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Biological Control

Biology and Biological Control of Purple Loosestrife

Linda M. Wilson, Mark Schwarzlaender,
Bernd Blossey, and Carol Bell Randall

Forest Health Technology Enterprise Team—Morgantown

USDA U.S. Department of Agriculture

Forest Service

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Biology and Biological Control of Purple Loosestrife

Linda M. Wilson[1], Mark Schwarzlaender[1], Bernd Blossey[2], and Carol Bell Randall[3]

In cooperation with Gary Piper (Washington State University)
and Eric Coombs (Oregon Department of Agriculture)

[1] Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, Idaho
[2] Department of Natural Resources, Cornell University, Ithaca, New York
[3] USDA Forest Service, Coeur d'Alene, Idaho

For additional copies of this publication, contact:

Carol Bell Randall
USDA Forest Service
Coeur d'Alene
Idaho
(208) 765-7343
crandall@fs.fed.us

Richard Reardon
FHTET, USDA Forest Service
Morgantown
West Virginia
(304) 285-1566
rreardon@fs.fed.us

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INTRODUCTION

Overview

Purple loosestrife, *Lythrum salicaria* (Figure 1), is a member of the Loosestrife family (Lythraceae). It is a large, perennial, wetland plant that can grow up to 9 feet (3 m) tall. It was introduced to the northeastern United States and Canada in the 1800s from Europe, probably as seed contained in soil used as ship's ballast. It was also brought to North America for ornamental and medicinal purposes.

Figure 1. Purple loosestrife. (UGA1291003)

Once established in North America, this invasive weed quickly spread in wetlands and waterways. It is reported in nine Canadian provinces and in the United States it occurs in all states except Florida and Hawaii (Figure 2). Although purple loosestrife is declared noxious in 23 states and 5 Canadian provinces, it is still sold as an ornamental plant in some places.

Figure 2. Map of purple loosestrife distribution in the United States and Canada.

In its native range, purple loosestrife occurs from the United Kingdom west to western Russia and from Finland in the north to southern Europe and northern Africa in the south.

Purple loosestrife invades natural and disturbed wetlands, such as stream banks, lakeshores, marshes, bogs, fens, sedge meadows, canals, drainage ditches, reservoirs, riparian meadows, wet prairies, and sub-irrigated pastures. Established plants can tolerate drier sites, posing a threat to agricultural lands and pastures.

Once established, purple loosestrife quickly crowds out most native vegetation, such as sedges, rushes, cattails, and other wetland plant species. At high densities, purple loosestrife can create near-monocultures. In addition to the loss of native biodiversity, purple loosestrife harms waterfowl nesting habitat, has negative impacts on some amphibians and algal communities, reduces water flow and quality, inhibits transportation, and degrades hunting and fishing areas.

Successful management of purple loosestrife is an intensive process. Herbicides approved for use around water provide short-term control of small infestations or isolated plants, but may be impractical and uneconomical against large infestations. Pulling small, individual plants is feasible; pulling large plants is very difficult. Plowing or disking small infestations is also possible, but will require repeated treatments as plants will sprout from cut root pieces. Plowing large infestations is difficult and generally ineffective. Mowing, burning, and flooding can be effective management strategies, but are difficult, expensive, and time-consuming, and may have severe negative, long-term consequences for wetland communities.

The biological control program for purple loosestrife began in 1985 at the International Institute of Biological Control in Switzerland with the search for, and testing of, potential biological control agents. In 1992, two European leaf-feeding beetles and a root-feeding weevil were imported and released. An additional flower-feeding weevil was subsequently introduced in 1994, completing the complex of four insects for biocontrol of purple loosestrife in the United States and Canada. Today, biological control of purple loosestrife is one of the most widely implemented biocontrol of weeds programs in North America.

Biological Control of Weeds

Most invasive weeds in the United States are not native: they arrived with immigrants and commerce from Europe and, to a smaller extent, South Africa, Australia, and Asia. Some plant species were brought intentionally for medicinal purposes or to adorn gardens; others arrived as stowaways in ship's ballast or clinging to livestock, clothing, and other goods. Exotic plant species continue to be introduced accidentally as contaminants of agricultural goods or purposefully through travel and commerce (particularly for gardening), and some of these species will likely prove to be invasive in the future. These immigrant plants are generally introduced without their natural enemies, a complex of plant-eating organisms that are specialized to feed only on a particular plant species. Lack of control by its natural enemies is one of the main factors explaining why non-native plant species become major pests in the new area of introduction.

