i>	Toothed spurge	Annual	Forb/herb	AL, AR, AZ, CA, CO, GA, IA, IL, IN, KY, LA, MD, MI, MO, MS, NC, NE, NJ, OH, OK, PA, SC, TN, TX, VA, WV
<i>Euphorbia discoidalis</i>	Summer spurge	Perennial	Forb/herb	AL, FL, GA, LA, MS, TX
<i>Euphorbia eriantha</i>	Beetle spurge	Annual	Forb/herb	AZ, CA, NM, TX
<i>Euphorbia exserta</i>	Coastal Sand spurge	Perennial	Forb/herb	FL, GA, NC, SC
<i>Euphorbia exstpulata</i>	Squareseed spurge	Annual	Forb/herb	AZ, CA, NM, OK, TX, UT, WY
<i>Euphorbia floridana*</i>	Greater Florida Sprge	Perennial	Forb/herb	AL, FL, GA, MS
<i>Euphorbia gayeri</i>	No common name	Perennial	Forb/herb	MN
<i>Euphorbia haeleeleana</i>	Kauai spurge	Perennial	Tree	HI
<i>Euphorbia helleri*</i>	Heller's spurge	Annual	Forb/herb	LA, TX
<i>Euphorbia heterophylla</i>	Mexican fireplant	Annual/Perennial	Forb/herb	AL, AZ, CA, FL, GA, HI, LA, MS, NM, PR, TX, VI,
<i>Euphorbia hexagona</i>	Sixangle spurge	Annual	Forb/herb	AR, CO, DE, IA, IL, KS, MN, MO, MT, NE, NM, OK, SD, TX, WY
<i>Euphorbia innocua</i>	Velvet spurge	Perennial	Forb/herb	TX

* Pemberton's subgenus *esula*.

Table 2, continued. North American native *Euphorbia* species identified in the PLANTS database.

Scientific name	Common name	Life strategy	Plant type	Distribution (United States)
<i>Euphorbia inundata</i> *	Florida pineland spurge	Perennial	Forb/herb	AL, FL, GA, MS
<i>Euphorbia ipecacuanhae</i>	American ipecac	Perennial	Forb/herb	CT, DE, GA, MD, NC, NJ, NY, PA, SC, VA
<i>Euphorbia longicuris</i> *	Wedgeleaf spurge	Annual	Forb/herb	AR, OK, TX
<i>Euphorbia macropus</i>	Huachua mountain spurge	Perennial	Forb/herb	AZ, NM
<i>Euphorbia marginata</i>	Snow on the mountain	Annual	Forb/herb	AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NY, OH, OK, PA, RI, SC, SD, TN, TX, UT, VA, WI, WV, WY
<i>Euphorbia mercurialina</i>	Mercury spurge	Perennial	Forb/herb	AL, DC, FL, GA, KY, MD, NC, TN,
<i>Euphorbia misera</i>	Cliff spurge	Perennial	Shrub	CA
<i>Euphorbia nephradenia</i>	Paria spurge	Annual	Forb/herb	CO, UT
<i>Euphorbia oerstediana</i>	West Indian spurge	Perennial	Forb/herb	PR, VI
<i>Euphorbia palmeri</i> *	Woodland spurge	Perennial	Forb/herb	AZ, CA, NV, UT
<i>Euphorbia palmeri</i> var <i>subpuens</i>	Woodland spurge	Perennial	Forb/herb	AZ
<i>Euphorbia peplidion</i> *	Low spurge	Annual	Forb/herb	TX
<i>Euphorbia petiolaris</i>	Manchineel berry	Perennial	Tree/shrub	PR, VI
<i>Euphorbia pinetorum</i>	Pineland spurge	Perennial	Forb/herb	FL
<i>Euphorbia polyphylla</i>	Lesser Florida spurge	Perennial	Forb/herb	FL, LA
<i>Euphorbia pubentissima</i>	False flowering spurge	Perennial	Forb/herb	AL, AR, DC, FL, GA, IL, KY, LA, MD, MO, MS, NC, NH, OK, SC, TN, TX, VA, WV
<i>Euphorbia purpurea</i> *	Darlington's glade spurge	Perennial	Forb/herb	DE, OH, MD, NC, NJ, PA, VA, WV
<i>Euphorbia radians</i>	Sun spurge	Perennial	Forb/herb	AZ, NM, TX
<i>Euphorbia roemeriana</i> *	Roemer's spurge	Annual	Forb/herb	TX
<i>Euphorbia schizoloba</i> *	Mojave spurge	Perennial	Forb/herb	AZ, CA, CO, NM, NV
<i>Euphorbia spathulata</i> *	Warty spurge	Annual/Perennial	Forb/herb	AL, AR, AZ, CA, CO, DC, FL, GA, IA, ID, IL, IN, KS, KY, LA, MD, MI, MN, MO, MT, NC, ND, NE, NM, OH, OK, OR, PA, SC, SD, TN, TX, UT, VA, WA, WI, WY, WV
<i>Euphorbia strictior</i>	Panhandle spurge	Perennial	Forb/herb	NM, TX
<i>Euphorbia telephioides</i> *	Telephus spurge	Perennial	Forb/herb	FL
<i>Euphorbia tetrapora</i> *	Weak spurge	Annual	Forb/herb	AL, GA, LA, OK, TX
<i>Euphorbia texana</i>	Texas spurge	Annual	Forb/herb	LA, OK, KS, TX
<i>Euphorbia trichotoma</i> *	Sanddune spurge	Perennial	Subshrub/Forb/herb	FL
<i>Euphorbia wrightii</i>	Wright's spurge	Perennial	Forb/herb	TX

* Pemberton's subgenus *esula*.

Table 3. Invasive *Euphorbia* species in North America identified in the PLANTS database.

Scientific name	Common name	Life strategy	Growth habit	States infested	Biological control target	Declared noxious
<i>Euphorbia cyparissias</i> *	Cypress spurge	Perennial	Forb/herb	AL, AR, CA, CO, CT, DE, GA, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NY, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	Yes	CO, CT
<i>Euphorbia dentata</i> <i>E. dentata</i> var <i>dentata</i>	Toothed spurge	Annual	Forb/herb	AL, AR, AZ, CA, CO, DE, GA, IA, ID, IL, KS, KY, LA, MD, MI, MO, MS, NC, NJ, OH, OK, PA, SC, TN, TX, VA, WV	No	ID
<i>Euphorbia esula</i> <i>E. esula</i> var <i>esula</i> *	Leafy spurge	Perennial	Forb/herb	AZ, CA, CO, CT, DE, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MO, MT, ND, NE, NH, NJ, NM, NV, NY, OH, OR, PA, RI, SD, UT, VA, VT, WA, WI, WV, WY	Yes	AL, AZ, CA, CO, CT, HI, ID, IA, KS, MN, MT, NE, NM, NV, ND, OR, SD, UT, WA, WI, WY
<i>Euphorbia esula</i> var <i>uralensis</i> *	Russian Leafy spurge	Perennial	Forb/herb	CO, CT, KS, MI, MN, MT, NE, PA, WY	Yes, same as leafy spurge	AL, AZ, CA, CO, CT, HI, ID, IA, KS, MN, MT, ND, NE, NM, NV, OR, SD, UT, WA, WI, WY
<i>Euphorbia myrsinites</i>	Myrtle spurge	Biennial/perennial	Forb/herb	CA, CO, ID, NM, OR, UT, WA	No	CA, OR, WA
<i>Euphorbia oblongata</i>	Eggleaf spurge	Annual	Forb/herb	CA, OR, WA	No	CA, WA
<i>Euphorbia serrate</i>	Serrate spurge	Perennial	Forb/herb	CA	No	CA
<i>Euphorbia terracina</i>	Geraldton Carnation Weed	Perennial	Forb/herb	CA, PA	No	CA

* Subgenus *esula*.

Leafy spurge

Species *Euphorbia esula* L.

Synonyms

Euphorbia virgata Wald. & Kit.,
Euphorbia podperae Croiz., *Euphorbia*
x pseudovirgata, *Euphorbia waldsteinii*
 = *E. virgata*

Common names

Leafy spurge, Tithymal, Faitour’s grass

Family

Euphorbiaceae (Spurge)

Taxonomy

North American leafy spurge is considered a “complex” of leafy spurge subspecies from multiple introductions, and may represent as many as 20 subspecies and/or hybrids of subspecies that are difficult to differentiate. Each subspecies comes from a different part of Europe and Asia. Two subspecies of *Euphorbia esula*, *E. esula* var (L.) *esula* and *E. esula* var. (L.) *uralensis* (Fisch ex Link) Dorn (common name Russian leafy spurge), are listed in the PLANTs database (Table 4).



Figure 12. Single stem of leafy spurge plant. USDA-APHIS.

Table 4. Classification of leafy and Cypress spurge.

	Leafy spurge	Cypress spurge
Superdivision	Spermatophyta – seed plants	Spermatophyta – seed plants
Division	Magnoliophyta – flowering plants	Magnoliophyta – flowering plants
Class	Magnoliopsida – dicotyledons	Magnoliopsida – dicotyledons
Subclass	Dicotyledoneae	Dicotyledoneae
Superorder	Rosidae	Rosidae
Order	Euphorbiales	Euphorbiales
Family	Euphorbiaceae – spurge family	Euphorbiaceae – spurge family
Tribe	Euphorbieae	Euphorbieae
Genus	<i>Euphorbia</i> L. – spurge	<i>Euphorbia</i> L. – spurge
Species	<i>Euphorbia esula</i> L.	<i>Euphorbia cyparissias</i> L. -Cypress spurge

Description

A deep-rooted perennial forb that normally grows 60 to 100 centimeters (2 to 3 feet) tall from a woody crown that is below the soil surface (Fig. 12, above). Each crown produces several upright stems, giving the plant a clump-like appearance (Fig. 13). Leafy spurge spreads by underground roots, shoots, and seeds.

Roots

An extensive, rapidly growing root system consists of numerous coarse and fine roots, occupying a large volume of soil (Fig. 14). The roots are most abundant in the top 1 foot of soil, but can reach a depth of at least 6 meters (18 feet) and spread laterally 5 meters (15 feet) per year. Roots may be as large as 0.5 inch in diameter in the upper foot of soil and decrease to the size of a pencil lead with increasing soil depth. The root system contains a large nutrient reserve that can sustain the plant for years, allowing it to survive long periods of drought and most conventional control methods used to manage the weed.



Figure 13. Clump of leafy spurge stems. USDA-APHIS.



Figure 14. Leafy spurge root system. USDA-APHIS.



Figure 15. lateral shoot with buds. USDA-APHIS.



Figure 16. Leafy spurge leaves. USDA-APHIS.



Figure 17. Leaves of leafy spurge showing characteristic bluish-green color and alternate arrangement. USDA-APHIS.

Lateral shoots (rhizomes)

Occur below the soil surface and have numerous buds that give rise to new stems, allowing leafy spurge to quickly spread within a site (Fig. 15).

Leaves

Leafy spurge plants bear numerous narrow, linear-shaped leaves, 2.5 to 10 centimeters (1 to 4 inches) long with smooth margins (Fig. 16). The leaves are alternate and have a characteristic bluish-green color that turns to yellow or reddish-orange in the fall (Fig. 17). When damaged, leafy spurge leaves exude a toxic, milky latex that can be very irritating to the skin, and especially so to the eyes and mouth. If consumed, the latex may irritate the digestive tract, and can cause vomiting, abdominal pain, and diarrhea.

Stems

Thickly clustered, the stems are hairless, pale blue-green, and can grow up to 80 centimeters (32 inches) tall (Fig. 18). They originate from crown buds and roots, and begin to grow between March and April, making leafy spurge one of the first plants to emerge. Stems exude milky latex when damaged.



Figure 18. Leafy spurge stem. Norman E. Rees, UGA 0025049.

Flowers

Flat-topped clusters of showy, yellowish-green, petal-like bracts surround the true flowers (Fig. 19). The bracts appear in late spring and early summer, giving the plant the appearance of “blooming.” However, the true flowers, which are inconspicuous, small, and green, do not develop until mid-June (Fig. 20). The flower produces sticky pollen and nectar that attract bees.



Figure 19. Leafy spurge flower and bracts. USDA-APHIS.

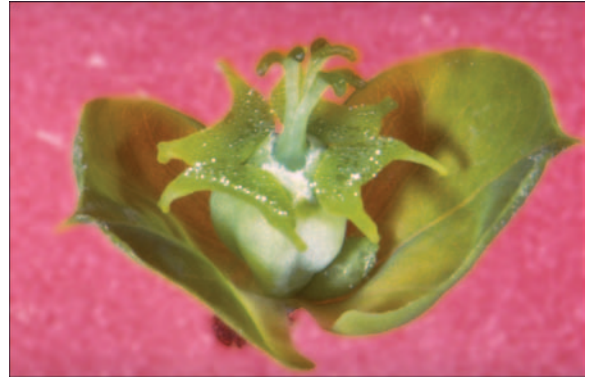


Figure 20. Leafy spurge flower. USDA-APHIS.



Figure 21. Leafy spurge seed capsule. USDA-APHIS.

Seeds

Oblong and vary in color from gray to brown to mottled. Seeds are borne in a three-celled capsule, with each cell containing one seed (Fig. 21). When seeds are mature (about 30 days after pollination), the capsules burst, throwing the seeds up to 5 meters (15 feet) from the parent plant. Further dispersal can be aided by wildlife, wind, water, and humans. On average, 140 seeds are produced per stem, and seeds may remain viable in the soil for eight years.

Seedlings

The peak period of seed germination is late May through early June, but seeds can germinate and seedlings become established throughout the growing season (Fig. 22). Optimal soil depth for seed germination is 1 to 5 centimeters (0.5 to 2 inches), but seeds may emerge through 10 centimeters (4 inches) of soil. Seedlings have a remarkable capacity for vegetative reproduction; they can reproduce



Figure 22. Leafy spurge seedling. USDA-APHIS.

in this manner from underground tissue as early as 4 to 11 weeks after germination. Typically, seedlings do not flower during the first year.

Biology and ecology

Leafy spurge is one of the first plants to emerge in the spring. It emerges during March in Iowa and Wisconsin, in early April in North Dakota, and in late April in Saskatchewan. The early and rapid growth gives this plant a competitive advantage over crop and pasture plants.

Most seeds germinate between late May, when temperatures are still near freezing, and early June. With adequate moisture, seeds can germinate throughout the entire growing season. Sixty to 80 percent of seeds are viable and can remain so for up to 8 years. Of those that germinate, 95 percent will do so within two years after maturation. Germination is promoted by alternate freezing and thawing, wet and dry periods, and prolonged dark periods.

Although seedlings have a very high capacity for regeneration, seedling mortality may be as high as 80 percent. The number of buds produced by seedling roots is limited; the roots of mature plants can form up to six times as many root buds as seedling roots can produce. Root bud formation will limit the growth of the seedling. Every root bud has the potential to produce new shoots. Stems grow rapidly as daily temperatures increase from May through June. Inflorescences form on the main stem from May to the end of July. The yellow-green bracts develop first, followed in about two weeks by the true small green flowers. Flowering is usually completed by the end of July and seed maturation and dispersal continue into early August. The plant usually stops growing during hot dry weather in August but resumes growth in fall.

In the fall, plant color changes to golden-yellow or reddish-yellow before the leaves fall from the plant. The plant's woody stems persist through the winter and remnants often can be found at the base of new shoots in the spring. Light is a limiting factor. In low light situations, plants fail to flower, plant density decreases, and plant height increases. Plant densities can reach to 20 stems per square foot (200 stems per square meter) in light sandy soils, and up to ten times that number in heavy clay or clay-loam soils.

Leafy spurge flowers are pollinated by a large variety of insects attracted to the pollen and nectar.

Leafy spurge can reproduce vegetatively, allowing the weed to increase densities and spread rapidly. Vegetative reproduction occurs from both root crown buds and root buds, but plants must overwinter before new shoots can emerge in spring. The root crown of leafy spurge, located right under and at the soil surface, develops a large number of both buds, from which new stems emerge every year, and new roots, which contribute to the persistence of leafy spurge plants.

Leafy spurge is long-lived, but it is not known how long individual plants can live. High genetic variability, early and late seasonal growth at times when many competing plant species are dormant, and the extensive deep root system help leafy spurge to out-compete and out-shade native and/or desirable plant species.

Research indicates leafy spurge may be able to limit the translocation of herbicides.

Distribution

Leafy spurge has been observed in 35 states in the U.S. and six provinces in Canada. Leafy spurge has been declared a noxious weed in 21 U.S. and in all six Canadian provinces (see Fig. 2, page 1). The most extensive infestations are in the southern portions of the prairie provinces of Canada, and the midwestern and western states of Minnesota, North Dakota, South Dakota, Nebraska, Colorado, Idaho, Montana and Wyoming.

Notes

The many genotypes of leafy spurge now established in North America may have affected the success of weed control programs, especially those that use biological control agents. Typically, insects for biological control are collected from a relatively small geographic area to ensure that a homogenous population is used for host-range testing. Because leafy spurge seed came from many different regions of Eurasia, biological control agents introduced in North America may not be well adapted to all leafy spurge genotypes.

Cypress spurge

Species *Euphorbia cyparissias* L.

Synonyms

Galarhoeus cyparissias (L.) Small ex Rydb

Tithymalus cyparissias (L.) Hill

Common names

Cypress spurge, Bonaparte's Crown, Graveyard Moss

Family

Euphorbiaceae (Spurge)

Taxonomy

North American Cypress spurge is considered a “complex” of Cypress spurge subspecies from multiple introductions (see Table 4, “Classification of leafy and Cypress spurge,” page 14).



Figure 23. Cypress spurge. Richard A. Casagrande, UGA 0886040.

Description

Cypress spurge is a perennial forb and overwinters as root and crown tissue or seeds. In early spring, shoots develop from the crown and root buds. They grow in masses and normally to heights of about 40 centimeters (16 inches) before flowering in mid spring (Fig. 23). Cypress spurge reproduces by seeds and lateral root buds.

Roots

Cypress spurge produces an extensive underground root system that allows the plant to reproduce with lateral root buds. The root system consists of two types of roots: long indeterminate roots that spread horizontally and vertically, and short determinant roots

that spread only horizontally. The taproot may reach lengths in excess of 3 meters (9 feet) and give rise to lateral roots, which produce adventitious buds.

Leaves

Cypress spurge leaves are green, stalkless, alternate, narrow, linear to lance-shaped, approximately 1 to 3 centimeters (0.5 to 1.5 inches) long and 1 to 2 millimeters (0.06 to 0.125 inch) wide. When damaged, Cypress spurge leaves exude a toxic, milky latex that can be very irritating to the skin, and especially so to the eyes and mouth. If consumed, the latex may irritate the digestive tract, and can cause vomiting, abdominal pain, and diarrhea.

Stems

Cypress spurge stems are hairless, green to yellowish green, and branched in the upper portions (Fig. 24). The thickly clustered stems originate from crown buds. Roots begin to grow in early spring between March and April, making Cypress spurge one of the first plants to emerge. Stems exude milky latex when damaged.

Flowers

Appear in early spring. Flowers are tiny, lime green to white, and clustered in bunches at the ends of the stems. Flowers are surrounded by yellowish-green bracts (Fig. 25). The flower produces a sticky pollen and nectar that attracts a variety of insects.



Figure 24. Cypress spurge stems. USDA-APHIS.



Figure 25. Cypress spurge flowers and bracts. USDA-APHIS.

Seeds

The seeds are borne in a three-celled capsule and each cell contains one seed (Fig. 26). When the seeds mature the seed capsule bursts explosively and ejects the seeds.

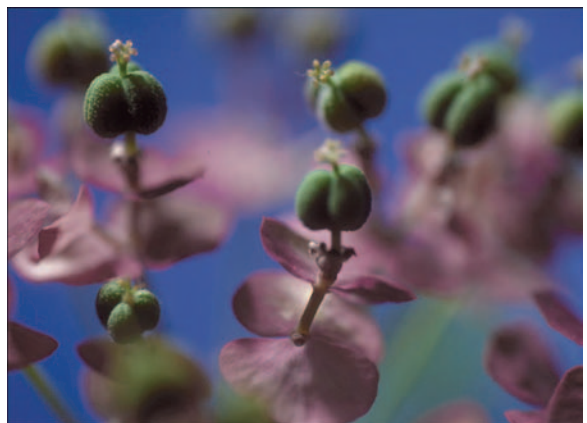


Figure 26. Cypress spurge seed capsules. Richard A. Casagrande, UGA 0886041.

The PLANTS database

The PLANTS Database provides standardized information about the vascular plants, mosses, liverworts, hornworts, and lichens of the United States and its territories. It includes names, plant symbols, checklists, distributional data, species abstracts, characteristics, images, crop information, automated tools, onward Web links, and references. This information primarily promotes land conservation in the United States and its territories, but academic, educational, and general use is encouraged. PLANTS reduces government spending by minimizing duplication and making information exchange possible across agencies and disciplines.

PLANTS is a collaborative effort of the USDA NRCS National Plant Data Center (<http://www.npdc.usda.gov>), the USDA NRCS Information Technology Center (<http://www.itc.nrcs.usda.gov>), and many other partners.

For more information visit the PLANTS Database Website: <http://www.plants.usda.gov>. The core U.S. vascular plant checklist, distribution, and attribute data integrated into PLANTS have been provided by John Kartesz, Biota of North America Program (<http://www.bonap.org>).

Biology and ecology

Cypress spurge is a weedy plant that invades disturbed areas. It can reproduce vegetatively and invade an area very quickly, and can re-grow from roots following destruction of above ground parts.

Cypress spurge commonly occurs in dry to moderately moist meadows, pastures, forest edges, roadsides, rights-of-way, cemeteries, and gardens. Generally, it does not occur on intensively cultivated soils.

Plants overwinter as seed, root or crown tissue. Root buds develop on indeterminate roots. New shoots emerge and seeds germinate, each spring soon after the snow melts. Flowering begins mid spring. Seeds may mature as early as June. A second flowering often occurs in late summer or early fall.

Distribution

Cypress spurge is found throughout much of North America, but economic losses are primarily restricted to the northeastern United States (Fig. 27).

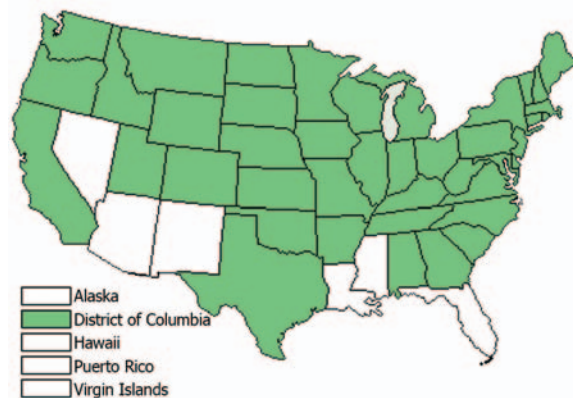


Figure 27. Distribution of Cypress spurge in the United States. PLANTS database.

4. Commonly confused non-native and native spurge species

Leafy spurge is often confused with two other non-native spurges: Cypress spurge, *E. cyparissias*, (described above) and Siberian spurge, *Euphorbia seguieriana*, which is sold as an ornamental in the United States.

There are several native spurge species that may be confused with leafy spurge in North America, including: Horned spurge, *E. brachycera* (formerly *E. robusta*); Toothed spurge, *Euphorbia dentate*; Tinted woodland spurge, *E. commutate*; Warty spurge, *E. spathulata*; and Darlington's glade spurge, *E. purpurea*. In the western states, horned and warty spurge are very similar in appearance and are commonly found in the same habitat as leafy spurge.

CHAPTER 2: BIOLOGY OF LEAFY SPURGE BIOLOGICAL CONTROL AGENTS

DR. DENISE OLSON, DR. RICH HANSEN

1. Basic insect biology

Insects are a very large and diverse class of animals. An understanding of basic insect biology and anatomy will help land managers recognize and identify biological control agents of leafy spurge.

Leafy spurge biological control insects have complete metamorphosis, a life cycle with four distinct stages: egg, larva, pupa and adult (Fig. 28). Adult insects have an exoskeleton; a segmented bodied divided into three regions: head, thorax, and abdomen; three pairs or six segmented legs; and most have one or two pairs of wings. The head of the adult insect has one pair of compound eyes and antennae, or “feelers” (Fig. 29).

Immature insects have an exoskeleton which must be shed, or molted, for immature insects to grow to the next stage. Larval stages between molts are called “instars.” Larvae eat and generally complete three to five instars before they molt into the pupal stage. During the pupal stage the insect changes from a larva to an adult. Insects do not feed during the pupal stage.

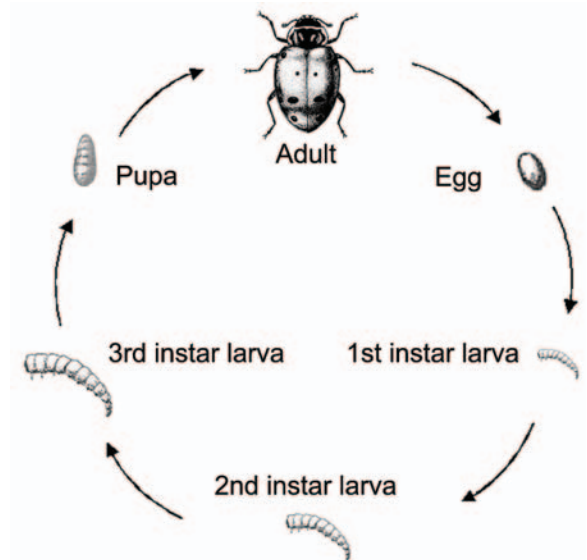
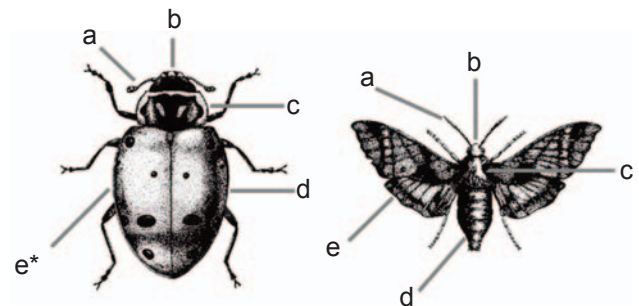


Figure 28. Complete metamorphosis.