Biological control of weeds is the deliberate use of naturally occurring organisms to limit the distribution and abundance of a target weed. *Classical* biological control uses host-specific, natural enemies from the plant's native range (the terms "biological control" or "biocontrol" used throughout this manual refer to classical biological control). Natural enemies (also referred to as "biocontrol agents," "bioagents," and "biological control organisms") can directly kill or severely damage plants by destroying seeds, roots, foliage, or stems. Their damage may limit weed reproduction or facilitate secondary infection from pathogens. These stresses reduce the weed's ability to compete with other plants. The aim of weed biocontrol, therefore, is to restore at least a part of the ecological balance that limits the competitive ability of an invasive plant species in its native range.

There are a number of advantages to classical biological control of weeds. Biocontrol is selective of a specific weed or closely related group of weeds; it can provide long-term control; and the biocontrol agents are self-perpetuating, thus avoiding recurring acquisition, rearing, and reintroduction costs. The disadvantages of biocontrol of weeds are the high initial costs of a program and the uncertainty that the agents will be effective. Additional disadvantages include the risk of unintended, adverse impacts on other plant species (*non-target effects*) and that, once released, biological control agents cannot be retrieved. Because biocontrol agents are irretrievably introduced into the environment, they must be carefully selected and extensively studied before being approved for release.

Natural enemies used in classical biological control of weeds include insects and mites, and sometimes nematodes and fungi. Beetles, flies, and moths are among the most commonly used insects. To be considered for release in the United States, insect biological control agents must eat and develop only on the target plant, and in some cases, on only a few closely related plant species. Also, the insect's lifecycle should be closely matched, or *synchronized*, with the target plant's. For example, when properly synchronized, foliage-feeding insects would be in the feeding stage when the weeds are actively growing, and root-feeding insects would be in the feeding stage when resources in below-ground tissues are at their maximum.

The most important precondition for an insect to be used as a biocontrol agent is that it will die without the target plant. This is known as *host specificity*, and is the ecological cornerstone of classical biological control of weeds. Potential biocontrol agents often undergo more than five years of rigorous testing to ensure that host-specificity requirements are met. These studies are important in order to:

- Have effective, safe biocontrol agents
- Introduce biocontrol agents that damage only the target plant
- Protect non-target plant species

The United States Department of Agriculture–Animal and Plant Health Inspection Service–Plant Protection and Quarantine (USDA-APHIS-PPQ) is the federal government agency responsible for authorizing the importation of biocontrol agents into

quarantine. All states have their own final approval process to permit field release of plant biocontrol agents. The Technical Advisory Group (TAG), an expert committee of volunteers representing federal land management and environmental protection agencies, reviews all petitions to import new biocontrol agents, and makes recommendations to USDA-APHIS to aid their decision making process. Effective laws and regulations are in place to minimize the risks to native plant and animal communities associated with the introduction of exotic organisms to manage weeds. Weed biocontrol researchers work closely with USDA-APHIS-PPQ and TAG to further maximize the environmental safety of weed biocontrol programs.

Although weed biocontrol is an effective and important ecological management tool, it does not work in all cases and is not expected to completely eradicate a weed. For example, the expectation of classical biological control of purple loosestrife is to reduce the abundance of purple loosestrife to levels that do not damage the rest of the plant community. In general, classical biocontrol programs reduce a weed's ability to compete with native plants. Often, biological control can be integrated with other methods of weed control—e.g., chemical or cultural control and livestock grazing. For more information on classical biological control of weeds we recommend reading the literature listed in the Selected References section of this manual.

About This Manual

This manual provides background information on purple loosestrife biology and on each of the four insects selected for biocontrol of purple loosestrife. It also provides guidelines to establish and manage a purple loosestrife biocontrol program. The manual is divided into the following chapters:

Chapter 1 provides a detailed description of purple loosestrife, including scientific name, description of the leaves, stems, flowers, seeds, habitat, and occurrence in the United States and Canada. Photographs, and drawings are provided. Closely related *Lythrum* species, as well as unrelated plant species often confused with purple loosestrife are also described.

Chapter 2 describes the four purple loosestrife biocontrol agents: two leaf beetles and two weevils. Included is information on biocontrol agent identification, lifecycles, and biology. This chapter is particularly useful for identifying biocontrol agents in the field.

Chapter 3 includes detailed information and guidelines on how to plan, implement, monitor, and evaluate an effective purple loosestrife biocontrol program incorporating one or more of the four biocontrol agents. Included are guidelines and methods for:

- Planning a purple loosestrife biocontrol program
- Selecting and preparing release sites

- Collecting, handling, transporting, shipping, and releasing purple loosestrife biocontrol agents
- Monitoring of biocontrol agents and their effects on vegetation

Glossary defines technical terms essential to communicating specifics of purple loosestrife biological control.