Insect body parts

- a antenna
- b head
- c thorax
- d abdomen
- e wings













* Membranous wings under hard elytra.

Figure 29. Generalized adult insect anatomy.

2. Leafy spurge biological control insects

Twelve leafy spurge biological control species (seven beetles (Coleoptera), two flies (Diptera) and three moths (Lepidoptera) are permitted for release in the U.S. (Table 5).

Table 5. Biological control agents released in the United States for leafy spurge and Cypress spurge.

Type	Scientific name	Common name	Appearance	Leafy spurge	Cypress spurge
Beetle	<i>Aphthona abdominalis</i>			X	
Beetle	<i>Aphthona cyarissiae</i>			X	X
Beetle	<i>Aphthona czwalinae</i>			X	X
Beetle	<i>Aphthona flava</i>			X	X
Beetle	<i>Aphthona lacertosa</i>			X	X
Beetle	<i>Aphthona nigriscutis</i>	Leafy spurge flea beetles		X	X
	<i>Oberea erythrocephala</i>	Leafy spurge stem and root boring beetle/longhorn beetle		X	
Fly	<i>Spurgia esulae</i>	Shoot tip gall midge		X	X
Moth	<i>Hyles eurphorbiae</i>	Leafy spurge hawk moth		X	X
	<i>Chamaesphecia</i> spp. (two)	Clearwing moths		X	

Beetles (Order Coleoptera)

Most adult beetles are hard-bodied with tough exoskeletons. They have two pairs of wings. The two forewings, called elytra, are thickened and meet in a straight line down the abdomen of the adult insect, forming a hard protective covering. The two hind, membranous wings are used for flight, are larger than the elytra, and are folded under the elytra when not in use.

Beetle larvae are wormlike with three small pairs of legs. Most are pale white with a brown or black head.

Seven beetle species were released for biological control of leafy spurge in the United States, six *Aphthona* spp. flea beetles and *Oberea erythrocephala*. Most releases were between 1980 to 1989. Adult beetles feed on leafy spurge leaves; the larvae are root and stem feeders.

***Aphthona* spp.**

Order

Coleoptera

Family

Chrysomelidae

Common name

Leafy spurge flea beetles

Native distribution

- *A. abdominalis* northern and central Italy, Spain, France, southern Poland, Austria, eastern Europe, southwestern Asia, and northwestern Iran
- *A. cyparissiae* Europe
- *A. czwalinae* central and eastern Europe to central Asia and eastern Siberia; eastern Austria and northwestern Hungary are probably the southwestern limits of its range.
- *A. flava* Europe
- *A. lacertosa* Austria, Italy, and Eastern Europe
- *A. nigriscutis* Europe

Original sources

- *A. abdominalis* Europe
- *A. cyparissiae* Europe, Austria, Hungary, and Italy
- *A. czwalinae* Hungary
- *A. flava* Italy and Hungary
- *A. lacertosa* Eastern Europe
- *A. nigriscutis* Europe, including Hungary

First releases in United States

- *A. abdominalis* 1983 Montana and North Dakota
- *A. cyparissiae* 1987 Montana
- *A. czwalinae* 1987 Montana
- *A. flava* 1985 Montana
- *A. lacertosa* 1984 North Dakota
- *A. nigriscutis* 1989 Montana



Description

When disturbed, adult flea beetles use their enlarged hind legs to jump quickly. *Aphthona* flea beetles appear shiny or “polished” and are black, brown, copper, or gold (Fig. 30). Adults of most of the leafy spurge flea beetle species are 3 to 4 millimeters long. Eggs of *Aphthona* spp. are approximately 0.7 x 0.4 millimeter in size, initially pale yellow, and mature to yellow-brown. The larvae of all six species are about 1 to 5 millimeters long, creamy-white, and have brown head capsules and very short legs.

Life history

All leafy spurge flea beetle species have a similar life cycle (Fig. 31). Adult flea beetles emerge from the soil beginning in May and emergence continues until late June or late July. Adults can live for 45 to 65 days. About 3 to 5 days after emergence, females begin to lay their eggs near the base of leafy spurge stems, at or just below the soil surface. Newly emerged larvae burrow into the soil where they feed on the fine root hairs of leafy spurge. As the larvae grow they feed on progressively larger roots (Fig. 32) and eventually enter the root buds (Fig. 33). Larvae stop feeding and hibernate when the soil temperatures drop below 4°C (40°F). They re-

Figure 30. Adult *Aphthona* flea beetles: a) *A. abdominalis*; b) *A. czwalinae*. USDA-APHIS.

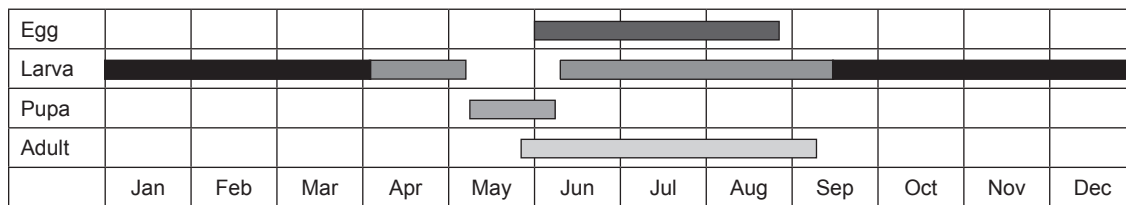


Figure 31. Life cycle of *Aphthona* spp. flea beetles used for the biological control of leafy spurge. Bars indicate the approximate length of activity for each of the life stages. The black bars represent the overwintering period for that life stage.



Figure 32. *Aphthona* spp. larvae feeding on large roots. USDA-APHIS.



Figure 33. *Aphthona* spp. larva feeding on bud tissue. USDA-APHIS.

sume feeding in the spring. Mature larvae move to just under the soil surface where they pupate. Generally, *Aphthona* spp. have one generation per year. *Aphthona abdominalis* appears to have at least three generations in southern Europe. The number of generations that *A. abdominalis* has in North America has not been documented.

Feeding stages and host impact

Adult flea beetles feed on leafy spurge foliage and flowers; at higher populations they can defoliate leafy spurge plants (Fig. 34 and Table 6). Adult feeding has little or no impact on leafy spurge growth and development. Larvae do the greatest damage to leafy spurge. Larvae feeding on the roots create wounds that can allow plant pathogens to enter, further deteriorating the root system.



Figure 34. *Aphthona* spp. adult defoliation. USDA-APHIS.

Habitat preference and areas of establishment

The different flea beetle species have different habitat preferences (Table 7). Site characteristics that are generally common to flea beetle establishment and population development include sunny and drier sites with sandy loam to loamy soils, and moderate densities of leafy spurge. For optimal biological control, a variety of flea beetle species should be released in a variety of environments. Thereafter, the beetles will decide their preferred habitats.

Availability

Since the early 1990s, millions of *Aphthona* spp. flea beetles have been collected for annual redistributions. Sources for obtaining flea beetles can be found in chapter three.

Comments

In 1995, five species of *Aphthona* beetles, *A. cyparissiae*, *A. flava*, *A. nigriscutis*, *A. czwalinae*, and *A. lacertosa*, were collected from leafy spurge sites in Montana and North Dakota and released in the eastern United States as biological control agents for leafy spurge and Cypress spurge (*Euphorbia cyparissias*). The *Aphthona* species now present in North America provide good control of leafy and Cypress spurge in open grassland habitats, but have not been as successful at controlling spurge in high moisture or shaded habitats.

Native flea beetles in the genus *Glyptina* have been encountered occasionally in leafy spurge stands. There are about 12 *Glyptina* spp. described in the United States, with most occurring east of the Rocky Mountains. Little is known about the biology of *Glyptina* flea beetles. Like most flea beetles, larvae probably feed on plant roots and adults probably feed on foliage. Host plants probably include a number of native plants, and there is some evidence that at least one *Glyptina* sp. may feed on leafy spurge.

Table 6. Leafy spurge biological control insects and their damage.

Common name	Species name	Generations/yr	Adult	Egg	Larva	Over-winter	Damage
Leafy spurge hawkmoth	<i>Hyles euphorbiae</i>	1 to 2	Light brown with white, tan, brown, and pink to red markings; 2-3 cm long, 5- to 7-cm wing-span.	Singly or clusters on spurge foliage.	Greenish-yellow and mature to combination of black, white, red, and yellow and 10 cm long.	Pupae in the soil.	Larvae defoliate the leafy spurge stems.
Leafy spurge clearwing moths	<i>Chamaesphecia</i> spp. (two)	1	Dark brown or black with yellow and cream striping; 1-1.2 cm long with a wingspan of 1.6-2.2 cm. Wings devoid of scales.	<i>C. crassicornis</i> : in groups of 2-4 along the stems. <i>C. hungarica</i> : singly on flower bracts.	Early instars feed in stem just under epidermis or in cortex below root crown, older instars mine center of roots.	<i>C. crassicornis</i> pupae in lower part of stem; <i>C. hungarica</i> larvae in roots.	Larval feeding destroys roots.
Leafy spurge flea beetles	<i>Aphthona</i> spp. (six)	Most species have one. <i>A. adominalis</i> has 3 or more in Europe.	Small (3-4 mm) black, brown, copper, or gold colored beetles which appear shiny or "polished".	Singly or clustered at or below soil surface at base of spurge stem.	Small (1-3 mm), white, worm like grubs in the roots of spurge plants.	Larvae in roots.	Larval feeding destroys roots.
Leafy spurge stem and root boring beetle	<i>Oberea erythrocephala</i>	1	Gray body, red head and yellowish-brown legs, 10 to 12 mm long.	Singling in the leafy spurge stem.	White and legless, 20 mm long.	Larvae in the crowns or roots.	Larvae feed in the crowns and roots.
Shot tip gall midge.*	<i>Spurgia esulae</i>	3 to 5	Small mosquito-like fly with reddish abdomen, 1 to 2 mm long.	Clustered on the apical bud.	Orangish and legless.	Larvae in galls.	Reduced seed production when the leafy spurge plant produces a gall at the apical bud in response to larval feeding.

* A second gall midge, *Dasineura capsulae*, has been permitted but not yet released in the United States.

Table 7. A comparison of *Aphthona* spp. flea beetle adults used in the biological control of leafy spurge and their habitat preferences.

Common name	Species name	Adult	Habitat preference
Leafy spurge flea beetles	<i>Aphthona abdominalis</i>	Head and front of thorax is reddish-yellow, anterior of thorax and abdomen is gray to black, outer wings are transparent and straw-colored.	Moist habitats in areas that receive 30 to 48 cm of rain per year and that have high relative humidity during the egg stage.
	<i>Aphthona cyparissiae</i>	Body is yellowish-brown or bronze in color and dark brown on the underside, only females with a dark brown spot behind the head at the center of the wing covers.	Open prairie sites with moist sandy loam soil, 50 to 125 leafy spurge stems/m ² and over 50 cm tall.
	<i>Aphthona czwalinae</i> , <i>Aphthona lacertosa</i>	Metallic blue black in color body, hind femur in <i>A. lacertosa</i> is yellowish to bronze in color whereas it is black in <i>A. czwalinae</i> .	<i>Aphthona czwalinae</i> prefers sites with mesic moisture loam soil and leafy spurge mixed with other vegetation, <i>Aphthona lacertosa</i> is adapted to a wide range of habitats including mesic-dry to moist conditions but appears intolerant of floods, mixed populations of <i>A. czwalinae</i> and <i>A. lacertosa</i> may coexist at several release sites.
	<i>Aphthona flava</i>	Orange-brown in color giving it a copper-like appearance.	Hot temperatures, light shade, moist soil and spurge infestations of widely spaced plants.
	<i>Aphthona nigricutus</i>	Yellowish brown or bronze in color and darker brown on the underside, brown spot behind the head at the center of the wing covers.	Hot and drier open sites with spurge greater than 50 cm tall.

Adults of these beetles may be confused with *Aphthona* flea beetles released as biological control agents of leafy spurge. *Glyptina* adults are very small beetles that exhibit the typical flea beetle “jumping” behavior. *Glyptina* adults are light brown or copper-brown, and are difficult to distinguish from *Aphthona nigricutus* or *A. cyparissiae* adults. Generally, *Glyptina* adults have a lighter brown coloration than do the *Aphthona* beetles; however, the best way to distinguish the two is by their comparative body sizes. *Glyptina* beetles are very small (body length = 1 to 2 millimeters) and only about half the size of a typical *Aphthona* adult (body length = 3 to 4 millimeters). These native flea beetles may be similar in size to *A. abdominalis* adults, but this latter insect is not known to be established in the United States and probably won’t be encountered.

Remember: Insect adults do not grow. Once they emerge from the pupal stage, the size of adult beetles remains constant. Thus, the small, native *Glyptina* flea beetles should not be considered as “immature” *Aphthona* adults that will eventually “grow” to their normal size.

Oberea erythrocephala (Schrank)

Order

Coleoptera

Family

Cerambycidae

Common name

Root Boring Beetle

Native distribution

Europe

Original sources

Europe, Italy, and Switzerland

First release in United States

1982 Montana

Description

Adult *O. erythrocephala* (Fig. 35) are 10 to 12 millimeters (0.02 to 0.05 inches) long with dark antennae, a gray body, red head, and yellowish-brown legs. The underside of the body has gray and reddish markings. The egg is 1.8 to 2.0 millimeters long, yellow, and matures to a pale pink. The white larvae are legless with a yellowish to brown head. Fully grown larvae are approximately .02 millimeter long.



Figure 35. Adult *Oberea erythrocephala*. Inset, larva in root. USDA-APHIS.

Egg												
Larva												
Pupa												
Adult												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Figure 36. Life cycle of *Oberea erythrocephala*. Bars indicate the approximate length of activity for each of the life stages. The black bars represent the overwintering period for that life stage.

Life history

Oberea erythrocephala has one generation per year and overwinters in the larval stage. Two years may be required for this beetle to complete its life cycle in the Northern United States. Adults can be found during early to mid-summer (Fig. 36.) The female girdles the upper part of the leafy spurge stem and chews a hole just above the girdle (Figs. 37, 38). Females deposit a single egg in the hole and cover the hole with latex. Each female can lay up to 40 eggs. Newly emerged larvae tunnel through the pith of stems to the root crown just below the soil surface. Larvae feed in the crowns and roots (Fig. 39) until they hibernate for the winter. They resume feeding during early spring until mid- to late May, when they form cells in the crowns and pupate. Adult emergence usually occurs when the leafy spurge is at peak flowering. Adults mate after feeding for about two weeks. Adults live 3 to 8 weeks, depending on temperature and moisture conditions.



Figure 37. Leafy spurge stem girdled by female *Oberea erythrocephala* during egg laying. USDA-APHIS.



Figure 38. Egg hole made by female *Oberea erythrocephala*. USDA-APHIS.

Feeding stages and host impact

Adults feed on leafy spurge foliage, flowers, and stem tissues but have little impact on the health or vigor of leafy spurge (Table 6, page 30).

Larvae feed in the stem pith as they tunnel down to the root system (Table 6, page 30). Generally, larvae feed in the crown and lateral roots, causing extensive damage. *O. erythrocephala* exhibits slow population growth requiring several years to reduce a leafy spurge infestation. The impact *O. erythrocephala* has on leafy spurge infestations has not been sufficiently documented.

Habitat preference and establishment areas

Oberea erythrocephala is found in well drained areas in leafy spurge-infested grasslands (Table 7, page 31). Populations of *O. erythrocephala* are scattered across the United States.

Availability

Populations of *O. erythrocephala* generally are too small to provide enough individuals for redistribution to new release sites. However, this agent may be available through your local land management agencies, universities, or commercial providers.

Comments

The root-boring activity of *O. erythrocephala* larvae does not appear to have the dramatic impact on leafy spurge demonstrated by *Aphthona* flea beetles. Unlike *Aphthona* flea beetles, *O. erythrocephala* populations grow slowly and appear to require several years to reduce a leafy spurge infestation.



Figure 39. *Oberea erythrocephala* larval root feeding. USDA-APHIS.

True flies (Order Diptera)

Adult true flies are usually small, soft-bodied insects. They are easily distinguished from other groups of insects by their single pair of membranous wings. Larvae of most true flies are legless and wormlike and are called maggots. Many insects have the word “fly” in their common name. “Fly” is written as a separate word in the common names of true flies (for example, house fly), to distinguish them from other orders of insects which use fly in their common name (for example, butterfly (order Lepidoptera) and mayfly (order Ephemeroptera)).

Only one species of fly has been released in the United States as a biological control agent for leafy spurge. Another fly has been permitted for release, but no live adults have been reared successfully in quarantine. Both are midges in the family Cecidomyiidae.

Spurgia esulae Gagné

Order

Diptera

Family

Cecidomyiidae

Common name

Leafy spurge shoot tip gall midge

Native distribution

Italy

Original source

Italy

First releases in United States

1985 Montana and North Dakota

Description

The small adult fly (1 to 2 millimeters) is mosquito-like in appearance (Fig. 40 above). The body is dark-gray with a reddish abdomen. Eggs are 0.35 millimeter long, orange, and become darker with maturity. Larvae are light orange and legless, and are found within galls at the tips of lateral branches (Fig. 41). Light red pupae form inside larval cocoons. The galls appear as miniature cabbages or artichokes that have overlapping warty, pale green leaves (Fig. 42). Galls vary in length from 6 to 40 millimeters (0.16 inch).



Figure 40. *Spurgia esula* adult. USDA-APHIS.



Figure 41. *Spurgia esula* larvae. USDA-APHIS.

Life history

Spurgia esulae has 2 to 5 generations per year in the northern United States. They overwinter in the larval stage (Fig. 43). First generation adults appear as soon as early April, depending on the weather conditions and the availability of new leafy spurge shoots, but live for only 24 to 36 hours. Adults are active only during the calm, cool periods of the day. Females deposit eggs in groups of 20 or more on leafy spurge stems or on leaves near apical buds (Fig. 44). Each female will lay more than 70 eggs during her short life. Eggs hatch in 4 to 5 days. Newly emerged larvae tun-



Figure 42. Gall made by *Spurgia esula*. USDA-APHIS.

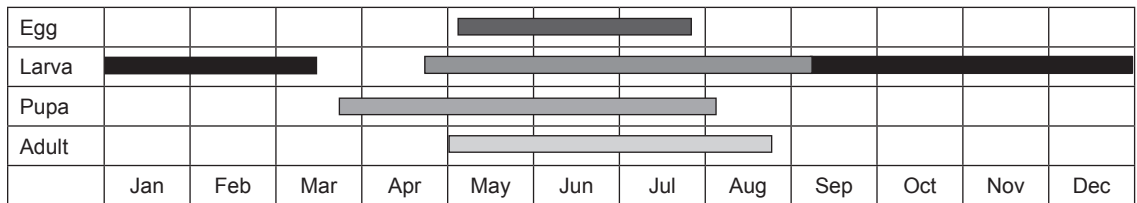


Figure 43. Life cycle of *Spurgia esulae*. Bars indicate the approximate length of activity for each of the life stages. Black bars represent the overwintering period for that life stage.

nel into and feed in the terminal buds. Larval feeding causes the leafy spurge plants to produce galls from terminal bud tissues. Larvae continue to feed and develop inside the galls. Mature larvae pupate inside silk cocoons they construct inside the galls. Adults emerge in 2 to 3 days. The last generation larvae exit galls, drop to the ground, and overwinter in the soil. Overwintering larvae pupate the following spring and adults emerge from the soil during early spring.



Figure 44. *Spurgia esula* eggs. USDA-APHIS.

Feeding stages and host impact

Spurgia esula larval feeding causes leafy spurge plants to produce galls at the terminal buds. The galls do not kill the plants, but galled plants are unable to produce flowers, and seed production can be reduced significantly when gall densities are high (Table 6, page 30). As the attacked tips die, the plant produces new shoots from below the attacked shoots. These new shoots are then attacked by the next generation of midges. Midge-produced galls are also believed to deplete nutrient production and storage in host plants.

Habitat preference and establishment areas

The leafy spurge gall midge appears to prefer south-facing slopes in cooler climates with relatively dense and shady stands of leafy spurge (Table 7, page 31). This delicate fly also may prefer leafy spurge infestations that are near wind breaks, where they will be protected from the wind. *Spurgia esula* populations are small in many of the states in the U.S. where it was released.

Availability

To date, established *S. esula* populations have provided only enough galls for research or demonstration purposes. Where *Spurgia esula* populations occur, small numbers of galls would be available for redistribution purposes.

Comments

In 1995, *Spurgia esula* was collected from sites in Montana and North Dakota and shipped to the eastern United States for use in biological control efforts on leafy spurge and Cypress spurge. As a result, galls are found in New York, New Hampshire, and Rhode Island, though damage to leafy or Cypress spurge is not yet apparent.

The introduction of *Spurgia esula* has not resulted in significant reductions in leafy spurge infestation size or density. However, *S. esula* may prove effective in suppressing leafy spurge growth if applied after other biological control agents, such as *Aphthona* flea beetles, have reduced infestations of this weed.

Dasineura* sp. nr. *capsulae

Order

Diptera

Family

Cecidomyiidae

Common Name

None widely accepted

Native distribution

Italy

Original source

Italy

First release in the United States

None. Though permitted in 1991, because of very high parasitism rates no live adults from foreign collections have been reared in quarantine for release.

Comments

Dasineura capsulae is a gall midge that attacks the seed producing part of the leafy spurge plant. Larvae overwinter in the soil and pupate in early April. Adults emerge between mid-April and mid-May. Eggs are deposited in flower clusters between the bracts where the larvae feed. The flower structures form a gall in response to larval feeding. As with *S. esulae* galls, *D capsulae* galls do not kill leafy spurge plants. Galled plants are unable to produce flowers, causing a reduction in seed production.

To date, researchers have been unable to rear this agent in quarantine, so no field releases of this agent have been made.

Moths (Order Lepidoptera)

Most moths are distinguished from other insects by their four membranous wings, usually covered with powder-like scales, and their coiled mouthparts which can be extended to siphon nectar and sap from plant flowers. The clearwing moths have no powder-like scales on their wings. Adult moth colors can be either brilliant or dull. The moth larva (caterpillar) is usually the plant-damaging stage and feeds internally or externally on its host plants.

Biological control agents from two families of moths, a hawk moth (family Sphingidae) and two species of clearwing moths (family Sessidae), were introduced for leafy spurge biological control in the mid 1960s.

Hyles eurphorbiae (L.)

Order

Lepidoptera

Family

Sphingidae

Common name:

Leafy Spurge Hawk Moth

Native distribution

Southern and central Europe, northern India, and central Asia

Original source

France, Germany, Hungary, and Switzerland

First release in United States

Mid-1960s in Idaho, Montana, Oregon, Utah, and Washington



Figure 45. Underside of *Hyles eurphorbiae*. USDA-APHIS.

Description

Hyles eurphorbiae is a large insect. The adult measures 2 to 3 centimeters (0.8 to 1.2 inches) long with a 5 to 7-centimeter (2 to 2.8-inch) wingspan. Its body is olive brown with white and black markings. The forewings are patterned with olive brown; the hindwings are pink with black markings. It is readily distinguished from native *Hyles* species by its underside (ventral surface), which is predominately pink (Fig. 45). Larvae develop through five instars. Newly emerged larvae are black (Fig. 46). The second and third instars are greenish-yellow and the fourth and fifth instars are a combination of black, white, red, and yellow (Fig. 47). Mature larvae can be 10 centimeters (4 inches) long. Pupae are 4 to 5 centimeters (1.6 to 2 inches) long and are light tan with short, fine, dark lines.



Figure 46. Newly emerged *Hyles euphorbiae* larva. USDA-APHIS.



Figure 47. Mature *Hyles euphorbiae* larva. USDA-APHIS.

Life history

The leafy spurge hawk moth has one to two generations per year. Pupae overwinter in the soil (Fig. 48). First-generation adults emerge in early to midsummer; if a second-generation of adults occurs, it will do so sometime from late August through September. Each female will lay 70 to 110 eggs singly or in clusters on leafy spurge foliage. Larvae emerge in 1 to 2 weeks depending on temperatures. First-generation larvae can be found during June; if a second generation occurs, it will in August. Mature fifth-instar larvae drop to the ground and enter the soil to pupate.

Egg												
Larva												
Pupa												
Adult												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Figure 48. Life cycle of *Hyles euphorbiae*. Bars indicate the approximate length of activity for each of the life stages. The black bars represent the overwintering period for that life stage.

Feeding stages and host impact

Leafy spurge hawk moth larvae feed on leafy spurge foliage. Although larvae may defoliate individual plants, larval feeding appears to have little impact on leafy spurge’s density (Table 6, page 30). This is thought to be due to leafy spurge’s extensive root reserves, which are able to compensate for the loss of foliage.

Predation and disease tend to keep the moth’s population levels too low to be effective.

Habitat preference and areas of establishment

Leafy spurge hawk moth prefers disturbed meadows and valleys with dense stands of leafy spurge (Table 7, page 31). Moth populations can be found in Idaho, Minnesota, Montana, New York, North Dakota, Oregon, and Wyoming.

Availability

Populations tend to be too small to support collection for redistribution to other spurge infestations. Moths can be obtained through commercial sources.

Comments

The hawk moth was released in the eastern United States for biological control of Cypress spurge in 1976, but it failed to establish at all sites except in Warren County, New York. The population in Warren County increased from 180 to about one million insects within five years and caused defoliation in some areas. Even where populations were high, hawk moth damage was not significant, because Cypress spurge plants easily tolerate defoliation.

Populations of *H. euphorbiae* are occurring at higher frequencies in leafy spurge infestations in the upper mid-west states. *H. euphorbiae* is now found on leafy spurge in numerous counties in North Dakota.

Chamaesphecia crassicornis* and *C. hungarica

Order

Lepidoptera

Family

Sessidae

Common name

Leafy spurge clearwing moth

Native distribution

Eastern Austria, Romania, and southern Slovakia

Original source

Romania

First release in United States

1994 in Montana

Comments

The wings on the clearwing moths are transparent to clear, hence the common name. The establishment rate for the *Chamaesphecia* spp. has been very low to none; therefore, the biology of the clearwing moths is not well documented for conditions in North America (Figs. 49 above, 50). No information is available on the impact of the clearwing moths on leafy spurge in the United States. They have the potential to be effective biological control agents, because the larvae feed within the leafy spurge roots and root-feeding injury has the greatest impact on leafy spurge.



Figure 49. Clearwing moth, *Chamaesphecia* sp. USDA-APHIS.

Egg												
Larva												
Pupa												
Adult												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Figure 50. Hypothetical life cycle of *Chamaesphecia* spp. in North America. Bars indicate the appropriate length of activity for each of the life stages. The black bars represent the overwintering period for that life stage.

Biological control agent host specificity testing

Host specificity is the most important consideration for a natural enemy to be used as a biological control agent. Host specificity means that the biological control agent cannot survive on plants other than the target weed. Potential biological control agents often undergo more than five years of rigorous testing to ensure that host specificity requirements are met. These tests are necessary to ensure that the biological agents are both effective and safe, and that they will damage only the target weed.

Leafy spurge has been a target of biological control since 1961. Between 1961 and 1994, tests measuring the host specificity of several species of biological control agents were conducted by Centre for Agriculture and Biosciences International (CABI) International Institute of Biological Control (IIBC) (Delemont, Switzerland), and the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) European Biological Control Laboratory (Montpellier, France). These tests estimated the range of selected natural enemies to the host-plant genus level. Insects considered for release in the United States were subjected to a second round of tests at the USDA, ARS laboratory in Albany, California. Results of these tests are summarized below:

***Aphthona* spp.**

Based on field surveys in Europe and a variety of host-specificity experiments, the host ranges of all six *Aphthona* spp. released in the United States appear to be restricted to *Euphorbia* spp. in the subgenus *Esula* (Refer to Chapter 1 for more information). Native spurges in the subgenus *Esula* are at greatest risk for potential nontarget feeding, but few of these plant species were included in pre-release host-specificity tests.

There are 17 native *Euphorbia* spp. in the subgenus *Esula* found in the continental United States; five annuals and twelve perennials. Annual spurges cannot support *Aphthona* spp. larval development. Of the twelve perennials in the *Esula* subgenus, only six might occupy the same habitats as leafy spurge infestations. Hence, they are at highest risk of non target feeding from *Aphthona* spp. released for the biological control of leafy spurge. Only one of these perennial species, *Euphorbia brachycera* (formerly *E. robusta*), has been screened for non-target feeding. *Aphthona nigriscutis* can feed and develop on this native spurge, but its overall impact on *E. brachycera* is uncertain and might even be positive.

Oberea erythrocephala

Adults have been collected from at least five European *Euphorbia* spp. hosts. Laboratory host-specificity experiments indicated that adult feeding, stem girdling, and oviposition occurred on *Euphorbia* spp. from several subgenera, but successful larval development occurred only on a subset of spurges from the subgenus *Esula*.

Established populations of *O. erythrocephala* are scattered across the United States, but it appears that no effort has been made to examine any potential utilization of native *Euphorbia* subspecies *Esula* plants under field conditions.

Spurgia esula

In Europe, *S. esulae* galls have been recorded only on *Euphorbia* spp., and the literature lists at least six host spurges. In no-choice and host-choice laboratory experiments, *S. esulae* completed its life cycle only on Eurasian spurges in the subgenus *Esula*; development was unsuccessful on two *Euphorbia* spp. native to the United States, neither of which is classified in the subgenus *Esula*. Additional tests with native North American *Euphorbia* spp. documented oviposition by female flies on seven of 11 tested species; five of the seven were in the subgenus *Esula*. Complete larval development and normal gall formation occurred on four native spurges in the subgenus *Esula*: *E. incisa*, *E. palmeri*, *E. brachycera*, and *E. spathulata*. However, *E. purpurea* and *E. telephioides*, two rare native spurges in the subgenus *Esula*, did not support normal gall or larval development. Testing indicates *S. esula* has a narrow host range restricted to some *Euphorbia* spp. in the subgenus *Esula*. Established populations of the gall fly are scattered across the United States, but it appears that no surveys of galling on non-target *Euphorbia* spp. have been undertaken.

Hyles euphorbiae

In Europe, field surveys found larvae feeding on several *Euphorbia* spp. in the subgenus *Esula*. In laboratory experiments, larvae could successfully develop on several subgenera of *Euphorbia* spp. Larvae have been observed feeding on the native spurge *E. brachycera*, subgenus *Esula*, in Montana.

3. Summary

Twelve insect species have been permitted for release into the United States for biological control of leafy spurge (see Table 5, page 26). Seven of these were subsequently released for biological control of Cypress spurge in the eastern United States. Table 6 (page 30) compares the leafy spurge biological control insects and their damage. Table 7 (page 31) compares the *Aphthona* flea beetle species and their habitat preference.

CHAPTER 3: DEVELOPING, IMPLEMENTING, AND MANAGING A BIOLOGICAL CONTROL COMPONENT OF AN INTEGRATED LEAFY SPURGE MANAGEMENT PROGRAM

DR. ANDREW NORTON, DR. ROB BOURCHIER, DR. RICH HANSEN

There is no universal recipe for the biological control of leafy spurge. Conditions and circumstances vary markedly from one management area to another, so you will have to develop a biological control program to address the management needs unique to your area. The following steps can help you do that.

1. Defining your weed management goals and objectives

This is the first and most important step in developing a biological control program. By defining what you want to achieve, you will be able to determine if, when, and where you should use biological control.

The first thing to do is define as precisely as possible what will constitute a successful leafy spurge management program. For example, a noticeable reduction in leafy spurge density over the next 10 years is perhaps achievable, but a 50 percent reduction in leafy spurge stems over the next three years would be more precise.

Biological control might be an appropriate leafy spurge weed management tool if your goal is to reduce the abundance of leafy spurge. However, by itself biological control will not eradicate or completely remove leafy spurge from the landscape. If your goal is to eradicate leafy spurge, then you should plan to employ other weed control techniques in addition to biological control (see Chapter 4 for more details).

2. Taking stock of your leafy spurge infestation and your management options

Before embarking on leafy spurge management activities, you need to understand the scope of your leafy spurge problem, identify areas of special concern, and review and understand all weed management tools at your disposal.

Your first step should be to develop a map of leafy spurge infestations at a scale that will allow you to address the weed problem in a manner consistent with your land management objectives and your weed management resources. For example, in large management areas with significant leafy spurge infestations and limited resources, aerial mapping of large patches of leafy spurge may be sufficient to start planning a weed man-

agement strategy. In other management areas with small, discrete leafy spurge patches, or where a leafy spurge infestation affects your ability to meet management objectives, intensive mapping and characterization of leafy spurge infestations (location, size, density, cover) may be necessary to develop an appropriate weed management strategy.

Review the leafy spurge management tools available, including herbicides, mechanical, cultural, and biological controls, and determine the conditions (when, where, if, etc) under which it might be appropriate to use each tool. Consult your agency or university biological control expert, cooperative weed management area, or county weed coordinator/supervisor to learn about other leafy spurge management activities, underway or planned, in your area and the level of control to be achieved by each.

Identify the resources that will be available for weed management activities, and determine if these resources will be available consistently until you meet your weed management program objectives. If not, what will happen if the weed management activities are not implemented?

With a map of leafy spurge distribution in your management area, an understanding of your land management objectives, and a list of the weed management tools available and the level of control you can realistically expect from each, you can identify the sites where biological control would be a good fit.

3. Developing, implementing, and managing your leafy spurge biological control program

Things you should consider when planning your leafy spurge biological control program include selecting appropriate release sites, obtaining and releasing insects, and monitoring the success of the program. The following sections will help you work through the whole process.

- Before you begin.
- Selecting biological control agent release sites.
- Choosing which biological control agent(s) to release.
- Obtaining and releasing leafy spurge biological control agents.
- Documenting, monitoring and evaluating a biological control program.

(In addition, we have included Appendix B: Troubleshooting Guide; When Things Go Wrong.)

Before you begin

There is a fair amount of preliminary work to do before you can implement a biological control release program. The following points will help you get it all done.

- Develop a distribution and density (percent cover or stems/acre) map for leafy spurge infestations targeted for biological control.
- Many biological control programs do not result in visible weed reduction for a

number of years (3 to 5 years or longer). Make sure that you can make a long-term commitment to the program.

- Decide how you are going to assess the success of your biological control activities and how long you can let biological control activities continue if weed control goals are not being met.
- Discuss your biological control program plans with neighboring landowners and land managers. Ask local weed managers about their experiences with biological control. Determine which agents they have used, alone or in combination with other weed control tools, and what level of control they achieved. Would their level of control be acceptable for your management area? Talk to neighboring managers about any activities, such as herbicide, grazing, or mowing programs, they have planned on their land. They could have a direct impact on your proposed biological control activities.
- Visit your prospective sites, determine if they are suitable for biological control (e.g. lack of disturbance, stable ownership) and record the following:
 - Density of leafy spurge.
 - Associated vegetation.
 - Presence of other invasive weeds.
 - Habitat conditions at the site (e.g., moist or dry, presence of shade, soil type).
 - Names of other biological control agents present.
- Set short- and long-term goals. For example, a short-term goal might be to release and determine establishment of biological control agents; a long-term goal might be to reduce leafy spurge density by 50 percent within 10 years.
- Determine what resources will be consistently available for 5 to 10 years for implementing, monitoring, and assessing your biological control program.
- Establish support logistics:
 - Commit resources for field equipment and supplies.
 - Recruit and train personnel.
 - Identify sources of biological control agents.

Selecting biological control agent release sites

A number of factors should be considered when evaluating potential biological control agent release sites. In general, sites that have large, contiguous leafy spurge infestations of moderate density, and that are situated on fairly dry, flat, unshaded sites, should be favorable for most biological control agents. Sites should be accessible and relatively free from disturbance for at least 3 to 5 years after release. (More specific site selection factors are addressed below.)

An overriding issue for land managers to consider is that leafy spurge infestations, for which biological control might be the most desirable or only management option, may not be suitable for establishment and efficacy of biological control agents. For example, tall, dense leafy spurge stands in shaded riparian habitats may have few if any control options other than biological control; unfortunately, few if any of the available leafy spurge biological control agents will work in under those conditions.

Establish goals for your release site

The overall management goals for a given site must be considered when evaluating its suitability for the release of biological control agents. In other words, is biological control compatible with the site management plan? Are there likely to be regular and/or significant disturbances to the site, such as grazing, logging, road building, burning? If so, biological control may not be an appropriate management choice.

Finally, additional factors must be evaluated even after deciding that biological control is appropriate for a given site. Suitability factors will differ, depending whether the release is to be:

- A general release, where agents are simply released for leafy spurge management;
- A field insectary (nursery) release, primarily employed for production of biological control agents for distribution to other sites, or;
- A research release, used to document biological control agent biology and/or the agent's impact on the target weed and nontarget plant community.

Presence of leafy spurge biological control agents

Examine your prospective release sites to determine if one or more leafy spurge biological control agents is present. If an agent you are planning to release is already established at a site, you can either release it at that site to augment the existing population, or release it at another site. You should re-evaluate the release of the planned species if another biological control agent is present. For example, it is probably not a good idea to initiate an insectary site for *Spurgia esulae* at a location where the flea beetle, *Aphthona*

It is probably not a good idea to initiate an insectary site for *Spurgia esulae* at a location where the flea beetle, *Aphthona lacertosa*, is already established, because the flea beetle population might reduce the abundance and vigor of leafy spurge stems so significantly, *S. esulae* would not be able to establish itself and become effective.

lacertosa, is already established, because the flea beetle population might reduce the abundance and vigor of leafy spurge stems so significantly, *S. esulae* would not be able to establish itself and become effective.

Leafy spurge infestations

In reality, a leafy spurge infestation can't be too large for biological control releases. However, it can be too small. Small, isolated patches may not allow agent populations to build up and persist, and may be better suited for other control

tactics, such as herbicide applications. Infestations should cover at least 0.4 hectare (ha) (1 acre) but preferably more, especially for field insectaries. Infestations should be contiguous; relatively uniform weed populations are preferable to scattered patches over a given area. Most biological control agents do best in leafy spurge stands of moderate density (about 70 to 110 large stems/m², or 7 to 10 large stems/ft²). As a rule of thumb, you should be able to see at least patches of the soil surface when looking down at a leafy spurge population.

Moderately tall plants (35 to 75 centimeters, or 14 to 30 inches) are preferable to very tall or very short plants.

Other site characteristics

Generally, all leafy spurge biological control agents do best on warm, sunny sites. Sites with a flat topography or a south-facing exposure are preferable to cooler, North-facing sites. Similarly, sites with an abundance of shade trees and large shrubs should be avoided; eventually, some agents might colonize shaded sites, but usually only after becoming established in adjacent, unshaded areas. Favorable biological control sites are generally dry to somewhat mesic. Avoid locations with a very high water table or standing water, or that are subject to occasional flooding. Loamy soils are preferable to high clay or sand soils. Such soils are especially important for the root-feeding flea beetles, *Aphthona* spp., because these beetles require an abundance of small, lateral roots within a few inches of the soil surface. Such conditions are seldom found on sites with very sandy soils. Other agents may be able to establish on locations with clayey or sandy soils, provided other site factors are optimal (Table 8, page 51).

Land use and potential for disturbance

Preferred release sites are those that experience little to no human or other disturbances. Fallow sites and natural areas are good choices for biological control agent releases. If a site must be disturbed (e.g., mowed or grazed) the activities should not take place during the spring and summer months when biological control agents are active above ground. Sites where insecticide applications are routine should not be used for agent releases. Such sites would include those near wetlands that are subject to mosquito control efforts; where grasshopper outbreaks routinely require chemical control; or near agricultural fields that receive regular insecticide sprays. Avoid sites prone to seasonal flooding. Do not use sites where significant conversion will take place, such as road construction, cultivation, building construction, mineral or petroleum extraction.

Ownership and access

In general, release sites on public land are preferable to sites on private land. If you must release biological control agents on private land, it is a good idea to select sites on land likely to have long-standing, stable ownership. Stable ownership will help you establish long-term agreements with a landowner permitting you access to the sites to sample or harvest biological control agents, and collect insect and vegetation data for the duration of a project. This is particularly important if you are establishing a field insectary (nursery) site, because 5 years or more of access may be required to complete insect harvesting or data collection. General releases of biological control agents to control leafy spurge populations require less-frequent and shorter-term site access; you may need to visit such a site only once or twice after initial release.

Five or more years of access may be required to complete insect harvesting or data collection.

You may wish to restrict access to release locations, especially field insectaries and research sites, and allow only authorized project partners to visit the sites and collect insects or plants. The simplest way to accomplish this would be to select only locations

that are not visible to, or accessible by, the general public. Being practical, most if not all of your sites will be readily accessible, so in order to restrict access you should formalize arrangements with the landowner or public land manager. These formalities will involve such things as posting trespassing restrictions and installing locks on gates, etc.

Another consideration is the physical access to a release site. You will need to drive to or near the release locations, so determine if travel on access roads might be interrupted by periodic flooding or inclement weather. You might have to accommodate periodic road closures by private landowners and public land managers for other reasons, such as wildlife protection.

Choosing which biological control agents to release

You should consider several factors when selecting biological control for release at a site, including agent efficacy, availability, and site preferences (Table 8).

Agent efficacy

Efficacy means the ability of the agent to directly or indirectly cause weed mortality and control leafy spurge. Most of the available data on efficacy (Table 8) is anecdotal, observational, or based on limited experimental data; however, it is clear from the data that leafy spurge flea beetles (*Aphthona* spp.), particularly *A. lacertosa* and *A. nigriscutis*, are the most effective biological control agents currently available. The potential efficacy of several agents that are not established in the U.S. is simply not yet known.

It is preferable to release only the most effective biological control agents. Consult with local weed biological control experts and neighboring land managers and landowners to identify the agent(s) that appear most effective given local site factors.

Agent availability

Several leafy spurge biological control agents do not have established, collectable populations in the U.S. and are not available for distribution (Table 8). Among established agents, insects that are readily available probably have at least several collectable populations in all states with leafy spurge infestations. These insects should be easy to obtain from intrastate and local sources. Leafy spurge agents with limited availability are likely to have collectable populations only in several states, and depending on the exact locations, might not be easy to access. Federal agencies and commercial biological control suppliers may be able to assist you in acquiring leafy spurge agents that are not available in your state (see “Obtaining and releasing leafy spurge biological control agents,” page 52). County weed managers, extension agents, or federal and university weed or biological control specialists should be able to recommend in-state sources for various leafy spurge biological control agents.

Release site characteristics

General physical and biological site preferences for each agent (Table 8) have been developed from anecdotal observations and experimental data. As noted above, *Aphthona* flea beetles and most other leafy spurge biological control agents seem to prefer leafy spurge stands of moderate density and height. Such sites also have well-drained soils, a mesic or dry moisture regime, and level ground or south-facing slopes. There is con-

Table 8. Summary of general characteristics and site preferences of leafy spurge biological control agents released in the United States (through 2005).

Agent					Release site characteristics				
Scientific name	Efficacy	Availability	Leafy spurge density	Leafy spurge stem ht.	Aspect	Shade from trees	Soil type	Moisture regime	Notes
<i>Aphthona abdominalis</i>	Unknown	Not available	Moderate (70 to 110 stems/m ²)	Intermediate (40 to 50 cm)	Level or South-facing	None	Sandy loam or loam	Mesic	Avoid soils with high sand or clay content
<i>Aphthona cyparissiae</i>	low to moderate	Limited, available in a few states.	Moderate (70 to 110 stems/m ²)	Intermediate (40 to 50 cm)	Level or South-facing.	None	Sandy loam	Mesic to somewhat dry	Avoid soils with very high sand or clay content
<i>Aphthona czwalinae</i>	High in mixed pop'ns with <i>A. lacertosa</i> .	Readily available in mixed pop'ns with <i>A. lacertosa</i>	Moderate (70 to 110 stems/m ²)	Intermediate (40 to 50 cm)	Level or South-facing.	None	Sandy loam or loam	Mesic to somewhat dry	Avoid soils with very high sand or clay content
<i>Aphthona flava</i>	Moderate	Limited, available in a few states	Moderate (70 to 110 stems/m ²)	Intermediate (40 to 50 cm)	Level or South-facing.	None	Sandy loam or loam	Mesic to somewhat dry	Avoid soils with very high sand or clay content
<i>Aphthona lacertosa</i>	High	Readily available	Moderate (70 to 110 stems/m ²)	Intermediate (40 to 50 cm)	Level or South-facing.	None	Sandy loam or loam	Mesic to somewhat dry	Avoid soils with very high sand or clay content
<i>Aphthona nigricutis</i>	High	Readily available	Low to moderate (<80 stems/m ²)	Short (<45 cm)	South-facing	None	Sandy loam	Dry	Avoid soils with very high sand or clay content
<i>Obera erythrocephala</i>	Moderate	Limited, available in a few states.	Moderate (70 to 110 stems/m ²)	Intermediate (40 to 50 cm)	Level or South-facing.	Can tolerate some shade	Loam	Mesic	
<i>Spurgia esulae</i>	Low to moderate	Limited, available in a few states.	Moderate to dense (>90 stems/m ²)	Intermediate to tall (>45 cm)	All but steep North-facing.	Can tolerate some shade	Sandy loam or loam	Mesic	
<i>Hyles euphorbiae</i>	Low	Limited, available in a few states.	Moderate to dense (>90 stems/m ²)	Intermediate to tall (>45 cm)	All but steep North-facing.	Can tolerate some shade	Tolerates most soils	Mesic	
<i>Chamaesphecia crassicornis</i>	Unknown	Not available	Moderate to dense (>90 stems/m ²)	Intermediate to tall (>45 cm)	Unknown	Can tolerate some shade	Unknown	Mesic	
<i>Chamaesphecia hungarica</i>	Unknown	Not available	Moderate to dense (>90 stems/m ²)	Intermediate to tall (>45 cm)	Unknown	Can tolerate some shade	Unknown	Mesic	
<i>Dasineura</i> sp.	Unknown	Not available	Unknown		Unknown	Unknown	Unknown	Unknown	

siderable overlap in site suitability factors among the flea beetle species. However, in general, *A. nigriscutis* populations seem to prefer relatively sparse spurge patches on dry sites, *A. lacertosa* is effective on moderately-dense infestations found on more mesic sites, and other *Aphthona* spp. are generally intermediate in their preferences.

A scarcity or absence of small lateral roots in the upper portion of the topsoil (within about 8 centimeters (3 inches) of the surface) is a common factor at sites where *Aphthona* flea beetles fail to become established and/or control leafy spurge. This condition appears to be common in heavy clay and very sandy soils. Young larvae utilize these fine roots after hatching from eggs deposited at the soil surface, so avoid releasing *Aphthona* spp. on sites with these soil characteristics.

Some agents can tolerate limited shade and northern exposures and may become established in moderately dense spurge stands. However, none of the agents currently available in the U.S. appear to be effective in very tall and dense leafy spurge stands or in infestations that are heavily shaded by trees and shrubs, such as riparian and forested habitats.

Obtaining and releasing leafy spurge biological control agents

You can obtain leafy spurge biological control agents either by collecting them yourself, having someone collect them for you, or by purchasing them from a commercial supplier. Typically, the last two methods will require packaging and shipping from the collection site to your release location (see “Collecting leafy spurge biological control agents,” page 54).

Here are some factors to consider when looking for sources of biological control:

- Always check a prospective release site for the presence of biological control agents. Don't be surprised if you already have an established population of at least one biological control agent at your release site. Several leafy spurge agents have been widely distributed throughout the U.S., with releases made over many years in some states, such as North Dakota and Wyoming. Also, all biological control agents have mobile adult, and sometimes immature, stages and are quite capable of local and even long-distance dispersal on their own.
- You do not need to take a “lottery approach” and release all types of biological control agents at a site. In fact, some biological control agents will not be available even if you want them, and some have shown to have little or no effectiveness even after released. The best strategy is to release the best agent! Most successful biological control efforts against leafy spurge have involved two species of flea beetles: *Aphthona nigriscutis* and *Aphthona lacertosa*. In some cases both agents were released and established, but in most cases only one beetle was involved. Ask the county, state, or Federal biological control experts in your state if one or both of these flea beetles would work at your location. If not, most likely they will recommend biological control agents that will work.

Remember: Interstate transport of biological control agents requires a USDA permit.

- If available, biological control agents from local sources are best. Using local sources increases the likelihood that agents are adapted to the abiotic and biotic environmental conditions present, and are available at appropriate times for release at your site. Local sources may include neighboring properties or other locations in your county and adjacent counties. Remember: Interstate transport of biological control agents requires a USDA permit (see “Regulations pertaining to leafy spurge biological control agents,” pages 63, 64). Get your permits early to avoid delays.
- Many states have “field days” at productive insectary sites (Fig. 51). On these days, land managers and landowners are invited to collect or receive freshly collected leafy spurge agents for quick release at their sites. These sessions are an easy and often inexpensive way for you to acquire biological control agents. They are good educational opportunities as well, because you can often see first hand the impacts of various agents on leafy spurge and plant communities. Typically, field days are conducted at several sites in a state and on several dates during the summer.
- Although designed primarily for intrastate collection and distribution, out-of-state participants may be welcome to participate. (Remember that USDA permits are required for interstate movement and release of biological control agents.) Almost all field days involve collection of *Aphthona* flea beetles, typically *A. lacertosa* or *A. nigriscutis*. Contact county weed supervisors, university weed or biological control specialists, or federal weed managers for information about field days in your state and/or adjacent states.

Many states have “field days” at productive insectary sites. On these days, land managers and landowners are invited to collect or receive freshly collected leafy spurge agents for quick release at their sites.



Figure 51. Field day in North Dakota, providing education about leafy spurge biological control and collection and distribution of *Aphthona* flea beetles. USDA-APHIS.



Figure 52. The “front” of advancing leafy spurge mortality caused by *Aphthona* spp. flea beetles (indicated by arrow). Note the dead spurge stems uphill from this front. USDA-APHIS.



Figure 53. Standard 15-inch-diameter insect sweep net with heavy canvas collecting bag. USDA-APHIS.

Collecting leafy spurge biological control agents

Leafy spurge flea beetles, *Aphthona* spp.

Collecting guidelines are generally the same for all flea beetles. The most productive area from which to collect flea beetle adults is on the “front” of advancing, beetle-caused spurge mortality, immediately adjacent to dead stems (Fig. 52). Using a canvas sweep net (Fig. 53), walk through the collection site, vigorously sweeping the net in a back-and-forth motion that covers about a 120°-arc through the upper half of the leafy spurge crown. Live leafy spurge plants in this zone may be somewhat stunted, have suffered considerable defoliation, and support obvious clusters of aggregating beetles (Fig. 54). Depending on the size and distribution of the leafy spurge infestation and the distribution and abundance of the flea beetle, these productive collecting areas may be at the periphery of a circular impact zone or scattered over a large area in no clear pattern. In any event, concentrate your collecting efforts on these areas. You may sweep an area several times if you wait 15 to 30 minutes between sweeps, thus allowing beetles knocked off plants during earlier sweeps to return to the plants.

Sweep nets may fill up quickly when adult flea beetles are very abundant (Fig. 55). If this happens, quickly shake the collected beetles into a ball at the bottom of the net, invert the net over a storage container, deposit the beetles, add a few leafy spurge sprigs as food and a climbing substrate, and seal the container. Containers should be vented and stored in a cooler with several ice packs, and kept out of the sun. Store the containers this way for a very short time only, meaning only until you can either sort and package the agents for transport to distant sites, or release them on local sites.