Selected References are provided from the large body of literature on purple loosestrife biology, ecology, and biological control. Also included is a comprehensive list of Internet web sites that provide a wealth of practical information.

Appendix provides a troubleshooting guide, examples of insect release and monitoring forms, and vegetation monitoring forms. (These forms may be reproduced for use as needed.) These are, in order of appearance:

Troubleshooting Guide: When Things Go Wrong

Purple loosestrife Biocontrol Release Form

Monitoring Plan Questionnaire

Form 1: Purple Loosestrife Biocontrol Monitoring – Site Location

Form 2: Purple Loosestrife Biocontrol Monitoring – Biocontrol Agents

Form 3: Purple Loosestrife Biocontrol Monitoring – Vegetation

Form 4: Purple Loosestrife Biocontrol Monitoring – Associated Vegetation

CHAPTER 1: GETTING TO KNOW PURPLE LOOSESTRIFE

Scientific name: *Lythrum salicaria* L.
Common names: Purple loosestrife, purple lythrum, spiked loosestrife
Family Lythraceae (Loosestrife)

Description: Purple loosestrife (Figure 3a) is an erect, herbaceous, perennial wetland weed that is commonly found along waterways and other wetland habitats. It was introduced from Europe during the late 1800s.

Leaves: Purple loosestrife leaves are 2-5 inches (5-12 cm) long and narrow with a rounded or heart-shaped base, and smooth-edged. The stalkless leaves are arranged opposite or alternate along the stem, and lower leaves often form a whorl around the stem.

Stems: One of the more recognizable features of purple loosestrife is its square-shaped stems. They are five- or six-sided (you can feel the edges of the stem when you roll it between your fingers), woody, and can be either smooth or covered with downy hairs. Stems have short, slender branches and evenly spaced nodes. Mature plants have up to 30 flowering stems, which can reach a height of 5-9 feet (1.5 to 3 m). Dead stems persist through the winter and often decay slowly over several years, and new shoots are produced each spring from buds on the persistent, woody rootstock.

Flowers: The inflorescence of purple loosestrife is a spike of numerous, showy, reddish-purple or magenta flowers set in clusters (Figure 3b). Each flower measures about 0.6-0.8 inches (15-20 mm) across, has five to seven petals, and a small, yellow center. Flowering occurs from mid-June to September, depending on location.

Seeds: Seeds are produced in rounded capsules about 0.24 inches (6 mm) in length. The capsules open to release more than a hundred tiny, light brown seeds about the size of poppy seeds (Figure 3c). Water, wind, wildlife, and humans easily spread the lightweight seeds that are shed throughout the winter. Purple loosestrife is a prolific seed producer; a single mature plant can produce several million seeds. When purple loosestrife densities are high, billions of seeds are produced per acre. Seed viability is greater than 90 percent and seeds can remain viable in the soil for many years.

Figure 3. Purple loosestrife: a) inflorescence (UGA1291004); b) flowers (UGA1291005); and c) seeds (UGA1291006).

Biology and Ecology: Seeds germinate in late spring and early summer in open, sunny places when soil temperatures reach 68°F (20°C). Seedlings lodge into moist soil and quickly grow to over 3 feet (1 m) tall in their first growing season, and many even flower (Figure 4). Even young plants develop a large taproot, and have an extensive rootstock by the end of the first growing season. The mature size of the plant will depend, in part, on growing conditions, soil type, water level, the genetic potential of the plant, and plant density. After the first extreme frosts in fall, all above-ground plant parts will die back. Plants resprout each spring from their large rootstocks (Figure 5).

Any sunny or partially shaded wetland is susceptible to purple loosestrife invasion. Although purple loosestrife generally prefers moist soils, mature purple loosestrife plants can tolerate a wide range of water levels, pH and climatic conditions, soil, and vegetation types. In flooded areas, the plant forms dense, fibrous rootmats.