Adult *Aphthona* beetles are optimally collected in early to mid-summer, around the time of their peak abundance. The actual dates for these peaks will vary significantly among locations, and may differ considerably from year to year, depending on weather conditions, elevation, latitude, and exposure. Generally, collections should be done from mid-June, on southern and/or low-elevation collecting sites to mid-

Generally, collections should be done from mid-June on southern and/or low-elevation collecting sites to mid- to late July on northern and/or high-elevation sites. Actual dates of peak abundance will vary among flea beetle species. The order, from earliest to latest, in which flea beetle populations will peak is: *Aphthona lacertosa* and *A. czwalinae*, *A. nigricutis*, *A. cyparissiae*, and *A. flava*.



Figure 54. Aggregating *Aphthona lacertosa* adults on a leafy spurge stem. USDA-APHIS.



Figure 55. Insect sweep net becoming filled with *Aphthona* spp. adults. USDA-APHIS.

to late July on northern and/or higher-elevation sites. Actual dates of peak abundance will vary among flea beetle species. The order, from earliest to latest, in which flea beetle populations will peak is: *Aphthona lacertosa* and *A. czwalinae*, *A. nigriscutis*, *A. cyparissiae*, and *A. flava*.

Adult flea beetles love the heat. Optimal collecting conditions include warm to hot temperatures, calm winds, and bright sunshine or scattered cloudiness. Avoid collecting agents during cool and damp evenings and early mornings, and on cool, overcast, or rainy days. Adult flea beetles are much less active under these conditions and often hide on spurge stems or in plant litter, making them difficult to collect.

Leafy spurge root-boring beetle, *Oberea erythrocephala*

Adult beetles are the collectable life stage for *O. erythrocephala*. When abundant, adults may be collected from leafy spurge plants by hand or with a forceps and placed directly into shipping containers containing leafy spurge sprigs. Adults are fairly conspicuous and are usually found on stems, foliage, or flowers on the upper parts of leafy spurge shoots. *O. erythrocephala* adults can be collected with a sweep net, but this is less efficient than collecting them by hand because

Depending on location, *O. erythrocephala* adults are most abundant from mid-June through mid-July.

adults usually do not aggregate in large numbers the way *Aphthona* flea beetles do, and drop off plants or fly away when disturbed. If you use a sweep net, sweep gently through the upper parts of the leafy spurge canopy throughout the collection site.

Depending on location, *O. erythrocephala* adults are most abundant from mid-June through mid-July. Collections should be made on warm, sunny, calm days. Avoid collecting on cool, overcast, or rainy days, or during mornings and evenings.

Leafy spurge bud gall midge, *Spurgia esulae*

The most efficient way to collect and transport *Spurgia esulae* is when galls contain mature larvae and pupae. *S. esulae* is multivoltine; however, you should concentrate on collecting in spring, during the first generation, which is generally much more developmentally synchronized than later generations. To assess developmental stage, select several larger galls and peel away the outer leaves until you start to see larvae and/or pupae (Fig. 56). Larvae are very small, bright orange, legless maggots. Pupae are also orange, but are contained within white silk cocoons, which are sometimes visible through the silk. Inspect the galls in this way once a week, beginning in early spring. Collect the galls when a majority of insects are in the pupal stage, and there are still some relatively large, mature larvae present. The optimal collection time generally runs from mid-May to mid-June, but can vary considerably from site to site and from year to year.

Spurgia esulae is multivoltine; however, you should concentrate on collecting in spring, during the first generation, which is generally much more developmentally synchronized than later generations.



Figure 56. *Spurgia esulae* larvae and pupae. USDA-APHIS.

Once *S. esulae* have reached a collectable stage, harvest the galls by clipping the terminal 15 to 18 centimeters (6 to 7 inches) of stems on which there is at least one terminal gall. Try to collect a variety of gall sizes, as the largest galls do not necessarily contain the greatest number of *S. esulae*. Bundle the clipped stems loosely with rubber bands in groups of 20 to 25 and place them in plastic bags; wrapping the base of a gall bundle in a wet paper towel will help maintain the quality of the galls. Place the bundles in a cooler with ice packs immediately after collection. Galls degrade rapidly after harvest,

which may cause *S. esulae* larvae and pupae to desiccate or exit the galls, so transport and release the gall bundles as soon after collection as possible. If necessary, you can refrigerate the galls overnight.

If possible, harvest the galls on cool, overcast days. Collecting in the morning will allow for same-day shipments to release locations (see “Releasing leafy spurge biological control agents,” page 64).

Leafy spurge hawk moth, *Hyles euphorbiae*

Although other life stages may be collected for distribution, the larval stage (caterpillar) of *H. euphorbiae*, is the preferred stage to transport. You can collect larvae of any age, but the larger, mature caterpillars are the most conspicuous and easiest to find (Fig. 57). Look for green, stick-like leafy spurge stems that have been defoliated by the insects. Gently remove the larvae from leafy spurge shoots by hand or with a forceps and place them directly in containers with a few leafy spurge sprigs. Caterpillars may regurgitate a thick, green liquid when handled this way. This is a defense mechanism but will cause no harm to the insect or the person handling it.

Hyles euphorbiae apparently has two generations per year throughout the U.S., so you can collect larvae twice during the summer. Depending on location and weather conditions, larvae are present in June and again in August or September. Caterpillars will be most active and conspicuous during the middle of the day.

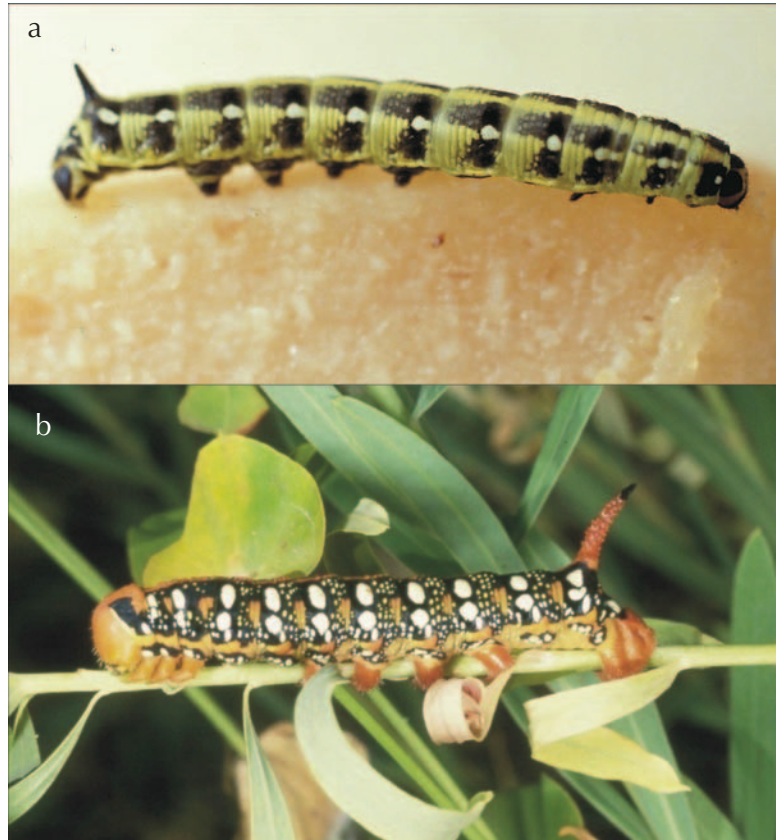


Figure 57. *Hyles euphorbiae* larvae: a) young; b) mature. USDA-APHIS.

Leafy spurge clearwing moths, *Chamaesphecia crassicornis* and *C. hungarica*

No collection methods have been developed for these insects, because there are no established populations in the U.S.

Sorting and packaging leafy spurge biological control agents

Collecting by hand can be monotonous, but it is the best way to remove unwanted insects, plant parts, soil, etc., from your collections. *Oberea erythrocephala*, *Spurgia esulae*, and *Hyles euphorbiae* larvae collected by hand should require no additional sorting before distribution. Containers of adult flea beetles, *Aphthona*, collected with sweep nets, are likely to contain a variety of other insects and plant debris, including seeds of leafy spurge and other plants. You must remove these contaminants before distributing your insects to other locations. There are several techniques for doing this.

Manual sorting

You should sort your collections manually whenever you intend to ship them any distance, and especially outside the state. It is a labor- and time-intensive procedure, but it is the best way to decontaminate *Aphthona* collections. To begin, open your containers to allow flying insects, such as wasps, flies, and moths, to leave. Generally, flea beetles will not fly from the container during this time. After several minutes, place the containers in a Plexiglas cage and allow the insects to distribute themselves (Fig. 58). If working indoors, place a light overhead to attract the beetles toward the top of the cage. Finally, use an aspirator or electric vacuum pump to select the beetles. Aspirators, powered by inhaling on a plastic tube, are cheap and useful in remote locations, but best suited for sorting relatively small numbers of flea beetles (Fig. 59). Electric vacuum pumps are better suited for sorting large numbers of *Aphthona* adults, but can be inconvenient in the field (Fig. 60). Some vacuum pumps can be powered by vehicle batteries, while others require a generator (Fig. 61). Consider using a hand-held counter to tally the number of beetles you collect, and record the number in your notes.



Figure 58. Clear Plexiglas cage used for sorting field-collected *Aphthona* adults. USDA-APHIS.



Figure 59. Aspirator used to collect small insects, including *Aphthona* flea beetle adults. The end of the rubber tube is placed in the mouth; a quick sucking action pulls insects into the metal collecting tube and then into the collecting vial. USDA-APHIS.

Passive sorting

Several passive tools have been developed to remove nontarget insects and plant parts from *Aphthona* adult collections. These devices exploit the size and dispersal differences among various insects. While they remove most contaminants, they cannot guarantee absolutely clean cohorts of leafy spurge flea beetles. Hence, they are best used for local or intrastate transportation of beetles. One type of passive sorter utilizes a length of PVC pipe, at least 10 to 15 centimeters (4 to 6 inches) in diameter and 46 centimeters (18 inches) long (Fig. 62). One end of the pipe is permanently sealed, the other has a removable cap. The pipe has many 3/16-inch holes or slits along its length. You dump the contents from the sweep nets into the pipes, place the pipes inside light-colored insect-mesh bags, thin laundry bags, or pillow cases (Fig. 63), and place the bags in the sun for about 30 minutes. The sun-loving beetles will crawl out of the pipes and collect in the bags, after which you can scoop them into containers. Plant debris and less mobile and larger insects will remain in the pipes.



Figure 60. Electric vacuum pumps for sorting collected *Aphthona* adults. USDA-APHIS.



Figure 61. Small collecting vacuum that is powered by a 12-volt car battery and used to sort *Aphthona* adults under field conditions. USDA-APHIS.



Figure 62. Passive *Aphthona* adult sorter, made from a length of 6-inch-diameter PVC pipe. Field-collected material is placed inside the pipe, and flea beetle adults crawl out the 3/16" holes. USDA-APHIS.



Figure 63. Passive *Aphthona* sorters inside fabric bags. Upper sorter has drilled holes; bottom sorter has saw cuts. USDA-APHIS.

Another passive sorter uses a variety of screens and baffles to remove most contaminants from the contents of containers and sweep nets and collects adult flea beetles in a separate, attached container (Fig. 64). This sorter works a bit more quickly than passive sorters made from PVC pipe (above), but materials from which they are made are more expensive and there is a significant amount of assembly required. (For details and instructions, see Appendix H.)



Figure 64. The “*Aphthona accelerato*” flea beetle sorter developed by TEAM Leafy Spurge. (a) the sorter as used in the field; (b) the collecting container at the bottom holding *A. lacertosa* adults. USDA-APHIS.



Figure 65. Plastic vials developed to volumetrically estimate numbers of collected *Aphthona* spp. adults. USDA-APHIS.

You can estimate the numbers of flea beetles you have collected from passive sorters as you place them into shipping containers. A 10-milliliter (10 cm³ or 0.34 fl. oz.) plastic vial will hold roughly 1,000 adult flea beetles (Fig. 65).

Unsorted, field-collected agents

Sorting field-collected material may not be necessary if you are transporting *Aphthona* flea beetles only a short distance, because other insect or plant contaminants you collect probably are already present at nearby sites. However, at most you should use unsorted agents for local or intra-county distribution.

Shipping containers for leafy spurge biological control agents

Your containers need to protect the biological control agents and prevent them from escaping. They need to be rigid enough to resist crushing, and ventilated to provide adequate air flow and prevent condensation. Unwaxed paperboard cartons are ideal for all leafy spurge biological control agents (Fig. 66). They are rigid yet permeable to air and water vapor and are available in pint, quart, half-gallon, and gallon sizes. However, they are becoming increasingly difficult to find, as most manufacturers have stop producing them. As an alternative, you can use either light-colored, lined or waxed-paper containers, or plastic containers, providing they are well ventilated. Simply cut holes in the container or its lid and cover the holes with a fine-mesh screen (Fig. 67). Untreated paper bags (lunch bags) work well for transporting agents across short distances. However, they are fragile and offer little physical protection for the agents within, and you must take care to seal them tightly to prevent the agents from escaping. Do not use glass or metal containers; they are breakable and make it difficult to regulate temperature, air flow, and humidity.

Fill the containers two-thirds full with fresh leafy spurge sprigs before adding either *Apthona* flea beetles, *Oberea erythrocephala* adults, or *Hyles euphorbiae* larvae. This plant material will provide food, a substrate for resting and hiding, and help regulate humidity. Leafy spurge sprigs should be free of seeds, flowers, and any hitchhiking insects. Do not place leafy spurge sprigs in water-filled containers; if the water leaks it will likely drown your agents. If you're shipping *Spurgia esula* galls, loosely wrap the galled stems in a plastic bag before placing them in a container.

Seal the container lids either with masking or label tape. If you are using paper bags, fold over the tops several times and staple shut.

Be sure to label each container with at least the biological control agent(s) name, the collection date and site, and the name of the person(s) who did the collecting.

Transporting leafy spurge biological control agents

Keep the containers cool at all times. If you sort and package the agents while in the field, place the containers in large coolers with frozen ice packs. Do not use ice cubes unless they are contained in a separate, closed, leak-proof container. Wrap the ice packs in crumpled newspaper or bubble wrap to prevent direct contact with containers. Place extra packing material in the coolers to prevent the ice packs from shifting and damaging the containers. Always keep coolers out of the direct sun, and only open them again



Figure 66. Unlined paper cartons used to store and transport leafy spurge biological control agents. USDA-APHIS.



Figure 67. Examples of vented plastic containers used to store and transport leafy spurge biological control agents. USDA-APHIS.

either when you are ready to remove the containers to place them in a refrigerator for overnight storage, or to release the agents.

If you sort and package your agents indoors, keep them in a refrigerator (no less than 4.5°C, or 40°F) until you transport or ship them.

Transporting short distances

If you can transport your biological control agents directly to their release sites within 3 hours after collecting them, and release them the same day or early the next, you need not take any measures other than those already described.

Shipping long distances

You might need to use an overnight delivery service (USPS, FedEx, UPS, or DHL) if your release sites are far from your collection sites, or you have to deliver your biological control agents to several sites. In such cases, the containers should be placed in insulated shipping containers with one or more ice packs, depending on the size of the packs. Some shippers have specially designed foam with pre-cut slots to hold ice packs. This construction allows cool air to circulate, but prevents direct contact between the ice and the containers (Fig. 68). Laboratory and medical suppliers sell foam “bioshippers” that are used to transport medical specimens or frozen foods (Fig. 69). If neither foam product is available you can use a heavy-duty plastic cooler.

Careful packaging is very important, regardless of the shipping container you use. Ice packs need to be wrapped in crumpled newspaper, wrapping paper, or bubble wrap, and should be firmly taped to the inside walls of the shipping container to prevent them from bumping against and possibly crushing the containers during shipping. Empty spaces in the shipper should be loosely filled with crumbled or shredded paper, bubble wrap, packing peanuts, or other soft, insulating material. Use enough insulation to prevent agent containers and ice packs from shifting during shipment, but not so much that air movement is constricted. Tape the container lids shut. Enclose all paperwork accompanying the agents before sealing the shipping container. For additional security and protection you may place the sealed shipping containers or coolers inside cardboard boxes.



Figure 68. Custom shipper designed to hold two one-quart paper cartons containing leafy spurge biological control agents. A frozen ice pack between the cartons keeps the agents cool during shipment. USDA-APHIS.



Figure 69. A large “bioshipper” used to transport many paper cartons containing leafy spurge biological control agents. The box is lined with foam panels to maintain cool temperatures. USDA-APHIS.

Other factors to consider

- Make your overnight shipping arrangements well before you collect your biological control agents, and make sure the carrier you select can guarantee overnight delivery.
- Plan collection and packaging schedules so that overnight shipments can be made early in the week. Avoid later shipments that may result in delivery on Friday, Saturday, or Sunday.
- Clearly label the contents of your containers and specify they are living insects.
- Check with a prospective courier to make sure that they can accept this type of cargo and will not X-ray or otherwise treat the packages in ways that could harm the biological control agents. If the courier cannot guarantee that such treatments will **not** occur, choose a different carrier. Contact personnel at the receiving end, tell them what you are shipping and when it is due to arrive, verify that someone will be there to accept the shipment, and instruct them not to open or X-ray the containers.
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Regulations pertaining to leafy spurge biological control agents

- **U.S.A., intrastate.** Generally, there are few if any restrictions governing collection and shipment of biological control within the same state; however, you should check with your state's department of agriculture or agriculture extension service about regulations governing the release and intrastate transport of your specific biological control agent.
- **U.S.A., interstate.** The interstate transportation of biological control agents is regulated by the U.S. Department of Agriculture (USDA), and an approved permit is required to transport living biological control agents across state lines. You should apply for a Plant Protection Quarantine (PPQ) permit as early as possible, ideally at least six months before actual delivery date of your biological control agent. You can check the current status of regulations governing intrastate shipment of weed biological control agents and obtain the permit application form PPQ Form 526 (Appendix I), at the USDA-APHIS-PPQ Website at <http://www.aphis.usda.gov/ppq/permits/>.
- **Canada.** Canada requires an import permit for any new or previously released biological control agents. Permits are issued by the Plant Health Division of the Canadian Food Inspection Agency. Redistribution of leafy spurge biological control agents within a province is generally not an issue; however, you should consult with provincial authorities and specialists prior to moving biological control agents across provincial boundaries. More information on spurge biological control agents and their biology is available online at: http://res2.agr.gc.ca/lethbridge/weedbio/index_e.htm#toc. (See also, DeClerk-Floate et al. 2006.)

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Purchasing leafy spurge biological control agents

A number of commercial suppliers provide leafy spurge biological control agents. County weed managers, extension agents, or university weed or biological control specialists may be able to recommend one or more suppliers. Make sure that a prospective supplier can provide the biological control agent you want, and can deliver it to your area at a time appropriate for field release. (You may want to know where and when the agents were collected.) Interstate shipments of leafy spurge biological control agents by commercial suppliers also require a USDA permit (see box above). Determine in advance whether you or the shipper is responsible for obtaining the permit.

Releasing leafy spurge biological control agents

The most important consideration is to release biological control agents as soon after collection as possible. Ideally, that would be immediately after you receive them, or at least during the same day. If you must delay the release, store the containers in a refrigerator at 4 to 10°C (40° to 50°F). Do not freeze the containers.



Figure 70. A painted steel fence post used to mark an *Aphthona nigricutis* release in Montana. USDA-APHIS.

General Guidelines for release

Permanently mark a release point. Use steel fence posts or plastic or fiberglass poles at least 1.2 meters (4 feet) tall as markers (Fig. 70). Avoid wooden posts; they are vulnerable to weather and decay. Markers should be colorful and conspicuous—bright orange, pink, or red, not yellow and green, which may blend into surrounding vegetation. If tall, conspicuous posts are not practical or suitable at your release site, because of too much human or large animal traffic or a high risk of vandalism, etc, mark your release sites either with short, colorful plastic tent or surveyor’s stakes, or steel plates that can be etched with release information and located later with a metal detector and GPS.

Record the latitude and longitude at the release point from a GPS unit. This should be done as a complement to, rather than a replacement for, a physical marker, and will help locate release points if markers are damaged or removed.

Prepare a map that describes access to your release site, including roads, trails, and relevant landmarks. The map should be a complement to, not a replacement for, a physical marker. It will be especially useful for

a long-lived project, in which more than one person will be involved or participants are likely to change. Maps are very important if release sites are in remote locations that are difficult to access.

Complete relevant paperwork at the release site before, or just after, releasing biological control agents. Your agency may have forms for you to fill out. Typically, the information you would provide would include a description of the site’s physical location, including GPS-derived latitude, longitude, and elevation coordinates; a summary of its biological and physical characteristics; the names of the biological control agent(s) released; date and time of the release; weather conditions during the release; and the names of the person(s) who released the agents. The best time to record this information is while it is fresh; don’t wait until you are back in the office to do it.

It is better to release as many individuals of an agent as you can at one site, than spread those individuals over two or more sites.

Photograph the release site either just before, or while you are releasing, the biological control agent. These photos will establish the pre-release condition of the leafy spurge infestation and other vegetation, and later can be compared to photographs taken at various “post-release” intervals. The

comparisons will provide a quick and descriptive way to qualitatively assess the impacts of the released agents (Fig. 71). Pre- and post-release photographs should be taken from roughly the same place and at the same time of year. Make sure each photograph includes your release point marker. When possible, include fixed reference points or landmarks, such as trees, large rocks, fence posts, etc, in the photos.

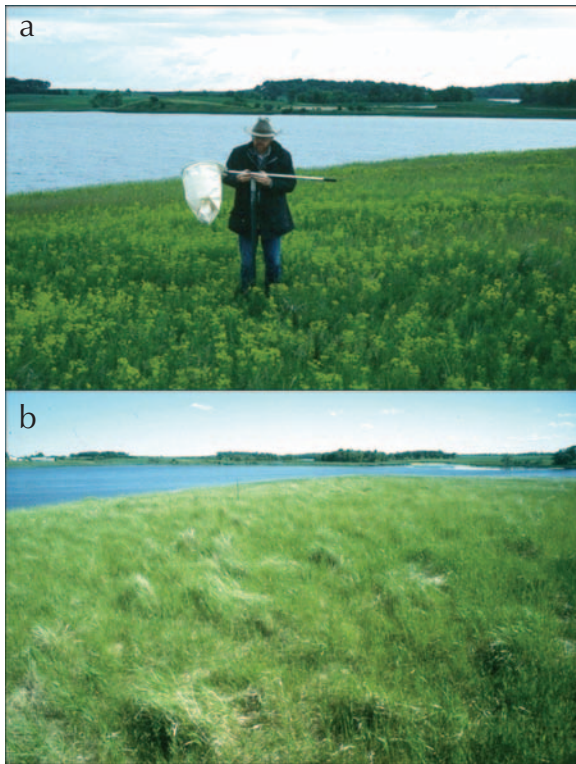


Figure 71. Photos of an *Aphthona nigriscutis* release site in Minnesota: a) before agent release; b) four years after beetle release. Note the steel fence post marking the release point. USDA-APHIS.

Release as many agents as you can. In reality, there is probably no maximum number of biological control agents that should be released; in other words, you can never release too many insects! So, as a general rule of thumb, it is better to release as many individuals of an agent as you can at one site, than spread those individuals over two or more sites. This will help ensure that adequate numbers of males and females are present for reproduction, and reduce the risks of inbreeding and other genetic problems. Guidelines for a minimum release size are uncertain for most agents. For *Aphthona* flea beetles, a minimum release of 1,000 beetles is recommended.

Unlike release strategies for *Aphthona* or *Oberea* beetles, you should scatter *H. euphorbiae* larvae over a broader area around a marked release point, rather than place them as a group.

Leafy spurge flea beetles, *Aphthona* spp.

Do not hold containers of beetles for more than a day or two. If possible, release the insects from mid-morning to late afternoon on warm, sunny days. Avoid making releases on cold or rainy days. If you encounter an extended period of poor weather, it is better to release the flea beetles than wait three or more days for conditions to improve.

Flea beetles should be released in a group at the marked release point, rather than scattered across a site. This will allow *Aphthona* adults to congregate and find mates. To release them, gently shake out the contents of containers over a clump of spurge stems.

Leafy spurge root-boring beetle, *Oberea erythrocephala*

Generally, *Oberea* adults should be released in one group near a marked release point rather than scattered over a larger area. Gently shake a container of beetles over one or two patches of spurge shoots; you may have to gently remove stragglers from the container with a forceps. Make sure no released beetles cling to your clothing as you leave the site. As with flea beetles, you should release root-boring beetles from mid-morning through late afternoon on warm, sunny days. Avoid periods of heavy rain. You can hold *Oberea* beetles for 1 or 2 days during poor weather conditions; however, if conditions do not improve, release them anyway.

Leafy spurge bud gall midge, *Spurgia esulae*

Each bundle of galled stems should be placed in a hole in the soil that is slightly wider than, but not quite as deep as, the bundle is long, or about 13 to 15 centimeters (5 to 6 inches) deep, with the *Spurgia* galls slightly above ground level. This will allow any mature larvae that exit the galls to enter the soil for pupation. Before adding the galls, water the holes thoroughly to provide moisture for the bundles. Place the gall bundles in the holes and gently fill the holes with soil to hold the bundles upright. Individual bundles should be “planted” about 20 to 25 centimeters (8 to 10 inches) apart, near a marked release point. If possible, select a release point that has at least a few taller leafy spurge plants to provide some shade for the galls and retard desiccation. If you are able to return to the site the day after release, gently water the soil around the gall bundles, especially if the weather is hot and dry.

Spurgia esulae larvae and pupae in galls are relatively fragile and short-lived, so release gall bundles as soon as possible. Weather conditions and time of day are not as important as prompt field release. In fact, cool and overcast conditions are probably optimal. The only conditions to avoid during or shortly after release are heavy downpours. If you must, you may hold bundles in a refrigerator for up to 24 hours before release.

Leafy spurge hawk moth, *Hyles euphorbiae*

Unlike release strategies for *Aphthona* or *Oberea* beetles, you should scatter *H. euphorbiae* larvae over a broader area around a marked release point, rather than place them as a group. This will allow plenty of food for each foliage-feeding larva, and compensates for their relatively poor innate abilities to disperse. Mid-morning releases on warm, sunny days are best. Avoid releasing agents during, or shortly before, heavy rains. Caterpillars need to be released quickly upon receipt, so weather conditions during release are less critical than timing. By hand or with a flexible forceps, gently place one or two large caterpillars, or five to ten smaller larvae, on spurge plants. Make subsequent caterpillar “plantings” about 1 to 1.5 meters (3 to 5 feet) apart in a fairly random pattern around the marked release point. Larger *Hyles* caterpillars may regurgitate a harmless, thick, green liquid when handled.

4. Documenting, monitoring, and evaluating your biological control program

Why monitor biological control releases?

Weed biological control does not result in the complete elimination of the targeted weed. Therefore, success must be measured by how well the biological control agents reduce the targeted weed to densities near or below a pre-determined threshold. Measurement of the status of the weed population, in relation to this threshold, will determine if your efforts have been successful. The effects of biological control agents take much longer to appear than those of herbicide and mechanical management strategies, and at least several years to have full impact on the targeted weed.

Documenting the outcomes (both successes and failures) will help create a more complete picture of biological control impacts, guide future management strategies at your and others' sites, and might serve a public relations function, as well. Documenting the initial conditions, coupled with data from periodic evaluations of the agent's establishment and impact, can indicate whether or not the program is working as desired, or if additional releases of the same or different species are needed. Likewise, it can provide critical information for other land managers elsewhere which can help them predict where and when biological controls might be successful.

The need for documentation

The value of monitoring and evaluation efforts will be greatly enhanced if the information you collect is recorded and is accessible by other land managers and researchers. Institutional memory is short, and documentation of initial conditions, release locations, successes and failures will provide critical information to those who will follow you. At the very least, it should help others avoid releasing biological control agents that don't work, and concentrate on those that do. Publicly accessible information on release locations, sizes, and outcomes can be extraordinarily useful information for biological control researchers and policy makers. Documenting successes and failures can help prioritize future research and collection efforts. Finally, other land managers need to know the location of your releases so that they can avoid engaging in activities, such as cultivating, mowing, and applying herbicides, that would harm your biological control agent populations.

Many federal and state agencies have electronic databases of information about biological control releases. We have included a standardized biological control agent release form and an example of a corporate database form, which, when completed, should provide sufficient information for inclusion in any number of databases (see Appendix C).

At the Federal level, the Animal and Plant Health Inspection Service (APHIS) maintains the Cooperative Agricultural Pest Survey (CAPS) database, which is part of the National Agricultural Pest Information System (NAPIS) (<http://ceris.purdue.edu/napis/>). Biological control release information can be entered into CAPS by a number of state and federal agency personnel who serve on the state's CAPS survey committee. Contact your local APHIS officials or state department of agriculture for more information.

The USDA Forest Service maintains a database that can store information on biological control agent releases on federal and non-federal lands. As of the writing of this document, biological control releases made on Forest Service lands should be entered into the FACTs database, and the infestation of the targeted weed should be entered into the TERRA database. Other agencies may maintain their own databases for this information. Many of the databases maintained by state and federal agencies have some safeguards in place to prevent undesirable uses of the information they contain.

For any weed biological control program, pre- and post-release monitoring is critical to determine if management goals have been achieved. Information on both biological control agent populations and the status of leafy spurge are collected during monitoring.

Populations of biological control agents

- Are biological control agents established at the release site?
- Are biological control agent populations increasing in size?
- How far beyond the initial release point(s) at a given site have biological control agents colonized?
- Has the population of biological control agents built up enough to be collected from the site and moved to other areas?

Status of leafy spurge and other plants

- Are the biological control agents causing damage to the target leafy spurge plants and/or nontarget vegetation?
- Has there been a change in the leafy spurge population since introducing the biological control agents?
- Has there been a change in desirable vegetation at the release site?
- Is there a change in undesirable plants, such as other noxious invasive exotic weeds, at the release site?

To address these questions, monitoring activities must be focused on biological control agents, their impacts (damage) on individual leafy spurge plants, the leafy spurge population, and the rest of the plant community in the vicinity of the release.

Assessing leafy spurge biological control agent populations

All biological control agents go through a population cycle of gradual increase, peak, and decline during the season. It is easier to assess insect establishment when populations are peaking, so we recommend you make multiple visits to a site throughout the season and sample when populations appear highest. For some biological control agents degree-day models (e.g. Legg *et al* 2004) are available that estimate the time to peak emergence based on local weather conditions and may allow you to better target your sampling with a single visit.

General agent surveys

If you wish to determine whether or not a leafy spurge biological control agent has become established after initial release, you may simply need to find the agents themselves and/or evidence (plant damage) of their presence.

Finding leafy spurge biological control agents

The easiest way to confirm biological control agent establishment in the years following release is to find one or more of the insect's life stages at the release site (Table 9). Begin looking for agents where they were first released. If you do not find any, continue to explore the area around the release site. Sometimes agents do not like the area where they were released and move to other patches of leafy spurge (see Appendix E).

Table 9. Life stages to look for when determining establishment of leafy spurge biological control agents.

Agent	Life stage	What to look for	When to look
Leafy spurge flea beetles, <i>Aphthona</i> spp.	Adult	Small beetles congregating and feeding on spurge foliage and stems.	Mid-summer (June through August, depending on location).
	Larvae	Small, white or yellowish larvae in, on, or near larger spurge roots and root buds.	Spring (April to June, depending on location).
Leafy spurge root-boring beetle, <i>Oberea erythrocephala</i>	Adult	Medium-sized beetles with long antennae, on the tops of spurge shoots or flying nearby.	Mid-summer (June through August, depending on location).
	Larvae	Cavities in root crown or largest lateral roots that contain legless, cream-colored larvae.	Spring, or almost anytime during the year (especially in northern areas).
Leafy spurge bud gall, <i>Spurgia esula</i>	Larvae and/or pupae	Very small orange maggots (larvae) or white silken cocoons (pupae) inside galls.	Spring through mid-summer.
Leafy spurge hawk moth, <i>Hyles euphorbiae</i>	Larvae	Large, brightly-colored caterpillars on spurge foliage and stems.	Mid- to late summer (June through September).
	Adults	Large moths hovering hummingbird-like near spurge plants or flowers of other plants.	Mid-summer.
Leafy spurge root-boring moths, <i>Chamaesphecia</i> spp.	Adults	Small day-flying moths on or near upper portions of spurge shoots.	Uncertain; probably June and July.

Leafy spurge flea beetles, *Aphthona* spp.

Adults are the easiest life stage to find. You can find them simply by examining leafy spurge plants at the release site, but this is often difficult when numbers are low. A more efficient way to find flea beetles is with a sweep net (Fig. 72). Starting at the marked released point, walk slowly through the leafy spurge stand, vigorously sweeping back and forth through the weed canopy. Examine the net contents after five to ten sweeps. If flea beetles are present, save a few as voucher specimens (see “Sweep net sampling,” page 74). Empty the net contents and resume sweeping, moving out from the release point in a widening circle.

You will have to look for flea beetle larvae if you are unable to visit the site when adults are present. The best time to do this is in the spring, when larger, mature larvae are preparing to pupate. Begin looking for larvae near the initial release point. Gently pull out or dig up some leafy spurge roots, making sure that at least some lateral roots and root buds are extracted. Examine the root material for the small whitish larvae; they may be feeding on the surface or



Figure 72. Using a canvas sweep net to sample *Aphthona* spp. adults. USDA-APHIS.

may be “mining” roots or buds (Fig. 73). Because larvae removed from the soil will probably not survive, limit this type of sampling during the first year after release, especially if the number of beetles initially released was small. Unfortunately, individual *Aphthona* species cannot be identified from the larval stage.

Phenological events in several plant species may serve as general but reasonably accurate indicators of peak agent abundance in the field, *Aphthona czwalinae*/*A. lacertosa* (mixed populations) and *A. nigriscutis*, would coincide with peak flowering of the native plants silvery lupine (*Lupinus argenteus*), Wood’s rose (*Rosa woodsii*), or common yarrow (*Achillea millefolium*). Peak abundance of leafy spurge flea beetle adults, *A. cyparissiae* and *A. flava*, would coincide with peak flowering of the exotic weed Saint Johnswort (*Hypericum perforatum*) and peak ripeness of fruits of the native shrub service berry (*Amelanchier alnifolia*) (Hansen 1999, and Hansen unpublished date).

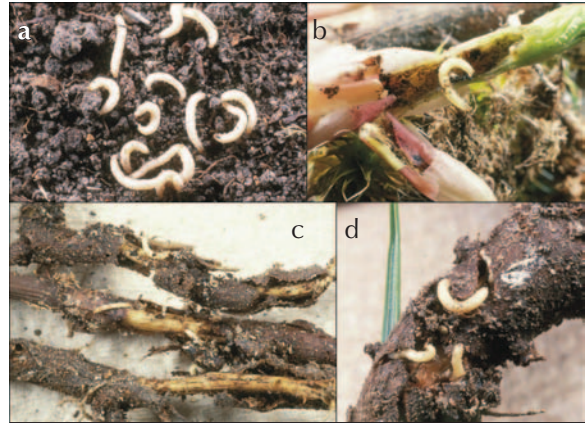


Figure 73. *Aphthona* spp. a) larvae in soil; b) feeding on leafy spurge root buds; c - d) feeding on leafy spurge roots. USDA-APHIS.

Leafy spurge root-boring beetle, *Oberea erythrocephala*

Adults are the easiest life stage to find, but they are flighty insects and often difficult to locate when numbers are small. Even when populations are established, this agent often persists at low numbers for at least several years following release; adults may be difficult to find and collect. You may find individual beetles by scanning the tops of leafy spurge stems during the summer, but the most efficient way to collect *Oberea* beetles is with a sweep net. Follow the procedures described above for leafy spurge flea beetles and concentrate your sweeping in the upper part of the leafy spurge canopy.

Finding larvae is even more difficult than finding adults. Look for spurge plants that have some evidence of root damage, such as stunted growth or foliar wilting. Gently pull out or dig up leafy spurge roots and slice open the root crown and large lateral roots to expose the larvae.

Leafy spurge bud gall midge, *Spurgia esulae*

Adult flies are very small, short-lived insects and are almost never seen in the field. The best way to document establishment of *S. esulae* is to find galls (see “Leafy spurge bud gall midge, *Spurgia esulae*,” page 74). The small larvae or pupae (in cocoons) may be found by gently pulling apart the gall “leaves.”

The completion of flowering by the introduced shrub common lilac (*Syringa vulgaris*) can be an indicator of peak first-generation pupal abundance for the leafy spurge bud gall midge. Thus, the optimal time for collecting this agent for distribution would be when lilacs are in full bloom. (Hansen 1999, and Hansen unpublished data).

Leafy spurge hawk moth, *Hyles euphorbiae*

The distinctive large, colorful, mature *Hyles* larvae (caterpillars) are easy to spot when they are feeding on leafy spurge foliage or resting along a spurge stem (see Fig. 57, page 57) . Young caterpillars are smaller and less colorful, but are often quite conspicuous, especially when groups are feeding on a single spurge stem. Adult moths are quite mobile, so you might find larvae some distance from the initial release point.

Hyles adults are large, colorful moths that are strong, fast fliers (Fig. 74). Moths are active during the day, and can be seen hovering over leafy spurge plants or at various flowers, feeding on nectar. However, other moths similar to *Hyles* in size, color, and behavior also fly during the day. So unless you can confidently identify the adults, the presence of moths alone may not be a reliable indicator of an established *H. euphorbiae* population.



Figure 74. *Hyles euphorbiae* adult. USDA-APHIS.

Leafy spurge root-boring moths, *Chamaesphecia* spp.

As of this writing, no established populations of these moths have been documented in the U.S., and no survey and sampling methods have been developed. If they were to be observed, adult moths would be the most likely life stage targeted for assessing establishment. These moths are generally active during the day, especially just before dusk. An examination of the tops of leafy spurge plants, or gentle sweeping through the leafy spurge canopy, may detect *Chamaesphecia* adults.

Identifying plant damage caused by leafy spurge biological control agents

The first indication that an agent has established may be visible plant damage. You may not be able to find the biological control agents themselves, but can find symptoms of their presence including: oviposition scars, where adults laid their eggs; missing or chewed foliage; and wilting stems which, when dissected, are filled with frass, a combination of chewed plant material and insect excrement. The symptoms will vary with the agent involved.

Leafy spurge flea beetles, *Aphthona* spp.

The earliest sign of leafy spurge flea beetle damage is injury to leafy spurge foliage caused by feeding adults (Fig. 75). When numbers are small, feeding damage often consists of small “shot-holes” on the leaves or rounded



Figure 75. Close-up views of adult *Aphthona* spp. feeding on leafy spurge leaves and inflorescences. USDA-APHIS.

“chunks” removed from leaf edges. As populations increase, adult feeding can result in the partial or complete defoliation of leafy spurge stems; remaining foliage typically has a ragged, grayish appearance (Fig. 76).

Eventually, large *Aphthona* populations may cause leafy spurge mortality. Characteristically, dead plants appear in “circles”, with dead, grey-brown stems remaining upright within the circle for some time (Fig. 77). Most living leafy spurge shoots around the edge of a circle will be stunted, and exhibit the feeding damage described above. During the summer, most adult flea beetles will be found at a circle’s edge.



Figure 76. Feeding damage on leafy spurge foliage by *Aphthona* spp. adults. USDA-APHIS.



Figure 77. Early, localized leafy spurge mortality caused by *Aphthona* spp. flea beetles, proceeding in a generally circular pattern from the initial release point (note marker). USDA-APHIS.

Leafy spurge root-boring beetle, *Oberea erythrocephala*

Oberea adults may feed on leafy spurge foliage or stems, but the damage is very difficult to detect or distinguish from casual feeding by other insects. You may be able to detect shoot damage caused by beetle oviposition, which is fairly characteristic for this agent (Fig. 78). An adult female girdles the upper part of leafy spurge stem by chewing around, but not completely through, the stem. It then chews a hole into the stem above the girdling damage and lays a single egg in that hole. The girdling often leads to a copious flow of white latex from the wound, though this may dry and disappear with time. Additionally, the portion of the stem above the girdling usually wilts or dies, a condition sometimes called “flagging.”



Figure 78. Leafy spurge stem damage caused by adult *Oberea erythrocephala* feeding. a.) stem girdling and resulting latex flow; b.) stem mortality above girdling and oviposition injury. USDA-APHIS.

Leafy spurge bud gall midge, *Spurgia esulae*

The only reliable indicator of the presence of the gall midge is the plant damage that it causes, i.e., the galls (Fig. 79). *S. esulae* galls are typically cabbage- or pineapple-shaped. Gall size varies from generally ranging from 1 to 3 centimeters (0.4 to 1.2 inches) long



Figure 79. Terminal stem galls caused by *Spurgia esulae*. USDA-APHIS.

and 0.5 to 2.5 centimeters (0.2 to 1 inch) wide. During the first (spring) generation, almost all galls are found at the tips of spurge shoots. Later, galls may be found on lateral branches. Typically, tight, light-green galls indicate that *S. esulae* larvae are still present within.

After the insect completes development, galls may persist but they open up and gall “leaves” begin to fall off. These older galls have a bleached appearance and are usually yellowish, tan, or grey.

You should begin your search for galls near the initial release point; however, it is quite possible that you will find galls some distance away, because *Spurgia esulae* adults are small insects and are easily dispersed by wind, often over long distances.

Leafy spurge hawk moth, *Hyles euphorbiae*

When populations are high, *Hyles* caterpillars may completely defoliate leafy spurge plants, leaving behind characteristic light-green “sticks” (stems) bearing almost no leaf material. This damage signature distinguishes *Hyles* from *Aphthona* adults which typically leave behind some dead, grayish leaf material, giving the stems a ragged appearance.

Quantifying populations of leafy spurge biological control agents

There are many possible ways to sample insect populations and make some quantitative estimates of population size. Several that have been used with leafy spurge biological control agents are described below. These have primarily been developed for leafy spurge flea beetles (*Aphthona* spp.), but they could be adapted for other leafy spurge agents. One type of sampling uses sweep nets and estimates population sizes based on the number of beetles netted per sweep. The other type attempts to collect all insects in a fixed area, resulting in an “absolute” population estimate for a specified unit area.

Sweep net sampling

USDA-APHIS-PPQ sweep net sampling protocols for adult leafy spurge flea beetles (*Aphthona* spp.) and leafy spurge root-boring beetle (*Oberea erythrocephala*). Many biological control agents spread out from the release point in an expanding circle. Therefore, you should start your post-release sampling efforts at your release point marker. Use a standard, 38-centimeter (15-inch) diameter canvas sweep net to collect agents. Do five sweeps along four lines projecting outward from the release point to the north, south, east, and west, for a total of 20 samples. For each line, begin as close to the release point as possible. Make four net sweeps in front of you, back and forth twice in roughly a 120° to 180° arc (see Fig. 72, page 70). Each sweep should proceed in a downward arc, so that the net moves vigorously through the vegetation as close to the ground as possible. Carefully examine the net and count the biological control agents

present, then empty the net to release counted insects. Record the number counted on a sampling form (see Appendix J). Move 2 meters (6.5 feet) out and repeat the sweeping at the next sampling point. You can estimate this distance by determining how much distance you can cover in with a single step. At normal walking speed 2 to 2.5 paces is about 2 meters (6.5 feet). Continue until you have sampled five points on a line, then repeat over the remaining lines.

It is a good idea to collect voucher specimens of the biological control agents you are sampling, especially during the first several years after release. These specimens can be used to confirm the identity of the agents. At some point during the sweeping process, collect 10 to 15 agents from the site and place them in 70 percent ethyl alcohol in a clear glass vial. If ethanol is not available, you may use isopropyl alcohol (rubbing alcohol). Place a label inside the vial, with the specimens. The label should be a small piece of paper listing release site location, sampling date, and the name of the person doing the sampling; this should be written in pencil, as most inks will dissolve in alcohol. Keep voucher specimens in a safe place for future reference.

Once agents have established and are starting to spread through the release patch, you may want to broaden your sampling effort to assess the agents' impact across the entire release patch. Use the sweeping technique described above and conduct your sweeps at specific points within fixed vegetation transects within the release patch (see "AAFC vegetation sampling protocols," page 79). Record the beetle densities at these points and compare them with changes in leafy spurge vegetation densities.

Vacuum sampling (estimating agent populations per unit area)

Agriculture and ArgiFood Canada (AAFC) sampling protocols for adult leafy spurge flea beetles (*Aphthona* spp.)

A garbage can with the bottom removed is used to delimit a fixed area. For example, for a bottom diameter of 41.5 centimeters (16.3 inches), the circumscribed area is 0.135 m².

Place the can over vegetation at a sampling point, and vacuum inside the can for 45 seconds using a modified leaf blower. Vacuuming vigorously, make sure to include the leafy spurge shoots, the sides of the can, and the ground around the plants, where beetles may have jumped or fallen. Remove the collected beetles from the fine-mesh stocking in the vacuum tube and place them in containers on ice for later counting in the lab. (This method is described in more detail in Kalischuk *et al.* 2003.) You can use the USDA-APHIS-PPQ sweeping protocol (above) to establish your vacuum points, i.e. every 2 meters (6.5 feet) out from a release point in each of the four cardinal directions. Or, as an alternative you can spread your sampling over the grid of vegetation transects to pair estimates of beetle densities with changes in leafy spurge densities (see "Monitoring vegetation: assessing the status of leafy spurge and other plants," page 77).

Vacuum sampling provides an estimate of the density of insect per unit area; sweep net sampling provides an estimate the sampling per unit effort. Vacuum sampling takes longer than sweeping but it is easier to standardize efforts among different samplers. Also, vacuum sampling may be less sensitive than sweep netting to weather conditions at the time of sampling. If it is cold beetles will not be sitting on the vegetation and available for sweeping, resulting in artificially low counts. That said, sweep sampling will be adequate for most people trying to assess establishment at a site.

(A slight variation in this methodology has been employed by USDA-APHIS-PPQ. A gasoline-powered Echo® PB-400 backpack leaf blower was modified to vacuum material into a collection basket attached to the suction tube (Fig. 80). The vegetation sampling area was confined using a 64 centimeters (25 inches) length of metal stove pipe, 20.3 centimeters (8 inches) in diameter; this confined a surface area of 0.03 m². Contents of the collection basket were examined at each sampling, and all collected *Aphthona* beetles were counted in the field and released. Twenty sample points were utilized, five along transects radiating in each cardinal direction from the initial release point as described above for sweep-net sampling.)

Quadrat sampling method for leafy spurge bud gall midge (*Spurgia esulae*) and leafy spurge hawk moth (*Hyles euphorbiae*).

These agents cannot readily be sampled using the sweep net and vacuum methods described for *Aphthona* flea beetles. You must use a quadrat sampling method, which will help you estimate the population size per unit area. Establish a linear transect in a leafy spurge infestation that is centered at the agent release point marker. Ideally, the transect should follow a given contour line across any slope that is present, rather than proceed uphill and downhill from the release point. Establish individual sampling points at regular intervals in each direction from the release point; possible intervals might be 2 to 5 meters (2 to 5 yards) or larger, depending on the size of the infestation and the number of sampling points employed. A minimum of 20 sampling points is recommended. At each sampling point, place a quadrat constructed of narrow PVC pipe (1-inch diameter) over the vegetation. Almost any quadrat size can be used, but fairly large ones are suggested; useful sizes include 1 x 1 meter (1 m²) or 1 x 0.5 meter (0.5 m² area). Make sure that quadrat placement relative to the transect (e.g. centered at the sampling point) is the same for all samples. Count all *S. esulae* galls or *H. euphorbiae* larvae found on leafy spurge within the quadrat at each sampling point. Collect a few *Spurgia* galls or *Hyles* larvae as voucher specimens, following the procedure described above for *Aphthona* spp.



Figure 80. Backpack vacuum sampler for collecting *Aphthona* spp. adults, based on a modified Echo[®] gasoline-powered leaf blower. Flea beetles are collected in the plastic basket attached to the vacuum hose. USDA-APHIS.

Monitoring vegetation: assessing the status of leafy spurge and other plants

The ultimate goal of a biological control program is to reduce the abundance of the target weed and enable the recovery of more desirable vegetation on the site. To determine the efficacy of biological control efforts there must be monitoring of vegetation attributes, such as target weed distribution and density, both before biological control efforts are started (pre-release), and at regular intervals after release.

The methods used in pre-release vegetation monitoring should enable land managers to determine later if they are achieving the objectives of the biological control program. Often, land manager use factors such as a reduction in leafy spurge patch size or density to gauge the success of weed management efforts. Pre-release estimates of leafy spurge stem density, flowering and vegetative stems, patch size and patch perimeter at the release sites are frequently measured to enable pre- and post-treatment comparisons.

Land managers may have a goal of changing the structure and composition of the vegetative community through biological control. Pre-release sampling techniques, which allow managers to describe pre-treatment vegetation, are integral to assessing progress towards this goal.

Pre-release monitoring should include the establishment of control plots where no insects will be released. These plots should be as similar as possible in habitat type (same soil type, aspect and exposure) to the release plots. Control sites should be far enough away from release sites so that it is unlikely they will be colonized by biological control agents at least during the monitoring period of the program. For consistency, the same data collection protocols should be used at control and release sites.

In order to measure biological control agent impact accurately, methods for assessing plant densities after biological control agents are released must be the same as the pre-release methods. Post-release assessments should be planned for at least 3 to 5 years after the initial agent release.

There are many ways to qualitatively (descriptively) or quantitatively (numerically) assess leafy spurge populations and other vegetation attributes at release sites.

Qualitative vegetation monitoring

Visual estimates of cover are made and recorded (see Appendix F).

Daubenmire quadrat sampling (USDA-APHIS-PPQ vegetation sampling protocols) (Daubenmire 1959).

In this strategy, one or more linear transects would radiate outward, usually in cardinal directions from the biological control agent release marker. (In large patches, transects may be established anywhere within the patch.) Transects should be established in flat areas or across slopes, such that the sampled areas are roughly the same in elevation and aspect.

The actual number of vegetation sampling points you have will depend on the size and shape of the leafy spurge infestation and how much time you can devote to data collection. However, we recommend a minimum of 20 sampling points, though larger numbers (40 to 50) may provide better long-term data. Place the points along the transects at intervals of 1 to 2 meters (3 to 6.5 feet, with equal numbers on either side of the agent release marker. If possible, permanently mark your sampling points with small metal or plastic stakes. If this is not possible, permanently mark the transect end points, collect your samples at fixed distances from the end points, and record those distances in your data logs.

At each sampling point, place a sampling frame over the vegetation. The sampling frame is a rectangle, 50 x 20 centimeters (20 x 16 inches), which encloses a sampling area of 0.1 m² (320 in²) (Fig. 81). Frames may be of metal, PVC pipe, or wood, with metal being the most durable. Divide the long axis of the frame into four equal segments of 12.5 centimeters (6 inches) and mark the increments with different-colored paints or tape.

Choose a final orientation to the sampling point, and use it consistently for all sampling points each time you sample the vegetation, regardless of whether or not the point is permanently marked. Place the frames with the long axes perpendicular to the transect. To minimize disturbance, walk and stand only on the side of transect opposite from the side on which you placed the frames.

Ideally, you want the frame to rest on the soil surface. This may be somewhat difficult to achieve in dense leafy spurge stands, and you may need to arrange plants so that all material outside the frame is excluded. The key point to remember is that only those leafy spurge stems and other plants that originate within the frame, i.e. that arise from the soil within the frame, are included in the sampling. Exclude all plants that originate outside the frame.