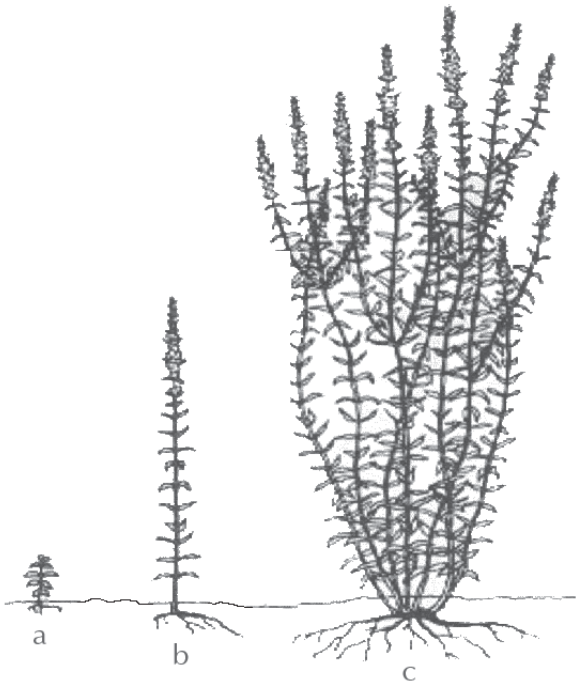


Figure 4. Purple loosestrife: a) seedling, b) one-year-old plant, and c) three-year-old plant. Adapted from Thompson et al. (1987).

Purple loosestrife spreads primarily by seeds. Seeds are dispersed by floating on streams, by birds, wildlife and livestock, or in the mud of vehicle tires or boots. In addition to spread by seeds, purple loosestrife also spreads vegetatively. Root fragments cut from the plant can produce new plants and stem pieces may generate new infestations when they float downstream and lodge against a streambank.

Comments: Although still sold and planted for its beauty as an ornamental, purple loosestrife's habit of devastating waterways and wetlands has caused its sale to become restricted or, in some states, illegal. It is especially important to know that the species called European wand loosestrife, *L. virgatum*, and widely sold as an ornamental, is the same species as purple loosestrife (*L. salicaria*). Thus, garden and pond plants that are being sold as *L. virgatum* are in fact *L. salicaria*, and may be illegal for sale in your area. Studies have shown that other 'varieties' of loosestrife sold as ornamentals are generally not sterile as advertised.

Figure 5. Purple loosestrife sprouting from rootstocks amid previous year's dead stems. (UGA1291009)

In addition to purple loosestrife, there are a number of other *Lythrum* species in the United States and Canada. Some species are native (see Table 1a) and some, like purple loosestrife, are introduced (see Table 1b).

Other plant species are often confused with purple loosestrife. While they are not related to purple loosestrife, they may be similar in appearance. They include hardhack (*Spirea douglasii*), fireweed (*Epilobium angustifolium*), and blue vervain (*Verbena hastata*) (see Table 2).

Table 1a. Species of *Lythrum* in the United States and Canada: native species.

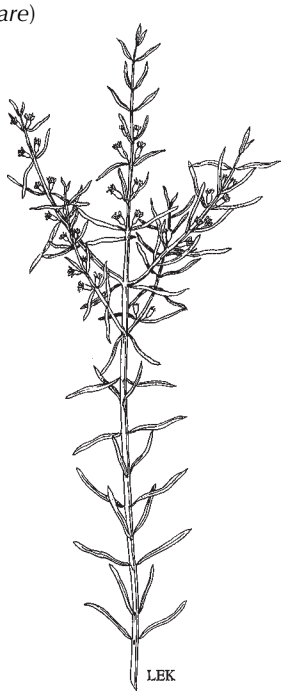
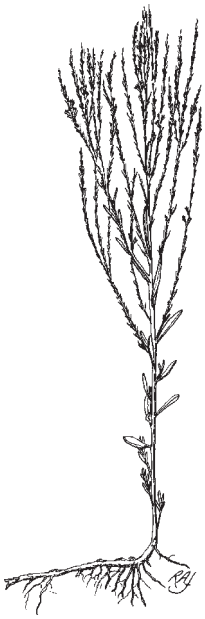
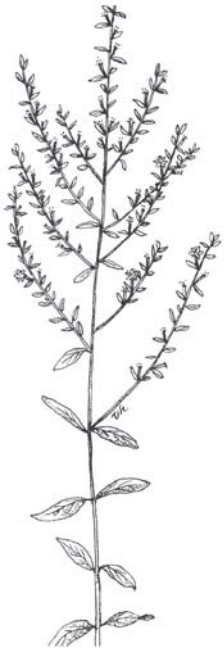
Wing-angled loosestrife (*Lythrum alatum*) 	Saltmarsh loosestrife (*L. lineare*) 	Florida loosestrife (*L. flagellare*) 
California loosestrife (*L. californicum*) 	Curtiss' loosestrife (*L. curtissii*) 	Low loosestrife (*L. ovalifolium*) 

Table 1b. Species of *Lythrum* in the United States and Canada: introduced species. Not shown: spatula-leaf loosestrife (*L. portula*) and thyme-leaf loosestrife (*L. thymifolia*).