Collect the following data for each frame:

- **Weed Density**—The total number of leafy spurge stems (weed density) entirely within the frame. You may count all stems present, including seedlings, or limit the count to a given size range of stems (e.g. stems taller than 15 centimeters [6 inches]). As an alternative, you may wish to organize your stem counts by categories, such as seedlings and mature stems. Each method will work, but neither will yield valid results unless you apply it consistently.
- **Average Height**—The typical or average height of leafy spurge stems within each frame.



Figure 81. Rectangular “Daubenmire” sampling frame (0.1-m²) used to assess vegetation changes associated with releases of leafy spurge biological control agents. USDA-APHIS.

- Cover Values**—Represent the relative amount (percentage) of the space contained within the frame, from the soil surface up, that is occupied by a given plant category. Typically, these values are recorded for leafy spurge, other forbs, grasses and grass-like plants (sedges), trees and shrubs, plant litter, and bare soil (Table 10). You may refine the categories to reflect other criteria, such as local management goals, or the varying levels of expertise among those doing the collecting to identify the plants. For example, you might want to subdivide the categories, “other forbs” and “grasses and grass-like plants,” into “native plants” and “exotic plants.” Or, you might want to track all individual plant species separately. (See Appendix K for a sample data form).
- Other data**—You might consider collecting are the presence/absence of a given plant species, or the presence/absence of a particular biological control agent on leafy spurge plants.

Table 10. Standard six class rankings used to describe vegetative cover during vegetation monitoring.

Cover class (score)	Percent (%) cover (range)
1	0 to 5
2	5 to 25
3	25 to 50
4	50 to 75
5	75 to 95
6	95 to 100

Note: classes may overlap, such that cover range may not add up to 100%.

AAFC vegetation sampling protocols; release stake scale, release patch scale.

You can assess the impact of biological control agents, primarily *Aphthona* beetles, at a low intensity release-stake scale and/or a higher intensity release-patch scale.

Release stake scale

This is a qualitative (descriptive) assessment and suitable for determining the impact of a newly established population of beetles. The focus of the assessment is to record the presence/absence at the release site of a “halo” of dead leafy spurge, a characteristic of established leafy spurge beetles. You would do this assessment on four transects, one for each cardinal direction, at intervals of 1, 3, 5, and 10 meters (3.2, 9.7, 16.2, and 32.4 yards) from the release point, and summarize the damage as:

- 1=No evidence of spurge thinning.
- 2= Some thinning to vegetative stems and some flowering stems.
- 3= Thinning to stems only.
- 4= Dead spurge with very few vegetative stems.

(Note: A halo does not always expand uniformly in all directions. For example, beetles tend to move up slope so halos tend to be head towards the tops of hills rather than down the slopes. If the beetles find the area around the stake where they were released to be unsuitable, they will likely leave the area entirely, and establish themselves elsewhere within the patch.)

Release patch scale

Sampling on release-patch scale occurs on a permanent transect grid that is designed to capture the clumped beetle distributions and subsequent leafy spurge impact that can occur within an entire release patch. If resources are available, your best approach would be to set up transects at the time of the release, because this will enable you to observe and record the changes in leafy spurge densities at the patch scale, later.

Set up the first transect running along the long axis (up to 50 meters (55 yards)) of the patch.

Create a sampling grid over the release patch by placing five, 20-meter (22-yard) cross transects at 90° to the long axis, crossing the long transect at 5, 25, 50, 25, and 5 percent of the patch area.

Place sample transects as a percentage of the long transect, such that samples between sites are comparable, even if long transects vary in length because of patch sizes. This transect grid contains between 100 and 150 points at 3-meter (3-foot) intervals, along the six transects for sampling.

Lay out a field tape with 1-meter (3-foot) increments along the long transect, and a similar tape on one of first of the crossing transects (5 percent).

Starting on the long transect, use a 20- x 0.5-cm (10- x 1/8-in) sampling slice to record the presence or absence of leafy spurge stems at 1-meter (3-foot) intervals. We use a wheel sampler that rolls at 1-meter (3-foot) intervals and pins the spurge stems down (Fig. 82). An L-shaped stick with a blade 20 centimeters (10 inches) long also works well. Place it on the ground at each point and record the number of leafy spurge stems it touches.

One simple way to measure change in leafy spurge patches is to compare the number of leafy spurge stems counted at each point from year to year.

You might also wish to categorize leafy spurge stems at each point. For example, you might divide your counts into old and new stems, and vegetative and flowering stems.



Figure 82. Wheel sampler used in vegetation monitoring. AAFC.

Depending on the time available to sample, you can either count the actual number of new and old leafy spurge stems that are trapped under each slice, or do a qualitative estimate.

If you use actual stem counts, you can assess the change in leafy spurge density by looking at either the change in the mean number of new stems per meter (39 inches), or the ratio of new stems to old stems. If you use qualitative estimates, categorize the stems at each point as follows:

- Old Stems: 0 = None; 1 = Some; 2 = Mature (can't see soil)
- New Stems: 0 = None; 1 = Light (1-14 stems); 2 = Medium (15-40 stems); 3 = Heavy (>40); 4 = Mat (can't see soil)

- Stem Type: 1 = Dominant type is vegetative; 2. Dominant Flowering; 3. 50/50 split between vegetative and flowering.
- You can assess change by comparing your data from year to year.

Assessing possible impacts of biological control agents on other plants

Sampling of vegetation other than leafy spurge should be included in order to assess the impacts of the biological control program. Specific methods will depend on the species targeted for sampling in particular areas.

Changes in abundance of other desirable or undesirable vegetation

Biological control agent releases can affect the presence and relative abundance of many other plant species even though the agents do not directly utilize them. If biological control works to remove leafy spurge at a site, it will create an empty niche to be filled by alternative, hopefully desirable, vegetation. These indirect effects are the result of changes in leafy spurge abundance. As leafy spurge becomes less abundant, the utilization of site resources is altered; some plants become more abundant, while others become less so. Within the overall management plan for your site, it may be important to document the changes in other vegetation after you release your biological control agents.

Depending on your program goals, you may need to document quantitative and/or qualitative changes for groups of plants, such as native forbs or exotic perennial grasses, or individual species, such as a rare plant or a food plant for a native butterfly. Plant species may be considered beneficial (e.g. native and introduced forage plants) or deleterious (e.g. other invasive weeds). One important management goal should be to avoid invasion of a site by another exotic weed after successful biological control of leafy spurge. That is, you don't want to replace one invasive weed with another. For this reason, we strongly recommend you monitor populations of other exotic weeds that are known to be problematic in your area.

You will need to clearly define site management goals and become familiar with the plant communities at your release location and nearby sites. You can easily modify the vegetation sampling procedures described above to monitor changes in density and/or cover for leafy spurge as well as other plant species, both before and after you release your biological control agents.

Direct impacts of biological control on nontarget plants

The host ranges of all currently available leafy spurge biological control agents are restricted to plants in the genus *Euphorbia* (see Chapter 2). Any concerns about potential utilization of non-target hosts can be restricted to native or other exotic spurges. Most other exotic *Euphorbia* spp. in the northern U.S. and Canada are considered weeds, so non-target feeding on these plants is not an issue. Most of the native spurges are found in the southern U.S. and are unlikely to be sympatric with leafy spurge infestations and releases of biological control agents. Thus, only a relatively few native *Euphorbia* spp. are at some risk of non-target utilization.

The first step in addressing possible non-target attacks on native spurges is to become familiar with the plant communities present at and around your release sites. A visual, pre-release survey may locate native spurges that are present. You may have to consult with a local botanist, if available, for advice on areas where these plants might be growing, what specific habitats they typically utilize, and how you can identify them. Herbarium records at a university or other research institution may provide guidance about the local or statewide distribution of native *Euphorbia* spp.

If you do find one or more native spurges at a potential biological control release site, you should not immediately cancel plans to release agents. The host ranges of the insects used for leafy spurge biological control are restricted within the genus *Euphorbia* subgenus *Esula* (Chapter 1); so native spurges may not be attacked or may experience only very limited feeding. That said, you should be concerned about two types of native spurges:

- Plants that are rare and perhaps listed as threatened or endangered; or
- Plants on which non-target feeding by a given agent has been documented under field conditions elsewhere in the U.S. For example, some feeding by *Aphthona nigricutis* has been observed on the native Rocky Mountain spurge, *Euphorbia brachycera* (= *E. robusta*), in several western states. *Hyles Euphorbia* also has been observed feeding on Rocky Mountain spurge (Fig. 83). However, feeding damage was found not to affect the population densities of this native species at one site (J. L. Baker, personal communication). In these situations, you should determine how much, if any, non-target feeding might be acceptable before you decide whether or not to release biological control agents.

Vegetation sampling procedures can be modified easily to monitor changes in density and/or cover of specific, known native spurge species, both before and after biological control agents are released. Concurrently, you may wish to collect additional data, such as the number of agents observed on non-target spurges, the amount of foliar feeding observed (for *Aphthona* flea beetle adults or *Hyles euphorbiae* larvae), or the presence of galls (for *Spurgia esulae*).



Figure 83. *Hyles euphorbia* on the native Rocky Mountain spurge, *Euphorbia brachycera* (= *E. robusta*). Dave Hanna, Nature Conservancy.

CHAPTER 4: THE BIOLOGICAL CONTROL COMPONENT OF AN INTEGRATED LEAFY SPURGE MANAGEMENT PROGRAM

DR. RODNEY LYM

1. Introduction

Integrated weed management (IWM) is a systems approach to management of undesirable plants. IWM is described in the Federal Noxious Weed Act as a system for the planning and implementation of a program, using an interdisciplinary approach, to select a method for containing or controlling an undesirable plant species or group of species using all available methods including:

- Education
- Prevention
- Biological control (insect or pathogens)
- Physical or mechanical
- Cultural (grazing, reseeding, etc.)
- Herbicides
- General long term land management practices

It is a multidisciplinary, ecological approach to managing unwanted plant species— weeds.

The goal of an effective weed management program is to replace undesirable plants that cause resource, economic, habitat, or aesthetic losses with a plant or plants that are beneficial to the environment.

An integrated and coordinated approach to weed management has two interdependent goals:

- The development of a long term plan that encompasses all land in a designated area, with landowners and managers working together towards effective management.

- The implementation of the most effective weed control methods for the target weed.

A program that integrates biological control methods with other weed control methods is far more likely to succeed, long-term, against leafy spurge than will any single control method used alone.

2. Integrating biological control methods

Classical biological control has been applied to many invasive weed species and there are many examples where both single- and multiple-agent introductions have successfully controlled the target weeds. The use of biological control agents alone to control weeds has been effective in about 30 percent of the attempts, and it may take 20 years or more reduce weed populations to manageable levels (McFadyen 2000). The success rate for classical biological control may increase when multiple species of biological control agent are used, so long as the different species attack the target weed at different locations on the plants or at different times during the growing season, or are released over a larger range of infestation.

Some agents, such as *Aphthona* spp., have successfully reduced leafy spurge densities, but biological control agents have not established in all areas where leafy spurge occurs (Fig. 84). Even when established, biological control agents do not eradicate the target weed. In ideal situations, biological control can maintain leafy spurge densities below economically significant levels. Biological control agents are not going to work every time at every site, and integration with other management tools, or simply using other tools, may be required.



Figure 84. Before and after shots of leafy spurge infestations where *Aphthona* beetles were released USDA-APHIS.

Land managers have learned that, to be successful, leafy spurge management must be cost effective, especially when applied to large infestations, and that because leafy spurge occurs in a wide variety of environments across western North America, no single control method would be successful in all leafy spurge infestations (Fig. 85). Land managers have also recognized that they must operate under social constraints which will limit the weed management tools they can use in sensitive areas, such as wilderness, near waterways, and on public lands. A wide variety of successful leafy spurge control methods, including herbicide mixtures, grazing, reseeding, and biological control agents, had been used by the late 1990s. Each method was initially used alone, but land managers have learned that long-term management is improved when various control methods were used in combination.

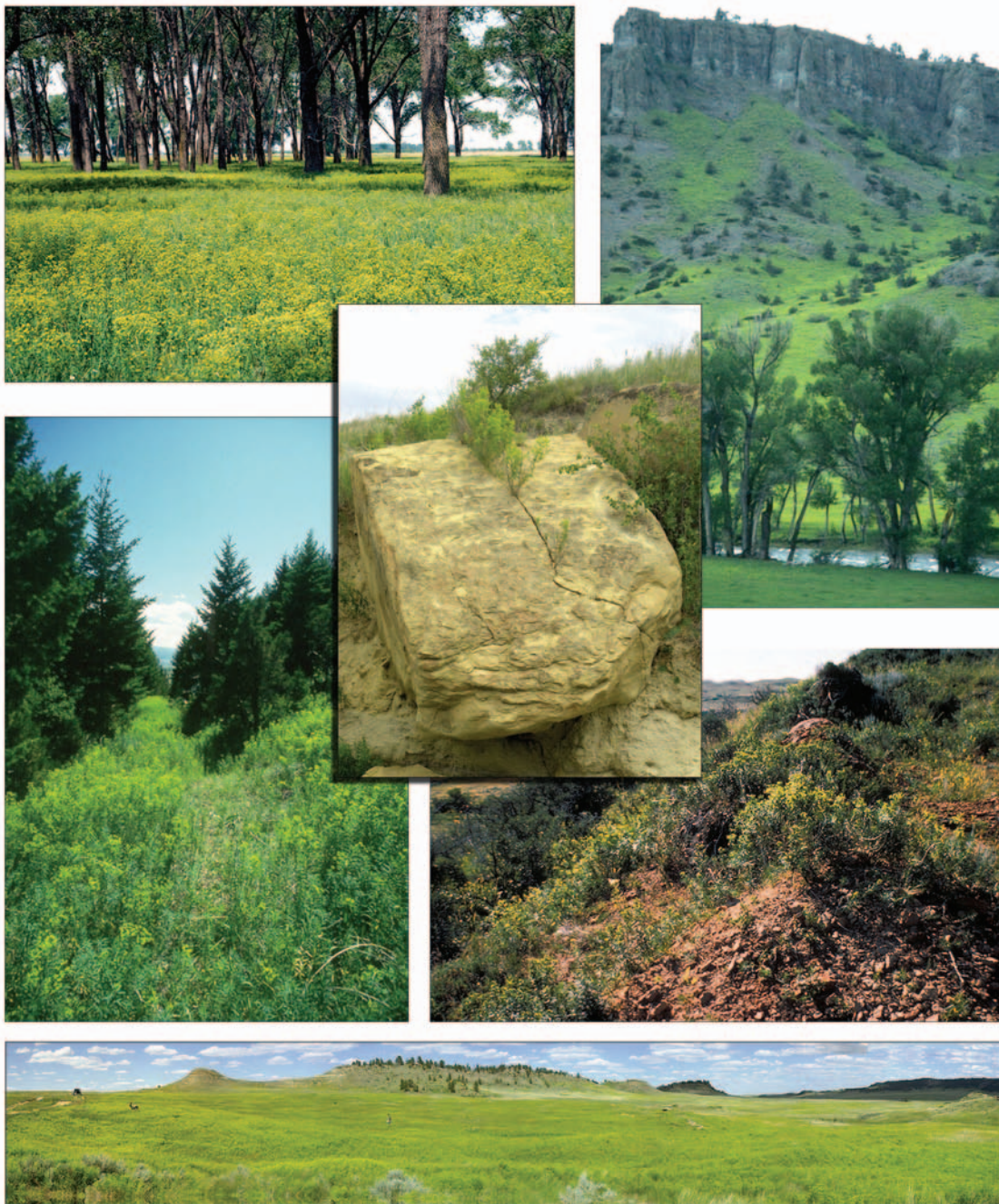


Figure 85. Various habitats in which leafy spurge can thrive. USDA-APHIS-ARS.

3. Weed control methods used to manage leafy spurge

There are seven commonly cited control methods: education, prevention, biological control methods, physical or mechanical methods, cultural methods, herbicide methods, and general land management practices.

Education

Education activities should aim to increase public awareness of noxious weeds and provide information on ways to combat the problem. Education efforts should be a significant component of any invasive weeds management (IWM) strategy and should be an important component of all IWM programs, regardless of the weed control methods employed.

Prevention

Prevention activities are aimed at areas not currently infested by leafy spurge, and are intended to keep weed-free areas weed free. Because leafy spurge is not already established in these areas, there is no opportunity to integrate biological control activities directly on these sites. Prevention activities are frequently combined with education efforts.

Exclusion

In this preventative weed control method, management constraints are designed to prevent the movement of leafy spurge seeds or plant parts into areas that are free of leafy spurge. Examples of exclusion efforts include weed-free forage programs, state seed laws, and mandatory equipment cleaning before entering a clean site (Figs. 86, 87).



Figure 86. Weed-free forage sign on NFS lands. Rick VanBebber, USDA FS, Region 4.

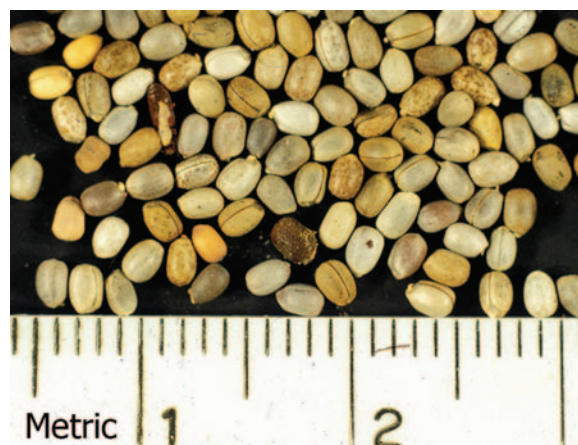


Figure 87. Spurge seeds. Rod Lym, NDSU.

Monitoring

Monitoring is a prevention method in which frequent surveys of clean areas are done to insure that leafy spurge is not invading (Fig. 88).



Figure 88. Technicians surveying a biological control release site in Rollag, Minnesota. USDA-APHIS.

Biological control

Biological control involves the use of living organisms, such as insects or pathogens, to control a weed infestation and recreate a balance of plant species with predators. Research has focused primarily on the introduction of natural predators from the weed's area of origin.

Physical or mechanical

This method uses physical means, such as hand pulling, hoeing, tilling, mulching, burning, and mowing to remove or control weeds, and is the oldest method of weed control.

Cultivation

Cultivation, including turning over or tilling the soil, will control leafy spurge if done on a timely basis. Cultivating twice each fall at approximately one month intervals often eliminates leafy spurge in a few years. Control is more rapid when an herbicide treatment precedes cultivation. Cultivation can be used to help establish competitive native species, such as grasses and forbs, in a leafy spurge infestation (Fig. 89). Once the grasses and forbs have established (usually 1 to 2 years after seeding), biological control agents can be released to control leafy spurge. Cultivation is generally not practical or desirable in wildlands and nature preserves.



Figure 89. Seeding operation. Rod Lym, NDSU.

Mowing

Cutting back the above-ground portion of a plant. Mowing will remove leafy spurge above-ground growth for a short period of time and can reduce leafy spurge seed production, especially if the plants are mowed frequently. Mowing does not injure the root system and long-term control is not possible unless an additional control method at-

tacks the roots. Mowing can be used to reduce non-target plant cover and litter prior to herbicide application or release of biological control agents. Because leafy spurge will be one of the first plants to re-grow in the spring, coverage of herbicides is improved and insects such as *Aphthona* flea beetles, that lay eggs on the soil, will have a higher success rate of larval survival, after mowing.

Prior to mowing, it is important to consider the life cycles of the biological control agents and where they will be on the plant. For example, mowing in the spring, when *Aphthona* flea beetle larvae are feeding in the roots, is compatible with, and perhaps even conducive to, biological control. However, during the summer when *Aphthona* flea beetle adults are feeding on the stems and leaves of the leafy spurge plants, mowing could kill large numbers of mating adults. Likewise, the effects of mowing on other leafy spurge biological control agents are predictable. Mowing is incompatible with the foliage feeding hawk moth and the shoot feeding midges. Adult stem boring beetles lay eggs in the early to mid summer on the middle of the leafy spurge stems. The eggs hatch and the larvae feed down the stem to the root: Fall mowing would be compatible but spring and summer mowing would not be. Clearwing moth larvae feed in the roots so mowing should not be a problem.

Cultural

Cultural methods of weed control, including seeding with competitive grass species, burning, and grazing, enhance the growth of desired vegetation, which may slow the invasion of leafy spurge onto a site. Regardless of which method is used, all cultural control methods are more successful when combined with other control methods, such as biological control and herbicide treatments.

Seeding competitive grasses

Leafy spurge control programs that include establishment of introduced and native perennial grasses have been more successful at increasing forage production and long-term weed control than any single method. Some perennial grasses can compete effectively with leafy spurge and provide a long-term reduction in density. The most competitive grasses include wheatgrass, wild rye, and smooth brome. However, the best variety to seed to compete with leafy spurge varies by region, so consult your local County Extension agent or NRCS representative. Control of leafy spurge prior to seeding grasses is important. (Roundup[®] (glyphosate) or Roundup[®] plus 2,4-D are applied once or twice during the summer to reduce leafy spurge vigor prior to a fall or early spring seeding.)

Incorporating biological control agents with re-seeding has been difficult, primarily because the methods used to establish a productive stand of competitive species are not always compatible with the establishment and survival of biological control agents. In order to establish a suitable site for re-seeding, either an area must be tilled to provide an acceptable seed bed, and/or herbicides such as glyphosate must be applied to reduce competition from leafy spurge. (Note: not only will these methods help establish the competitive grasses, they also will reduce the leafy spurge shoots on which biological control agents feed.)

Seeding of competitive species using a no-till planter would be less detrimental to an established leafy spurge biological control agent than conventional seeding techniques. Unfortunately, no-till seeding has been successful only when the site was mowed or burned prior to seeding and an herbicide was applied to control competitive grasses. The thick thatch of dead leafy spurge stems often found in old stands has been shown to reduce grass seedling establishment and ultimately may result in undesirable grass species replacing the remaining seeded grasses. The intensive management techniques needed to establish competitive species preclude the use of biological control agents until the seeded species have become established and the weed has begun to re-grow. Ongoing research seems to indicate that initial biological control with *Aphthona* spp., combined with no-till reseeding and herbicides, provided better control than either any single method used alone, or the combination of biological control plus herbicides. This may be a valuable treatment option in the future, especially where re-establishment of native plant stands is desired.

Prescribed fire

This method of plant control is used against many domestic and exotic plants. (Fig. 90). However, while prescribed burns have been ineffective at directly reducing leafy spurge infestations, they reduce the ground litter, which in turn results in uniform leafy spurge re-growth. Herbicides applied to these uniform leafy spurge infestations are more effective, and generally provide better long-term control, especially in very dense stands.

Controlled burns have aided in establishment of leafy spurge biological control agents in marginally suitable environments. For example, on a North Dakota wildlife refuge, establishment of *Aphthona nigriscutis* was higher on sites that had been burned prior to release of the agents than on non-burned sites (Fellows and Newton 1999). (Note: Most flea beetle populations established after a prescribed burn did not survive unless the habitat was otherwise suitable.)



Figure 90. Prescribed range land fire. Texas A&M, Texas Cooperative Extension, Rangeland Ecology and Management.

Controlled burns may also help increase the population of *Aphthona* spp. during the first few generations after establishment. Release of flea beetles in very dense leafy spurge infestations reduces the probability of establishment and subsequent weed control. A controlled burn in these dense stands prior to releasing the beetles can increase the survival rate for the larvae, because adults lay eggs at the soil surface rather than on several centimeters of plant thatch.

Biological control agents must be able to survive controlled burns that aid in returning native vegetation to leafy spurge-infested areas. Often, the timing of the controlled burn will determine if an agent survives. Established populations of *Aphthona* spp. will not be affected by fire as long as the burns are conducted in May or October when the agent is in the larval growth stage below the soil surface. *Aphthona* adults feeding on foliage during the summer would not survive a fire. The timing of a controlled burn would have to be adjusted for other leafy spurge control agents such as *Spurgia esula*, which has multiple generations per year.

Grazing

Sheep and goats can be used to control leafy spurge (Fig. 91). Grazing reduces the spread of the weed and allows grasses to be grazed by other animals. Grazing animals can reduce leafy spurge cover 55 to 85 percent in about three years and the land manager can recover some control costs through the sale of the animals. Sheep and goats may be best suited to control leafy spurge in large infestations or along waterways and forested areas where biological agents have not established well and chemical control is restricted

or is cost prohibitive. Grazing alone will neither reduce an infestation, nor kill leafy spurge roots. Even after 10 or more years of grazing, leafy spurge will begin to re-grow if the animals are removed. A second weed control method is needed to prevent rapid re-growth and re-infestation.



Figure 91. Sheep eating leafy spurge on the range. Rod Lym, NDSU.

Grazing combined with biological control agents can provide better control in wider variety of habitats than either method can provide alone. Integration of sheep with *Aphthona* spp. may provide a more rapid reduction of leafy spurge stem density and vigor than the biological control agents alone. Once grazing animals are removed, the biological control agents remain to prevent re-infestation. The combination of sheep grazing and *Aphthona* spp. may manage problematic leafy spurge infestations in riparian areas and shelter belts.

Grazing and *Aphthona* spp. Are used together throughout the season. However, some managers recommend grazing be done only in the spring and/or fall to insure an ample food supply for adult beetles in the summer. There is no data currently available which supports removing grazing animals when adult flea beetles emerge.

Herbicides

Herbicides are important tools for controlling noxious weeds and are available for leafy spurge control in a variety of environments. Herbicide recommendations vary by state and region. Please consult your local weed officer or county agent to learn which herbicides work best and when to apply them in your situation (see “Use Pesticides Safely,” page 91). A brief description of products most widely used follows.

Tordon® or Tordon® plus 2,4-D will provide residual control but cannot be used near water or trees. Plateau® is more selective than Tordon® and can be used under many species of shrubs and trees.

Landmaster® is a mixture of Roundup® plus 2,4-D that can be used in early summer to control leafy spurge in wooded areas or in areas with high water tables. The 2,4-D reduces grass injury expected when Roundup® is applied to grasses.