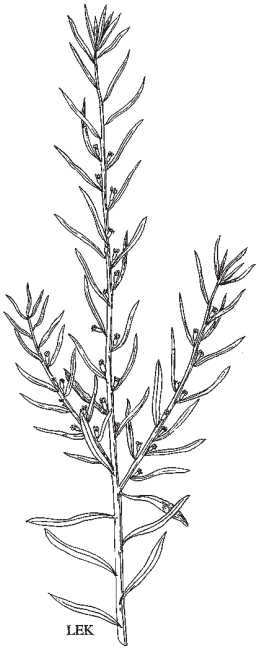
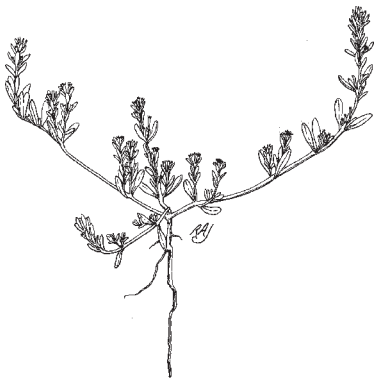
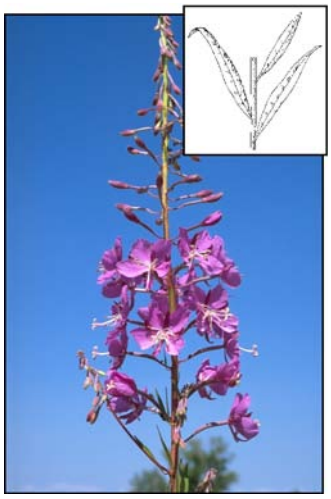
European wand loosestrife (*Lythrum virgatum* (= *L. salicaria*) 	Hyssop loosestrife (*L. hyssopifolia*) 
Punkamole (*L. maritimum*) 	Three-bract loosestrife (*L. tribracteatum*) 

Table 2. Comparison of purple loosestrife and similar looking plants. (Left to right, top to bottom: UGA1291040, UGA1291041, UGA1291042, UGA1291043)

Hardhack (*Spirea douglasii*) Family: Rosaceae Description: Stems are round; leaves are alternate, oval to oblong, on short petioles, with toothed margins from the midpoint to the tip, and whitish on the lower surface; 5-petaled, rose-pink flowers occur in a dense panicle, 5-15 cm long. 	**Fireweed** (*Epilobium angustifolium*) Family: Onagraceae Description: Stems are round; leaves are alternate, smooth, and lance-shaped, very short petiole; purple flowers borne in a loose, terminal raceme or in the axils of uppermost leaves. 
Blue vervain (*Verbena hastata*) Family: Verbenaceae Description: Stems are square; leaves are opposite, rough-hairy, narrowly oblong on short petioles, toothed or dissected; purple-blue flowers arise on slender, dense spikes—terminal flowers open first. 	**Purple Loosestrife** (*Lythrum salicaria*) Family: Lythraceae Description: Stems are square-sided (more than 4 sides); leaves are opposite or alternate, entire with a rounded, clasping (no petiole) base; flowers have 5-7 reddish-purple or magenta petals and a yellow center arranged in a loose spike. 

CHAPTER 2: BIOLOGY OF PURPLE LOOSESTRIFE BIOCONTROL AGENTS

Basic Insect Biology

Insects are a very large, diverse class of animals. Basic knowledge of insect anatomy and lifecycle will help in understanding insects, and recognizing them in the field.

Adult insects have several unique characteristics; an *exoskeleton* (outside skeleton), a segmented body comprised of a head, thorax, and abdomen, and three pairs of legs (Figure 6). Because insects have a skeleton on the outside, they must shed their skeleton as larvae in order to grow. This process of shedding the exoskeleton is called *molting*.

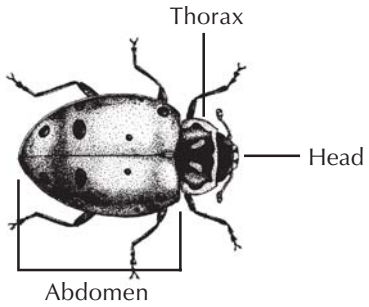


Figure 6. Diagram of beetle anatomy (a ladybird beetle is used as the example).

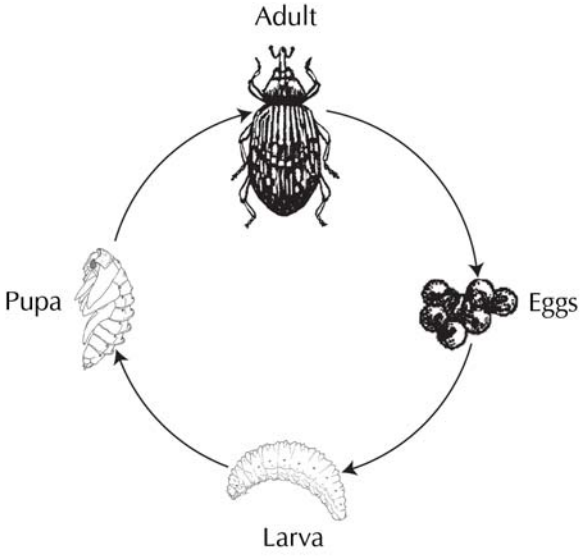


Figure 7. Insect lifecycle showing complete metamorphosis (a weevil is used as the example).