Paramount® is a very narrow spectrum herbicide that will control leafy spurge but has little activity on other broadleaf or grass species except field bindweed.

Roundup® can be used to control leafy spurge under trees, but is non-selective and will severely injure most grasses.

Often herbicides are mixed together to improve long-term control. Combinations of Tordon® plus 2,4-D plus Plateau® or Tordon® plus Overdrive® dramatically improve leafy spurge control compared to the herbicides used alone, but are expensive treatments.

For more information on the use of these herbicides, please contact your local weed control specialists (county weed superintendent, county extension agent) as herbicide recommendations and application methods change frequently.

Incorporation of herbicides with biological control agents for leafy spurge control began soon after the first insect species was introduced. Initially all biological agent releases were in isolated areas not exposed to herbicides or other control methods. In the fall of 1991, a population of the flea beetle *Aphthona nigricutis* established near Minot, North Dakota, was accidentally over-sprayed with Tordon plus 2,4-D. Although *A. nigricutis* had established two years before the herbicide application, the population was low and had not noticeably reduced the leafy spurge infestation. In the spring following herbicide treatment, it was observed that the leafy spurge density had declined by over

Use Pesticides Safely!

- Read the pesticide label- even if you have used the pesticide before. Follow the all instructions on the label.
- Wear protective clothing and safety devices as recommended on the label.
- Bathe or shower after each pesticide application.
- Be cautious when you apply pesticides. Know your legal responsibility as a pesticide applicator. You may be liable for injury or damage resulting from pesticide use.

(Peter M. Rice, 1993)

90 percent and more than one million *A. nigriscutis* adults were subsequently collected for redistribution.

Leafy spurge control is generally more rapid when herbicides are applied after *Aphthona* spp. have become established than when applied during the same season the insects are released. In addition, the combination treatment sometimes results in a rapid increase in the biological control agent population. The increase in *Aphthona* spp. population observed in Minot was likely due to a reduction in leafy spurge stem density, which led to an increase in egg laying at the soil surface rather than on litter, and increased movement of adults outside the initial release zone. Once leafy spurge stem density is reduced, the biological control agents often maintain a high level of control. Thus, a practical application of an integrated, herbicides/*Aphthona* approach, would be to apply herbicides after the flea beetles have been established for several years but have had limited impact. Such situations often occur in very dense leafy spurge stands. The herbicide reduces the stem density, thereby allowing the *Aphthona* population to establish and reproduce quickly.

Depending on the agent and the location, herbicides with biological control agents can be combined or used separately. For example, the leafy spurge gall midge, *Spurgia esulae*, can be used to reduce seed production in forested areas where herbicides generally cannot be used; in turn, herbicides can be applied away from trees to reduce leafy spurge spread.

Herbicide application must be timed to occur at a time least disruptive to the biological control agent. For *Aphthona* spp., herbicides must be applied in the fall after the adults have laid eggs and the larvae are feeding on the roots. The leafy spurge gall midge has

History and Development of Leafy Spurge Management Tools

Herbicides were the first line of defense for leafy spurge control beginning in the late 1930s, during which large quantities of various salts were applied. In the 1960s, Tordon[®], a small grain herbicide, was found to control leafy spurge at relatively high application rates. Tordon[®] applied alone and in combination with 2,4-D was the standard treatment for leafy spurge control from the early 1980s until about 2002, when new herbicides more specific to leafy spurge were introduced. Today, herbicides such as Plateau[®], Overdrive[®], and Landmaster[®] are used alone or in combination with Tordon[®] to control leafy spurge, in a wide variety of settings including near water and under trees.

Non-chemical methods other than biological control insects include cultivation or mowing, seeding with competitive grasses, and grazing with sheep and goats. Cultivation can reduce or eliminate leafy spurge, which is why the weed is seldom found in cropland. Seeding with competitive grass species can reduce leafy spurge, but is expensive and requires extensive land preparation. Grazing has been used successfully since the 1940s. It reduces the spread of leafy spurge but does not eliminate the root system. The use of pathogens as biological control agents of leafy spurge, evaluated since the early 1980s, has not been successful.

multiple generations from early spring until frost. Herbicides should be applied at the time best suited for each chemical; however, in order to insure that the insects maintain viable populations as the leafy spurge infestation is reduced, 25 percent of the area should remain untreated.

4. General land management practices

Leafy spurge has persistent growth characteristics and seed can remain viable in the soil for years. Therefore, you should implement long-term weed management programs. Long-term weed management includes re-treatment with herbicides or continued cultural, mechanical, or biological control practices to maintain low leafy surge populations. Range improvements, such as grazing systems, cross fencing, and water development, will help retard the invasion of many weed species including leafy spurge. Sites with no desirable species should be reseeded with a competitive plant species as part of the total management program (Table 11).

Table 11. Comparisons of leafy spurge management techniques.

Management Technique	Advantages	Disadvantages	Comments
Herbicides	Fast acting. High success rate for reducing leafy spurge densities. Rapidly enhances grass production.	Expensive for large areas. May harm desirable vegetation, especially broadleaf species. Many natural areas are inaccessible to spray equipment. Public resistance to chemical controls.	Best used on small patches when leafy spurge first appears or on the edges of a large infestation to keep it from spreading while other methods such as biological have time to establish.
Biological control	Very selective. Agents generally do not have to be reintroduced once established. Public acceptance is generally higher than with other weed control methods.	Some risk of undesirable effects on native plants. Permanent: cannot be undone. Biological control is not successful in all situations. Each agent only effective against one weed species. Measurable changes in weed densities may take several to many years.	Most economical option for large infestations. Will control leafy spurge in a variety of environments in which the weed occurs, especially if multiple agents are introduced.
Grazing	Allows use of the land even with heavy leafy spurge infestations. Can be used in combination with biological or chemical control methods.	Non-selective. Expensive. Cannot be used in many natural areas such as National Parks and Wilderness areas.	Will remove top-growth only, but does not reduce the root mass. The same areas must be grazed annually or leafy spurge will rapidly reestablish.
Mechanical/cultural	Very effective.	Not appropriate for natural areas/wildlands.	Not compatible with biological agents.
	Can be used to reseed native species	Expensive for larger infestations.	Best used when an areas is being "reclaimed."

GLOSSARY

abdomen	The last of the three insect body regions; usually containing the digestive and reproductive organs.
alternate	Leaves that are arranged singly at each node along a stem.
annual	A plant that flowers and dies within a period of one year from germination.
antenna	In arthropods, one of a pair of appendages on the head, normally many jointed and of sensory function.
apical buds	Buds located on the tip of a shoot.
aspirator	An apparatus used to suck insects into a collection container. The device can be simple (as in a mouth-aspirator or mechanical (as in a gasoline- or battery-powered vacuum aspirator).
beetle	A member of the very large and variable insect order Coleoptera; adults have hardened or leathery forewings (elytra) while larvae may be grub-like or mobile; beetles exhibit complete metamorphosis.
biennial	A plant that flowers and dies between its first and second years and does not flower in its first year.
biological control	The reduction in the abundance of a pest through intentional use of its natural enemies (predators, parasitoids, and pathogens); also called “biocontrol.”
biological control agent	A natural enemy of a target pest used in biological control efforts.
biotype	An informal subdivision of a species; generally, refers to a group of organisms that are morphologically identical to other individuals of a species but possess distinctive physiological characteristics (e.g., able to attack a normally resistant host, or not susceptible to a pesticide).
bolting	Plant stage at which the flower stalk begins to grow.
bract	A leaf, often modified or reduced, which subtends a flower or an inflorescence.

capsule	A pod or seed vessel made of two or more cells, that becomes dry and splits open to release mature seeds.
caterpillar	The larval stage of a moth or butterfly (Order Lepidoptera).
chemical weed control	Weed control strategies employing herbicides.
classical biological control	A biological control strategy employing the release of a pest's natural enemies imported from another region; typically directed against exotic pests, it uses natural enemies from areas where the pest is native.
competition	Negative interactions between individuals of the same or different species that utilize the same resource(s); if the resource is in short supply, one individual or species may survive and increase in number at the expense of the other(s).
complete metamorphosis	A type of insect development characterized by immature stages (larvae and pupae) that look quite different from the adults, and typically live in different habitats, eat different foods, and exhibit different behaviors than do the adults.
compound eyes	Paired eyes consisting of many facets, or ommatidia, in most adult Arthropoda.
community	A naturally occurring group of different species of organisms that live together and interact as a more or less self-contained "unit."
cover	The portion of the vegetative canopy in a fixed area attributable to an individual or a single plant species.
cultural weed control	Cultural methods of weed control that enhance the growth of desired vegetation, and which may slow the invasion of leafy spurge onto a site.
cyanthium	An inflorescence consisting of a cuplike cluster of modified leaves enclosing a female flower and several male flowers, as in the poinsettia.
defoliation	The loss of foliage due to insect or fungal activity or the actions of abiotic factors, such as hailstorms.
defoliator	An organism, usually an insect, that consumes plant foliage.
density	Number of individuals per unit area.

dispersal	The spread of animals and plants from any point; the redistribution of plant seeds, fungal spores, or insect eggs, larvae, and adults.
dormant	In a state of temporarily reduced metabolic activity.
elytron (pl. elytra)	Hardened forewing of a beetle.
emergence	Act of adult insect leaving the pupal case or reappearing after overwintering.
emetic	Promotes vomiting.
eradicate	To get rid of, as if by tearing up by the roots.
exoskeleton	A hard outer structure, such as the shell of an insect or crustacean, that provides protection or support for the organism.
exotic	Not native.
field insectary	An area where host plants or animals are abundant and biological control agents are released and propagated with or without additional human manipulation.
forb	A herbaceous plant that is not a grass nor grass-like in form.
frass	The excrement produced by insects, especially larvae, that contains feces and undigested plant material and is often used to detect individuals feeding inside plant tissues.
genera	A taxonomic category ranking below a family and above a species and generally consisting of a group of species exhibiting similar characteristics. In taxonomic nomenclature the genus name is used, either alone or followed by a Latin adjective or epithet, to form the name of a species.
genotype	The genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms.
head capsule	Hardened covering of the head of an immature insect.
herb	A plant whose stem does not produce woody, persistent tissue and generally dies back at the end of each growing season.

herbicide	A chemical substance used to destroy or inhibit the growth of plants, especially weeds.
homogenous	All of the same or similar kind or nature.
host	The plant or animal on which an organism feeds; the organism utilized by a parasitoid; a plant or animal susceptible to attack by a pathogen.
host range	The variety of hosts or host species that may be utilized by a plant- or animal-feeding organism.
host specificity	The dietary restriction of an organism to a single or limited food (for herbivores: the number of plant species accepted as food; the highly-evolved, often obligatory association between an insect and its host(s); the degree to which an organism is restricted to a particular number of plant or animal hosts.)
hybrid	The offspring of two parents of different species.
inflorescence	The flowering structure of a plant.
insect	A small arthropod animal that, as an adult, normally has six legs, three distinct body regions, one pair of antennae, and one or two pairs of wings.
instar	The period or stage between successive molts in a insect larva.
integrated weed management	A system for the planning and implementation of a program, using an interdisciplinary approach, to select a method for containing or controlling an undesirable plant species or group of species using all available methods.
invasive plant	An aggressive and dominant plant; likely to colonize and become established in new habitats; usually refers to weeds.
larva (pl. larvae)	Immature insect stage between the egg and pupa.
maggot	Immature larva of insects in the order Diptera (true flies).
mechanical weed control	Mechanical methods that employ physical means to remove or control weeds. Mechanical methods include such activities as hand pulling, hoeing, tilling, mulching, burning, and mowing.
metabolic sink	Any living cell, tissue, organ, or structure (e.g., gall) that is a storage depot of a metabolite.

membranous	Thin and transparent.
metamorphosis	The change from one life stage to another in insects, such as from larva to pupa.
molt	The process by which insects and other arthropods shed their exoskeleton (“skin”) as they grow and develop; among insects, molting is typically restricted to larval or nymphal stages (may also be spelled as moult).
monoculture	An area vegetated by a single plant species.
nectar	A sugary secretion produced by plants, usually in flowers, that attracts insect pollinators.
node	The position on a stem where leaves or branches originate; also known as a “joint.”
noxious weed	A weed whose control is mandated, and whose movement is regulated by federal or state law.
non-target	Not being the target of a control method, e.g., not a host or food source for a biological control agent.
ovary	In plants, the expanded basal portion of the pistil that contains the ovules.
oviposit	To lay or deposit eggs.
oviposition scars	Damage to plant tissues resulting from the act of ovipositing by insects.
pathogen	An agent, such as a bacterium or fungus, that causes disease.
perennial	A plant living more than two years.
pupa (pl. pupae) (v. pupate)	Non-feeding, inactive stage between the larva and adult in insects with complete metamorphosis.
phylogenetic	Relating to or based on evolutionary development or history.
purgative	Tending to cleanse or purge, especially causing evacuation of the bowels.
quadrat	A specific area used to sample vegetation (e.g., 1 square meter, or 1m ²).

qualitative	Measurement of descriptive elements (e.g., age class, distribution).
quantitative	Measurement of number or amount (e.g., number of seeds per capsule).
ramets	An individual member of a clone.
random sample	A sample obtained in such a way that all members of a population have an equal likelihood of examination or collection; an unbiased sample.
scours	Diarrhea in livestock.
senescence	The natural decline and death of a plant after reproduction or at the end of the growing season.
shrub	A woody plant of relatively low height, having several stems arising from the base, and lacking a single trunk; a bush.
species	A fundamental category of taxonomic classification, ranking below a genus or subgenus and consisting of related organisms capable of interbreeding.
subshrub	A low shrub; an undershrub.
subspecies	A taxonomic subdivision of a species consisting of an interbreeding, usually geographically isolated, population of organisms.
succulent	A succulent plant, such as a sedum or cactus.
sympatric	Occupying the same or overlapping geographic areas without interbreeding.
synchrony	Occurring at the same time.
TAG	Technical Advisory Group
taxon (plural taxa)	A taxonomic category or group, such as a phylum, order, family, genus, or species.
taxonomy	The classification of organisms in an ordered system that indicates natural relationships. The science, laws, or principles of classification; systematics.
thorax	Body region of an insect, behind the head, bearing the legs and wings.

transect	A straight line or path through an area.
tree	A perennial woody plant having a main trunk and usually a distinct crown.
USDA-APHIS- PPQ	United States Department of Agriculture- Animal and Plant Health Inspection Service- Plant Protection and Quarantine.
variable	A quantity that has any one of a set of values, i.e., plant height.
vegetative reproduction	Reproduction in plants other than by seeds, such as from rhizomes, stolons, and from nodes on lateral, often creeping, roots.
ventral surface	The lower surface of the body of an animal.
viability	The proportion of propagules (e.g., seeds) that are alive and can germinate.
voucher specimen	A specimen collected from a population of a biological control agents to confirm the identity of the species present.

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CHAPTER 2: BIOLOGY OF LEAFY SPURGE BIOLOGICAL CONTROL AGENTS

None

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APPENDICES

- Appendix A: Code of Best Practices for Biological Control of Weeds**
- Appendix B: Troubleshooting Guide; When Things Go Wrong**
- Appendix C: Leafy Spurge Biological Control Release Form**
- Appendix D: Leafy Spurge Monitoring Plan Questionnaire**
- Appendix E: Leafy Spurge Biological Control Insect Monitoring Form**
- Appendix F: Leafy Spurge and Cypress Spurge Biological Control Qualitative
Monitoring Form**
- Appendix G: Leafy Spurge Biological Control Vegetation Monitoring Form**
- Appendix H: Build Your Own Apthona Accelerator**
- Appendix I: PPQ Form 526 Permit Application to Transport Biological Control
Agents**
- Appendix J: Recovery and Sampling Report Form**
- Appendix K: Leafy Spurge Vegetation Sampling; Daubenmire Quadrats**

Appendix A: Code of Best Practices for Biological Control of Weeds

Code of Best Practices for Biological Control of Weeds

1. Ensure target weed's potential impact justifies release of non-endemic agents.
2. Obtain multi-agency approval for target weed.
3. Select agents with potential to control target weed.
4. Release safe and approved agents.
5. Ensure only the intended agent is released.
6. Use appropriate protocols for release and documentation.
7. Monitor impact on target weed.
8. Stop releases of ineffective agents, or when control is achieved
9. Monitor impacts on potential non-target species.
10. Encourage assessment of changes in plant and animal communities.
11. Monitor interaction among agents.
12. Communicate results to public.

Delegates and participants to the X International Symposium for Biological Control of Weeds, recognizing the need for professional standards in the subdiscipline of classical biological control of weeds, urge practitioners of the subdiscipline to voluntarily adopt the **CODE OF BEST PRACTICES FOR CLASSICAL BIOLOGICAL CONTROL OF WEEDS**, as published in the proceedings of the Symposium, and to adhere to the principles outlined in the Code.

J. K. Balciunas
USDA-ARS, Exotic and Invasive Weed Research Unit
800 Buchanan Street
Albany, CA 94710
USA

Appendix B: Troubleshooting Guide; When Things Go Wrong

This guide is intended to assist those who encounter problems when establishing or monitoring a biological control program for leafy spurge. It identifies the probable cause of common problems and offers solutions.

Problem	Probable Cause	Solution
Biological control agents unhealthy	Physical damage to agents.	Prevent collection containers from colliding; use crush-resistant containers. When aspirating, change vials often, avoid long exposure and crowding in vials. When sweeping, empty contents of net after 5 (for moths and flies) or 25 sweeps (for beetles).
	Drowning.	Do not put excess water in the collection containers.
	Excess or prolonged heat or cold.	Keep containers cool at all times; use coolers and ice packs; avoid exposure to direct sunlight while in transit.
	Starvation.	Put leafy spurge stems with foliage (no flowers, seeds, or roots) in container; minimize time agents are in containers.
	Release delay.	Transport or ship agents immediately after collection; release agents at new site immediately upon arrival or receipt of agents.
	Parasitism and/or disease.	Check source of agents when obtained from a supplier; ensure insect population is disease-free when collecting or receiving shipments.
Few insects collected	Wrong collection method used.	Refer to Chapter 3 for recommended collection time and technique.
	Collection done at wrong time.	Refer to Chapter 3 for recommended collection time and technique.
	Collection effectiveness.	Insects can be harmed during collecting; practice aspirating, sweeping, and sorting.
	Conditions at time of collection.	Collect in favorable weather.
	Population size.	Agent population still too low to collect.

Appendix B: Troubleshooting Guide; When Things Go Wrong, *cont.*

Problem	Probable Cause	Solution
Biological control agents not found after release.	Site is unsuitable.	Refer to Chapter 3.
	Site too small.	Select a larger site and dense stand of leafy spurge.
	Monitoring method.	Monitoring method is inappropriate or monitoring was done at the wrong time.
	Not enough agents released.	Repeat releases with a larger number of insects.
	Predation.	Agents may have been killed by ants, spiders, birds, etc.
	Bad luck.	Bad weather event, flooding, pesticides, or other event may kill many or all insects. Initiate another release.
Insects do not build up populations within years after release.	Existing populations may be too small.	Release additional insects.
	Site unsuitable.	Do not give up: repeat steps outlined above or try to assess which factors are critical and can be changed. If after assessing the situation and making possible changes the agents still won't establish, be prepared to drop this site.
	Bad weather.	Unfavorable weather conditions for several consecutive years can hamper insect population growth.
Cannot locate release site.	Permanent location marker not found.	Use bright-colored wooden, metal, or plastic stake; locate site with GPS coordinates.
	Map incorrectly or poorly drawn.	Check map, redraw with more detail, or add landmarks.

Appendix C: Leafy Spurge Biological Control Release Form

Released By: _____ Release Date: ___/___/___ State: _____ County: _____
(mm dd yy)

Biological Control Agents: _____	# Released: _____
Source of Agents: _____	Date Collected: ___/___/___ <small>(mm dd yy)</small>
Life Stage (circle): Larvae Adults	
Land Ownership (circle): Private County State USFS BLM COE BOR BIA/Tribe TNC Other (specify) _____	
Legal: T ___ R ___ Sec ___ Q ___ Lat: Deg ___ Min ___ Sec ___ Long: Deg ___ Min ___ Sec ___	
UTM: UTM Datum Zone: ___ UTM Year: ___ UTM Easting: _____ UTM Northing: _____	

ENVIRONMENT	
Temperature (°F): _____	Wind: Calm, Light, Moderate, Strong, Gusty Wind Direction: N S E W
Weather (circle): Clear Ptly Cloudy Cloudy Rain Snow	Release Time: _____ AM/PM
Site Aspect (circle): N, NE, E, SE, S, SW, W, NW	Elevation: _____
Site Slope: Flat (0-10%) _____ Gentle (10-30%) _____ Moderate (30-60%) _____ Steep (>60%) _____	
Topographic Position (circle): Valley Bottom Terrace Lower Slope Mid/Upper Slope Crest	
Disturbance: (check all that apply, circle most prevalent) Cultivation ___ Fire ___ Flood ___ Grazing ___ Logging ___ Roads ___ Mining ___ Recreation ___	

SITE CHARACTERISTICS	
Site Name: _____	Size of Infestation (acres): _____ Estimated % Weed Cover: _____
Est. Weed Height (cm): _____	Est. Weed Density (# per meter sq.): _____ Dominant Plant: _____
Distribution of Weed: Isolated ___ Scattered ___ Sc-Patchy ___ Patchy ___ Continuous ___ Linear ___	
Phenology: Seedling % ___ Rosette % ___ Bolt % ___ Bud % ___ Flowering % ___ Seed % ___ Dormant % ___	
Vegetation Type (check):	Estimate % Cover:
Grassland <input type="checkbox"/>	Tree _____
Pasture <input type="checkbox"/>	Shrub _____
Dry Meadow <input type="checkbox"/>	Forb _____
Moist Meadow <input type="checkbox"/>	Grass _____
Shrubland Steppe <input type="checkbox"/>	Litter _____
Conifer Forest <input type="checkbox"/>	Bare Ground _____
	Rock _____
Soil Texture: (check) Sand ___ Silt ___ Clay ___ Gravel ___ Loam ___	

Appendix C: Leafy Spurge Biological Control Release Form, *cont.*

CONTACT PERSON:

Name: _____
Address: _____
City: _____
State: _____
Phone: _____ - _____ - _____
e-mail: _____

LEGAL LANDOWNER:

Name: _____
Address: _____
City: _____
State: _____
Phone: _____ - _____ - _____
e-mail: _____

Road Map to Site

Site and Vegetation Map

Comments:

Appendix D: Leafy Spurge Monitoring Questionnaire

The following is a list of questions to be answered and documented prior to collecting data. Use the checklist as an outline for a monitoring plan.

What is the management objective of the biocontrol release site?

- a) Study site for long-term monitoring.
- b) Nursery site to increase numbers of agents for future collection and redistribution.
- c) Open release site, no additional monitoring is intended.

Notes: _____

What will be measured?

- a) Biological control agent presence/absence.
- b) Biological control damage to target weed.
- c) Plant community structure or composition.

Notes: _____

What equipment and supplies are needed? _____

What training is needed? _____

What is the cost of monitoring? _____

What is the interval between monitoring? _____

Appendix E: Leafy Spurge Biological Control; Insect Monitoring

Name: _____ Date: _____ Time: _____ am/pm _____

Location: _____ Site #: _____

Insect: _____ Year of release: _____

Cover Class by Plant Type						
	0%	1-5%	6-20%	21-50%	51-75%	76-100%
Leafy Spurge						
Annual Grasses						
Perennial Grasses						
Forbs						
Shrubs						
Trees						

Dominant Plants on Site:
Other Noxious Weeds:

Leafy spurge density class (check one)			
Flowering (plants/meter sq)		Leafy spurge distribution	
0		Isolated	
1-25		Scattered	
26-50		Scattered-Patchy	
51-75		Patchy	
>75		Continuous	

Phenology class at time of monitoring	
Leafy spurge stage	Estimated percent
Seedling	
Rosette	
Bolting	
Flowering	
Senescent	

Comments/Observations _____

Appendix E: Leafy Spurge Biological Control Insect Monitoring Form Instructions.

Materials needed: stopwatch, sweep net, monitoring form, pencils, clipboard, camera, and GPS unit to record site location.

General: The purpose of this monitoring activity is to estimate the abundance of leafy spurge biocontrol agents at your monitoring site. Conduct the monitoring when the biocontrol agents are at their peak emergence.

- 1) **Site information:** Fill out the site information at the top of the form.
- 2) **Insect counting:** To estimate the density of biocontrol agents, you need to count them. Carefully approach the site and avoid disturbing the vegetation. Adults often drop from the vegetation once you touch stems (or even as you approach the quadrat).

Timed Counting: Schedule monitoring to coincide with the adult stage of the biocontrol agent that you are sampling (or caterpillars of moths). Randomly walk at a steady pace within the observation area, staying within 32 yd (30 m) of the release point. Accuracy is increased if you avoid backtracking over the same area because both beetles and caterpillars are easily brushed off or fall from leafy spurge. Beginning at the release point marker, count all the agents you see, concentrating on the top portions of the plants. Count the insects for 5, 10, or 15 minutes, as appropriate, and record the number seen.

Sweep net sampling: Using a sturdy canvas net, sweep the upper 3 in (10 cm) of the plant along transects 64 ft (20 m) long. Sweeping lower will give you lots of broken plant parts, but will not necessarily yield any more beetles. Make 20 samples of five sweeps each, sweeping relatively gently through the upper parts of the leafy spurge plants. Open the net after the five sweeps, count any biocontrol insects collected, then either release the captured insects, or place them in alcohol-filled vials for identity confirmation. You may also empty your sweep net into a white tray for immediate counting, or dump the tray contents into a large plastic cup that you can cover for later counting. The 20 samples can be taken randomly through the leafy spurge infestation, or by walking in a straight line away from the release point along each cardinal direction (N, S, E, W). For the latter, do five to seven, five-sweep samples at regular intervals (e.g., 10-16 ft (3-5 m) apart) depending on the size of the leafy spurge patch.