The period between molts is called an *instar.* As larvae, insects grow a lot, often having to go through three to five molts.

Mature larvae molt into a *pupa*, the stage when the insect body changes from a larva into the adult. Insect development from an egg to adult is called *metamorphosis*. The insects we will be discussing in this manual go through complete metamorphosis, meaning they have four developmental stages: egg, larva, pupa, and adult (Figure 7). Once the insects become adults, they stop growing, and therefore stop molting.

Purple Loosestrife Biological Control Insects

All four of the insect species introduced for purple loosestrife biocontrol are beetles. Beetles are hard-bodied insects with tough exoskeletons. Adult beetles possess two pairs of wings: the front pair is thickened to form a hard covering, called the *elytra*, which meet in a straight line down the middle of the back and cover the larger, membranous hind wings, which are used for flight. Beetles that feed on plants, like those used for purple loosestrife biocontrol, have chewing mouth parts.

The four species used for purple loosestrife biocontrol belong to three different families of beetles: one family of leaf beetles (two species) and two families of weevils (one species each). Leaf beetles are small, foliage-feeding beetles; both adult leaf beetles and their larvae feed externally on plant foliage. Larval feeding often is more damaging, and can result in complete defoliation of plants. Weevils are generally plant-feeding beetles with long snouts bearing chewing mouthparts at the tip. They use the snout to chew and feed inside plant tissues or to notch out holes in which to lay their eggs. Similar to leaf beetles, weevil larvae (which feed internally in the stems, roots or flowers) often cause more damage to plants than adults. However, some weevil adults do cause significant feeding damage to foliage.

The leaf beetles are *Galerucella calmariensis* L. and *Galerucella pusilla* Duftschmidt; one weevil is a root-mining weevil, *Hylobius transversovittatus* Goeze and the other is a flower-feeding weevil, *Nanophyes marmoratus* Goeze (Table 3). Each species is described in the following sections.

Table 3. Purple loosestrife biological control insects. (Top and center: UGA1291044 and UGA1291045)

Insect Type	*Scientific Name* Common Name	Appearance
Leaf beetle	*Galerucella calmariensis* (L.) Black-margined loosestrife beetle *Galerucella pusilla* Duftschmidt Golden loosestrife beetle	 Actual length
Weevil	*Hylobius transversovittatus* Goeze Loosestrife root weevil	 Actual length
Weevil	*Nanophyes marmoratus* Goeze Loosestrife flower weevil	 Actual length

Galerucella calmariensis L.
Common name: Black-margined loosestrife beetle

Galerucella pusilla Duftschmidt
Common name: Golden loosestrife beetle
Order: Coleoptera
Family: Chrysomelidae

Galerucella calmariensis and *G. pusilla* are two nearly identical species of leaf-feeding beetles (Figure 8). Both beetles are light brown in color, but *G. calmariensis* (Figure 8a) usually has a black triangle or black line on the thorax while in *G. pusilla* (Figure 8b), the line is thin or nearly absent (these characters develop fully only in overwintered beetles). The beetles are the same size—about 0.15 to 0.3 inches (4 -6 mm) in length—and have the same habits. Released in 1992, these two leaf beetles were the second loosestrife insects to be introduced in the United States and Canada for biological control of purple loosestrife. They are now widely established in more than 30 states and 8 provinces where purple loosestrife is a problem.

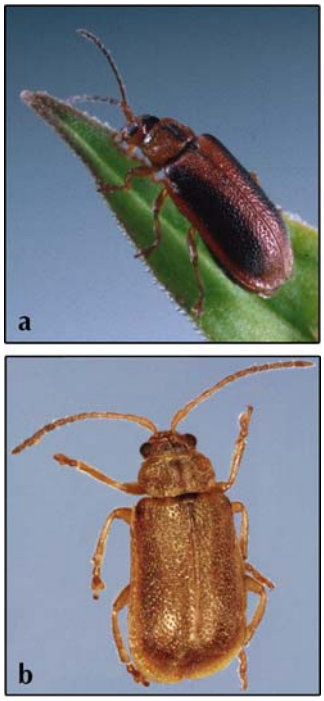


Figure 8. *Galerucella* adults: a) *G. calmariensis* (UGA1291008) and b) *G. pusilla*.

In early spring, adult beetles emerge from their overwintering sites in plant litter. They live eight to ten weeks after they emerge in the spring, feeding at first, and then mating and laying eggs. Adult feeding on loosestrife leaves and young shoot tips results in a characteristic "shothole" damage of the plants (Figure 9). Mating and oviposition begin in late May or early June. A single female can lay as many as 400 eggs in her lifetime. Eggs are laid in batches of one to ten on leaves and stems (Figure 10). Each egg is covered with a black line of *frass* (fecal deposit).


Figure 9. Adult *Galerucella calmariensis* feeding damage on purple loosestrife (UGA1291009).

Larvae hatch from eggs after about one week and move to leaf buds where they remain well-concealed as they feed. As they get older and larger, larvae openly feed on leaves and stems. The larvae of the loosestrife beetles look like tiny caterpillars with black heads and yellowish bodies (Figure 11). After completing three instars, mature larvae move into the litter beneath purple loosestrife plants to pupate. On flooded purple loosestrife they pupate in the spongy tissue (called *aerenchyma*) that develops on the flooded portion of the stem.

Figure 10. *Galerucella calmariensis* egg clusters on purple loosestrife. (Background: UGA12910010; inset: UGA1291011)

Figure 11. *Galerucella calmariensis* eggs and larvae on purple loosestrife. (UGA12910012)

Development time, from egg to adult, is 30-40 days (Figure 12). New adults emerge between mid-June and mid-July, feed for a limited time to accumulate body fat, and then seek overwintering sites in the leaf litter.

Both loosestrife beetle species usually have only one generation each year, but a partial second generation may occur in warm regions where the first new generation adults emerge before mid-June (before the summer solstice). At this time of year, warm temperatures and long day lengths may trigger beetles to mate and produce a second generation. If a second generation develops, new adults may emerge as late as the end of August.

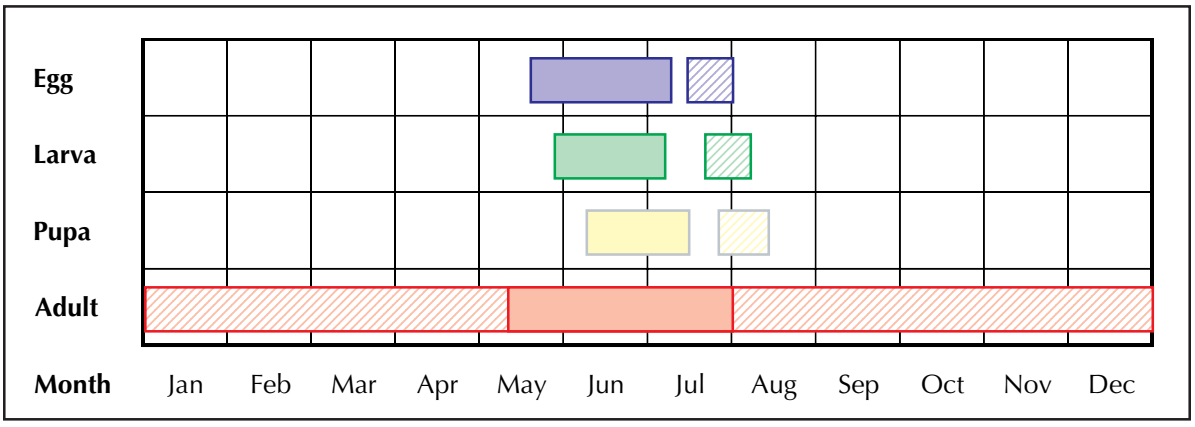


Figure 12. Schematic lifecycle of *Galerucella calmariensis* and *G. pusilla*. Solid colored bars represent the length of activity for each of the life stages. Short patterned bars for the upper three stages represent potential second generation activity, while the long patterned bars at the bottom represent adult overwintering.

Impact:

Adult loosestrife beetles are very good fliers and they can easily find new patches of purple loosestrife on which to feed and reproduce. Loosestrife beetle adults are known to disperse 2-4 miles a year. Although loosestrife beetles survive under a range of conditions, they do not thrive in shade or in areas where water levels fluctuate dramatically (e.g., dam reservoirs).