Tray sampling: This method is particularly useful when monitoring low to medium population densities of biocontrol beetles. Use a white or similarly light-colored plastic tray, pan, or other flat receptacle with sides. The tray should be rectangular or square (for ease of tipping collected insects into specimen vials or other containers), and 12-18 in (30-46 cm) on the longest side. Take 20 samples at each release location. For each sample, gently hold one leafy spurge stem or a handful of leafy spurge stems and shake or tap the upper stems 10 times over the collecting tray. This should dislodge at least some of the adults present; they can then be easily counted on the tray and released. Twenty to thirty samples may be taken randomly throughout the leafy spurge infestation, or you may collect tray samples walking away from the release point along each cardinal direction (N, S, E, W). For the latter, do five to seven 'beating' samples at regular intervals (e.g., 10-16 ft (3-5 m) apart), depending on the size of the leafy spurge patch.

Appendix F: Leafy and Cypress Spurge Biological Control Qualitative Monitoring Form

SITE: _____ STATE: _____ DATE: _____
year month day

Last name: _____ First name: _____

GPS: Lat N _____ ° _____ ' Long W _____ ° _____ '

UTM: UTM Datum Zone: __ UTM Year: ____ UTM Easting: _____ UTM Northing: _____

TIME: _____ TEMPERATURE: _____ WEATHER: _____

Biocontrol Insect: _____ Year of release: _____

Spurge Species (circle): Leafy Spurge Cypress Spurge

Cover class estimate by plant category							
	0%	1-5%	6-25%	26-50%	51-75%	76-95%	96-100%
Spurge							
Grasses							
Forbs							
Shrubs							
Trees							

Dominant plant species on site:
Other noxious weeds:

Estimate spurge density class (✓check one)			
Flowering plants/m ²		Spurge Distribution	
0		Isolated	
1-25		Scattered	
26-50		Scattered-Patchy	
51-75		Patchy	
>75		Continuous	

Spurge phenology class	
Spurge Stage	Estimated percent
Seedling	
Rosette	
Bolting	
Flowering	
Senescent	

Comments/Observations _____

Appendix G: Leafy Spurge Biological Control Vegetation Monitoring Form

SITE: _____ STATE: _____ DATE: _____
year month day

Last name: _____ First name: _____

GPS: N _____° _____' W _____° _____' Elevation: _____ ft m

UTM: UTM Datum Zone: ____ UTM Year: ____ UTM Easting: _____ UTM Northing: _____

TIME: _____ TEMPERATURE: _____ WEATHER: _____

Chart A: Cover Class	Class	% Cover	Class	% Cover	Class	% Cover	% Cover	Class
	0	0	2	6-25 %	4	51-75 %	6	96-100 %
	1	1-5 %	3	26-50 %	5	76-95 %		

Quad #	Leafy Spurge Stems					Cover Class (use Chart A)					
	# flowering stems	# non-flowering stems	Height (cm) of 4 tallest stems			Leafy Spurge	Grass	Forbs	Shrubs	Litter	Bare ground
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											

Notes: _____

Appendix G. Leafy Spurge Biological Control Vegetation Monitoring Form, *cont.*

Quad #	Leafy Spurge Stems				Cover Class (use Chart A)						
	# flowering stems	# non-flowering stems	Height (cm) of 4 tallest stems			Leafy Spurge	Grass	Forbs	Shrubs	Litter	Bare ground
21											
22											
23											
24											
25											
26											
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37											
38											
39											
40											

Notes: _____

Appendix G: Leafy Spurge Biological Control Vegetation Monitoring Instructions

Materials needed: 1 yard (1 meter) measuring stick, 3.5 ft² (0.1 m²) quadrat frame, data sheets, pencils, clipboard, camera, and GPS unit to relocate quadrats.

General: The purpose of this activity is to estimate the abundance of leafy spurge and other vegetation in the community, and to measure leafy spurge plants. Monitoring is easier with two people, one to make the observations and the other to record data.

- 1) **Site information:** Fill out the site information at the top of the form.
- 2) **Locate the transect and position the quadrat:** After you have completed the insect counts, locate the transect using the GPS coordinates and the permanent marker.
- 3) **Position the quadrat frame:** Position the quadrat frame along the transect, as close to the ground as possible, carefully positioning the quadrat frame along the transect line. Gently arrange vegetation so that plant parts are either within or outside, rather than underneath, the frame, but be sure not to damage the plants. The quadrat should be in the same location as the previous year's quadrat.
- 4) **Count stems:** Count the number of leafy spurge stems, beginning at one corner of the quadrat and working systematically across the quadrat. Count the number of flowering and nonflowering stems.
- 5) **Measure stems:** Select the four tallest leafy spurge stems in each quadrat (if there are fewer than four stems/quadrat, measure all that are present). Measure the stem height to the closest centimeter.
- 6) **Estimate percent cover:** Standing over the frame, estimate how much of the quadrat is covered by leafy spurge, expressed as a percentage. Then estimate the percent cover of grasses, forbs, shrubs, plant litter, and bare ground. Use the cover classes in Chart A to estimate percent cover.
- 7) **Other observations:** Record any general observations or useful information such as disturbances, grazing, fire, etc., for the sample quadrat or the site in general.

Appendix H: Build Your Own *Aphthona* Accelerator¹

Aphthona Accelerator Cost Estimates

Canning funnel	\$1.99	
Collection jar (small)	4.99	
Collection jar (large)	5.99	
Tomato cage (small)	0.75	
Tomato cage (large)	1.25	
Cable clips (4)	1.20	
Rubbermaid® Serving Saver - 3 qt.	3.99	
1/8" hardware cloth	1.70	@ \$2.59 per ft.
Plywood top	0.70	@ \$14 per sheet
'Boat glass'	5.00	@ \$10 per yard
Total Cost	\$27.56	



Additional required items and equipment required to build your own *Aphthona* Accelerator

rivets	quick drying hot glue	hot glue gun
primer & paint	silicone caulking	caulking gun
cable ties	jigsaw	rivet gun
stakes	Dremel® Tool	other common shop tools

Operating the *Aphthona* Accelerator



1. Collect large numbers of *Aphthona* flea beetles by sweeping!
2. Remove lid of sorting basket and deposit sweep collections. Don't overload the accelerator!
3. After several minutes *Aphthona* beetles will move through the hardware cloth to the interior funnel surface.
4. Occasionally tap the top of the accelerator to knock beetles into the collection jar.
5. Remove and replace collection jar when beetles



- become numerous in the bottom.
6. Transfer sorted *Aphthona* to a different container and place in a cooler to chill them down. This makes volumetric measuring much easier. Finally, transfer the beetles to shipping containers and keep them cool.

Prepared by USDA-APHIS-PFQ

Appendix I: PPQ Form 526 Permit Application to Transport Biological Control Agents

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0054. The time required to complete this information collection is estimated to average 0.17 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED
OMB NO. 0579-0054

No permit can be issued to move live plant pests or noxious weeds until an application is received (7 CFR 330 (live plant pests) or 7 CFR 360 (noxious weeds)).

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE PLANT PROTECTION AND QUARANTINE PERMITS AND RISK ASSESSMENT, UNIT 133 RIVERDALE, MARYLAND 20737 APPLICATION FOR PERMIT TO MOVE LIVE PLANT PESTS OR NOXIOUS WEEDS		SECTION A - TO BE COMPLETED BY THE APPLICANT					
1. NAME, TITLE, AND ADDRESS (Include Zip Code)							
3. TYPE OF PEST TO BE MOVED * <input type="checkbox"/> Pathogens <input type="checkbox"/> Arthropods <input type="checkbox"/> Noxious Weeds <input type="checkbox"/> Other (Specify) _____ This permit does not authorize the introduction, importation, interstate movement, or release into the environment of any genetically engineered organisms or products.							
2. TELEPHONE NO. ()							
A. SCIENTIFIC NAMES OF PESTS TO BE MOVED	B. CLASSIFICATION (Orders, Families, Races, or Strains)	C. LIFE STATES, IF APPLICABLE	D. NO. OF SPECIMENS OR UNITS	E. SHIPPED FROM (Country or State)	F. ARE PESTS ESTABLISHED IN U.S.?	G. MAJOR HOST(S) OF THE PEST	
4.							
5.							
6.							
7. WHAT HOST MATERIAL OR SUBSTITUTES WILL ACCOMPANY WHICH PESTS (Indicate by line number)							
8. DESTINATION		9. PORT OF ARRIVAL		10. APPROXIMATE DATE OF ARRIVAL OR INTERSTATE MOVEMENT			
11. NO. OF SHIPMENTS	12. SUPPLIER		13. METHOD OF SHIPMENT <input type="checkbox"/> Air Mail <input type="checkbox"/> Air Freight <input type="checkbox"/> Baggage <input type="checkbox"/> Auto				
14. INTENDED USE (Be specific, attach outline of intended research)							
15. METHODS TO BE USED TO PREVENT PLANT PEST ESCAPE				16. METHOD OF FINAL DISPOSITION			
17. <i>Applicant must be a resident of the U.S.A. I/We agree to comply with the safeguards printed on the reverse of this form, and understand that a permit may be subject to other conditions specified in Section B and C.</i>		SIGNATURE OF APPLICANT (Must be person named in Item 1)			18. DATE		
WARNING: Any alteration, forgery, or unauthorized use of this document is subject to civil penalties of up to \$250,000 (7 U.S.C. s7734(b)) or punishable by a fine of not more than \$10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. s1001).							
SECTION B - TO BE COMPLETED BY STATE OFFICIAL							
19. RECOMMENDATION <input type="checkbox"/> Concur (Approve) <input type="checkbox"/> Comments (Disapprove) <input type="checkbox"/> (Accept USDA Decision)		20. CONDITIONS RECOMMENDED					
21. SIGNATURE		22. TITLE		23. STATE	24. DATE		
SECTION C - TO BE COMPLETED BY FEDERAL OFFICIAL							
						25. PERMIT NO.	

PERMIT

(Permit not valid unless signed by an authorized official of the Animal and Plant Health Inspection Service)

Under authority of the Plant Protection Act of 2000, permission is hereby granted to the applicant named above to move the pests described, except as deleted, subject to the conditions stated on, or attached to this application. (See standard conditions on reverse side.)

* For exotic plant pathogens, attach a completed PPQ Form 526-1.

24. SIGNATURE OF PLANT PROTECTION AND QUARANTINE OFFICIAL	25. DATE	26. LABELS ISSUED	27. VALID UNTIL	28. PEST CATEGORY
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Appendix I: PPQ Form 526 Permit Application to Transport Biological Control Agents, *cont.*

Instructions for Completing PPQ Form 526
Application for Permit to Move Live Plant Pests or Noxious Weeds

Complete this form to request a USDA-APHIS-PPQ permit for the following activities:

1. **IMPORT** – Plant pests, including but not limited to the following living organisms; insects, mites, nematodes, snails, slugs, earthworms, microbes pathogenic to plants or invertebrates, honey bees and other pollinating bees, biological control organisms, parasitic plants, or Federal noxious weeds into the United States.
2. **SHIP INTERSTATE** - any of the above, EXCEPT honey bees and entomophagous insects. (Interstate shipment of entomopathogens does not require a permit unless the organisms were originally imported under a permit requiring containment. APHIS does not regulate interstate movement of pollinating bees, including honey bees.)
3. **RELEASE** - an organism, including those for biological control purposes, from containment **into the environment** of the United States.

DO NOT SUBMIT ANY GENETICALLY ENGINEERED PLANTS OR PLANT PESTS ON THIS FORM – PLEASE USE APHIS FORM 2000.

For additional information, visit the web site at <http://www.aphis.usda.gov/ppq/permits>

TYPE or PRINT legibly. Do not leave any boxes unfilled. If a box does not apply, enter “Not Applicable” or “N/A.” If you need to provide additional information or require more space, enter “See Attachment” and continue on a separate page. Label each page with “PPQ Form 526,” block number, your last name, and your affiliation.

HOW TO COMPLETE EACH BLOCK:

Block 1: Enter the complete name as shown on passports, legal documents, etc. Only one applicant is allowed per application and the applicant must be a U.S. resident. We strongly encourage academic advisors to apply for permits on behalf of their students as durations of permits issued to students will be restricted. The name of the applicant should be the person actually responsible for the requested organism(s) and permit conditions. Institutions and businesses should apply for permits under the name of the person using the organism(s) and not coordinators.

Block 2: Enter telephone number, including the Area Code and any extensions. Follow this with your facsimile number, including the Area Code. Please provide your e-mail address (optional).

Block 3: Indicate which type of pest is to be moved; Pathogens, Arthropods, Noxious weeds, or other. If you choose “other” enter the organism type on the provided line. Diagnostic laboratories would select “other” and enter “diagnostic” on the line and in blocks 4-6 list the type of organisms (e.g., phytopathogenic bacteria, plant viruses, or nematodes). Check “other” if pest status is unknown or not described in the categories provided (e.g., nematode-trapping fungi).

Invertebrate animals – e.g., insects, nematodes, snails, slugs, mites

Parasitic plants - plants that feed on other plants

Plant pathogens - e.g., fungi, viruses, bacteria, or related pests that infect plants

Entomopathogens - organisms that cause disease in insects

Noxious weeds - plants listed in CFR 360; visit the above website for this list

Biological Control Organisms - e.g., herbivores, parasites, parasitoids, predators and pathogens of invertebrate and microbial plant pests and of weeds.

Bees - USDA regulates only the importation of pollinating bees (honey bees, bumble bees, etc.) and not their interstate movement.

Blocks 4, 5, and 6: If not enough space is provided to list organisms, continue on a separate sheet and attach. Submittal of separate applications for arthropods and pathogens is encouraged. For large numbers of organisms, separate them into ‘related’ groups. Permit conditions for plant viruses, phytopathogenic bacteria, and arthropods differ and may require separate permits for scientific review if you request to inoculate plants.

Appendix I: PPQ Form 526 Permit Application to Transport Biological Control Agents, *cont.*

Columns A and B: Scientific Name and Classification: Enter the scientific name (genus and species) and the author, if known (e.g., *Cinara strobilifera* (Fitch) or *Baris lepidii* Germar). If sub-designations exist, list them, e.g. races, pathovars, subspecies, strains, or geographic isolates. If unknown enter as “unknown.” If the species is unknown, list the genus and, if possible, other identification such as a specimen or culture number. If sub-designations such as races, pathovars, subspecies or strains are desired, list the appropriate sub-designation. If these are not known or are undetermined, then enter “unknown.” Use correct spelling. Viruses should be identified using approved descriptive names.

Column C: Life Stages: For invertebrate animals use eggs, juveniles, larvae, nymphs, pupae, or adults. For fungi use spores, mycelia, fruiting bodies. For bacteria and viruses enter “N/A.” For plants use seeds, whole plants, or plant parts (such as leaves, stems, fruits, etc.)

Column D: Enter the number of specimens or units.

Column E: Enter the Country or State from which the organism(s) are originally being shipped.

Column F: Organism Establishment: If the requested organism/biotype/pathovar/isolate/etc. is of limited distribution in the United States, describe its distribution on an attached sheet.

Column G: Major Hosts: List the scientific name of the major hosts (or prey) of the organisms applied for, even when you do not intend to include them in shipments of the organisms for which you are applying. Enter “N/A” for non-parasitic noxious weeds.

Block 7: Media or host material accompanying the organism: List scientific name of host organisms or host plants that will accompany material in shipments. Be specific and accurate e.g. seeds, dried leaves, tissue cultures, fruits, stems, or soil. For nonparasitic weeds, enter “N/A.” For pathogens, list all components of the media. Describe the culture as pure or mixed with contaminants and identify known contaminants. If an application is only for ‘pure culture’ to be moved, the permit conditions will state that only ‘pure cultures’ are allowed, and if seeds, leaves and/or other materials are present in a shipment, then the entire shipment may be rejected at the PPQ Inspection Station.

Block 8: Destination: List the city and State where shipments will be received, housed, reared, or released. If the destination is the applicant’s address, list “Same as item #1.” If you intend to release the organism into the environment, list the exact location of the field tests including the county.

Block 9: Port of Arrival: List the desired port(s), otherwise enter as “unknown.” USDA will assign the port of entry. For interstate movement, enter “N/A”.

Block 10: Enter the estimated dates of shipments and/or releases, if known.

Block 11: Enter the approximate number of shipments. For multiple shipments, indicate the number per a given time period, e.g. 2 per year.

Block 12: Enter the supplier’s name and address.

Block 13: Method of Shipment: Check the appropriate box. For express deliveries, enter the company.

Block 14: Intended use: Be specific. Use a separate sheet to describe complex activities.

Describe the specifics such as handling, containment, disposal, use and purpose.

For microbes, do you plan to inoculate plants? Will the infected plants be housed in the laboratory (e.g. as tissue cultures or plantlets in sealed containers), in growth chambers, in greenhouses, or field tests? For field tests: Is the microbe you intend to release into the environment the same as those that occur naturally in the release area? What is the size (acreage or number of plants) of the proposed field test? How do you intend to reduce any inoculum in the local area following termination of the research?

Block 15: State the methods that will be used to prevent plant pest escape. If you stated that an organism is NOT established in the United States (see Box F), provide a detailed explanation on how this organism will be contained.

Block 16: Method of Final Disposal: List the proposed method(s) and date of final disposal of organisms, such as; autoclaving, freezing, double-bagging, and disposal in a land fill. Describe treatments in detail, e.g. temperature, pressure, or duration. For environmental release, enter “N/A.”

Block 17: The person named in Block 1 must sign the application.

Block 18: Enter the date the application was completed and signed.

Do not fill in or place other markings in boxes below Block 18.

SUBMIT this form with any attachments by mail to:

USDA-APHIS-PPQ, Permits, Registrations and Imports (PRI), Pest Permit Evaluations, 4700 River Road, Unit 133, Riverdale MD 20737

OR FAX TO 301-734-8700 or 301-734-4300.

Do not mail and fax the same application. Do not mail or fax the application to the State. APHIS-PPQ will notify the State during the review process.

Please call for help or information: 301-734-4393 or 301-734-8896.

Appendix I: PPQ Form 526 Permit Application to Transport Biological Control Agents, *cont.*

STANDARD SAFEGUARDS OF PERMIT

1. All pests must be shipped in sturdy, escape-proof containers.
2. Upon receipt of pests, all packing material media, substrate, soil and shipping containers shall be sterilized or destroyed immediately after removing pests.
3. Pests shall be kept only within the laboratory or designated area at the permittee's address.
4. No living pests kept under this permit shall be removed from confined area except by prior approval from State and Federal regulatory officials.
5. Without prior notice and during reasonable hours, authorized PPQ and State regulatory officials shall be allowed to inspect the conditions under which the pests are kept.
6. All pests kept under this permit shall be destroyed at the completion of the intended use, and not later than the expiration date, unless an extension is granted by this issuing office.
7. All necessary precautions must be taken to prevent escape of pests. In the event of an escape, notify this office.

Appendix J: Recovery and Sampling Report Form

USDA-APHIS-PPQ
Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT

Release (FIS) Location: _____ **Release code:** _____
 State: _____ County: _____
 Site name: _____
 USGS coordinates: T _____ R _____ Sect. _____ Qtr-sect. _____
 Latitude: _____ N _____ Longitude: _____ W (GPS derived? _____ YES _____ NO)

◆ **INSECT(S) RELEASED:** ◆

_____ Flea beetle *Aphthona nigriscutis* Year(s) of original release: _____
 _____ Flea beetle *Aphthona flava*
 _____ Flea beetle *Aphthona cyparissiae*
 _____ Flea beetle *Aphthona czwalinae/A. lacertosa*
 _____ Long-horned beetle *Oberea erythrocephala*
 _____ Gall midge *Spurgia esulae*

◆ **SAMPLING INFORMATION** ◆
 [Sampling procedures are described below]

SAMPLING DATE: _____ **SAMPLING TIME (approx.):** _____

Weather conditions: _____ Clear _____ Partly cloudy _____ Overcast _____ Rain _____ Other (specify: _____)
Air temp. (°F): _____ <60° _____ 60-70° _____ 70-80° _____ 80-90° _____ >90° (Actual temp., if measured: _____)
Wind: _____ Calm _____ Light _____ Moderate _____ Strong

Visual observation of insect, before sweeping? _____ YES _____ NO

Number of:

Net sweeps: _____
TOTAL number of insects swept (sum from reverse): _____
 ☞ For *Spurgia esulae* only, **number of galls observed:**
 _____ None _____ <25 galls _____ 25-100 galls _____ >100 galls

Observer: _____
Affiliation: _____

◆ **GENERAL SAMPLING INSTRUCTIONS** ◆

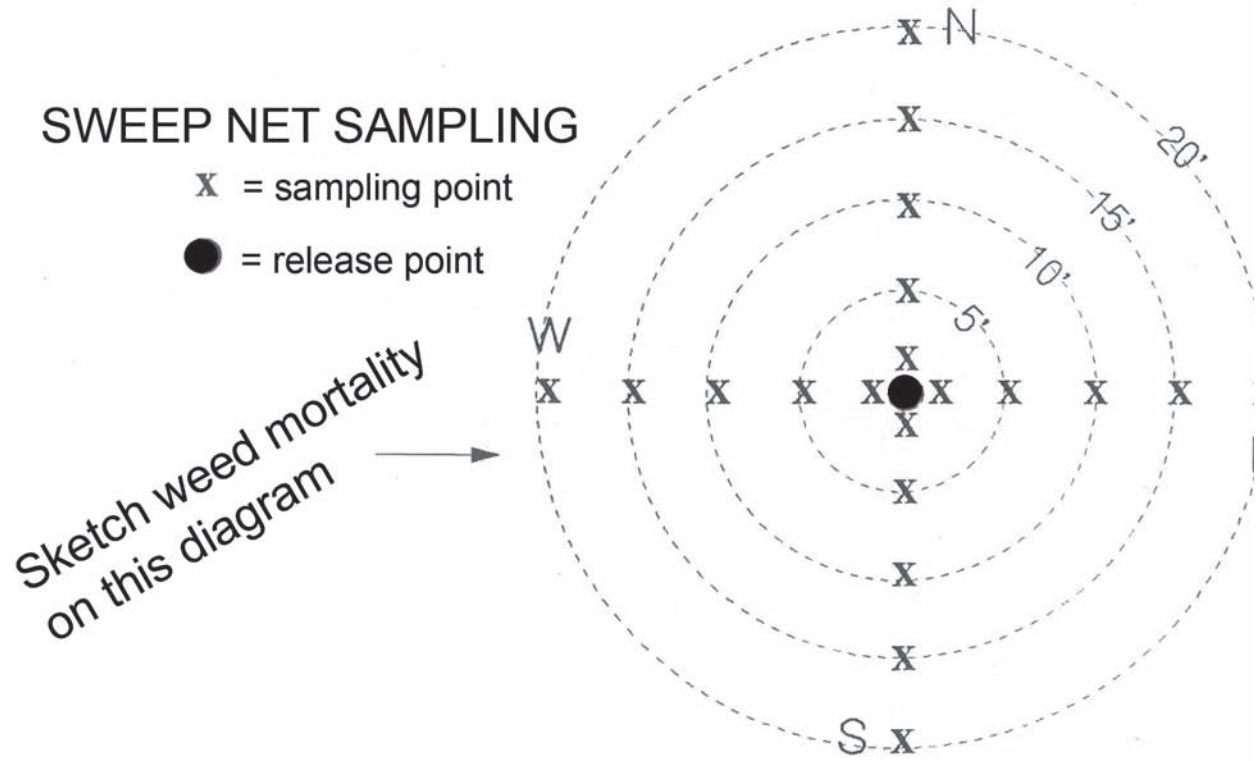
For *Aphthona* spp. and *Oberea erythrocephala*: First, look over the release area to see if biocontrol insects are visually apparent. Next, five sampling points will be swept along four lines in N, S, E, and W direction from release point (20 points total). For each line, begin as close to the release point as possible. Using a 15-in diameter sweep net, make **four** sweeps in front of you (back and forth twice). Each net sweep should proceed in a downward arc, so that the net moves vigorously through the vegetation as close to the ground as possible. Carefully examine the net and count the biocontrol insects present, then empty the net to release counted insects. Move 5 to 6 ft (2 paces) out and repeat above steps. Continue until five points have been sampled, then repeat over the remaining cardinal directions. A diagram of the sampling procedure and a chart on which to record insect counts is provided **on the back of this form**.

During the sweeping process, collect a **maximum** of 15 beetles from the site and place them in 70% alcohol in a **clear** glass vial. (NOTE: **If sampling an *O. erythrocephala* release, collect only one or two adult beetles.**) Place a completed label (provided by BBCF) into the vial, and send specimens to the Bozeman Biocontrol Facility along with this form. If no specimens are collected, return only the completed form.

Using the diagram on the back of this sheet, sketch out any spurge mortality or noticeable reductions in spurge density that are visible around the release point.

For *Spurgia esulae*: At *Spurgia esulae* releases, **do not use the sweep-net sampling procedure** described above. Instead, survey an area roughly 50-75 ft in diameter and centered at the original release point and note the relative abundance of galls on spurge stems. Up to five galls may be collected, placed in an alcohol-filled glass vial, and returned to BBCF to confirm identification. (R. Hansen, rev. 05/06)

Appendix J: Recovery and Sampling Report Form, cont. Sweep Net Sampling Diagram



INSECT COUNTS

	N	S	E	W
0				
5'				
10'				
15'				
20'				

Appendix K: Leafy spurge vegetation sampling; Daubenmire quadrats.

Site: _____
 State: _____
 Sampling date: _____

Site code: _____
 County: _____
 Sampler(s): _____

Cover class	% cover	Midpt.
1	0 – 5	2.5
2	5 – 25	15.0
3	25 – 50	37.5

Cover class	% cover	Midpt.
4	50 – 75	62.5
5	75 – 95	85.0
6	95 – 100	97.5

Sample	Leafy spurge			Grasses	Forbs	Woody	Litter	Bare	Notes
	Cover class	No. stems	Avg. ht (cm)	Cover class	Cover class	Cover class	Cover class	Cover class	
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
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