Larvae feed on the underside of leaves, stripping the photosynthetic tissue off while leaving the upper leaf cuticle and epidermis intact, creating a "window-pane" effect (see Figure 13). At lower larval densities, plants are less severely damaged. At high densities (greater than 4-5 larvae/inch stem or 2-3 larvae/cm of shoot length), stems can be defoliated. Large loosestrife beetle populations can defoliate 100 percent of the plants in an infestation many acres/hectares in size. While plants may recover after defoliation and flower late in the year, in some areas loosestrife beetle feeding has completely suppressed flowering. Plants that regrow after defoliation are often shorter and bushier than normal, unattacked plants.

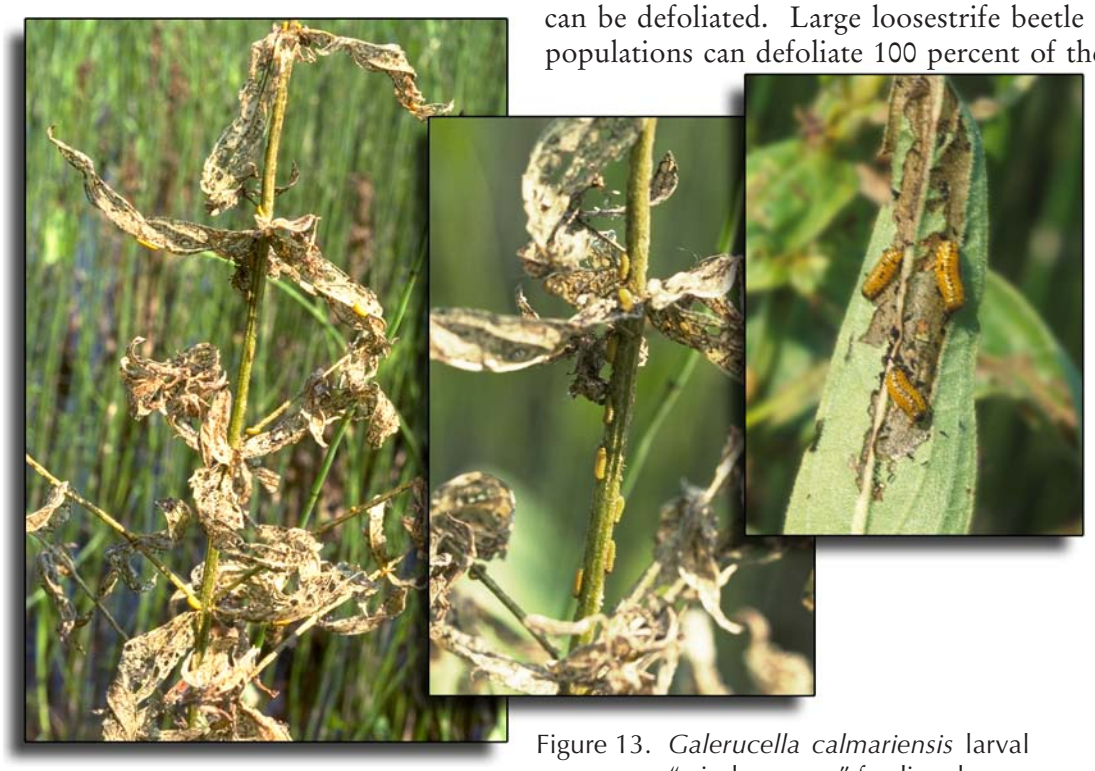


Figure 13. *Galerucella calmariensis* larval "window-pane" feeding damage on purple loosestrife. (Left to right: UGA1291013, UGA1291014, UGA1291015)

Predators and parasites of loosestrife beetles have also been reported in North America, although the specialist wasps that attack these species in Europe were carefully excluded when beetles were imported. Adult loosestrife beetles can be parasitized by a nematode that feeds and develops inside the beetle, eventually killing it. Other native predators of loosestrife beetles are ladybeetles, true bugs, predaceous beetles, spiders, and possibly birds, frogs, and lizards.

Hylobius transversovittatus Goeze

Common name: Loosestrife root weevil
Order: Coleoptera
Family: Curculionidae

Hylobius transversovittatus is a large, reddish-brown weevil, 0.4 to 0.6 inches (10 to 14 mm) in length (Figure 14). First released in 1992, it was the first of the loosestrife biocontrol insects to be introduced. It is now established at many sites across the United States and Canada. This weevil is nocturnal and long-lived as an adult (two to three years or longer).

Figure 14. Adult *Hylobius transversovittatus*. (UGA0002033)

In spring, overwintering adult weevils appear shortly after purple loosestrife shoots sprout. Loosestrife root weevils are most active at night, but can be found on their host plants in the early evening and morning hours or during cool, overcast, or rainy days. During warm, sunny days, they hide in the litter, often at the base of the plants.

Adults begin feeding on foliage and young stems, and within two weeks, mating and oviposition begin, which can last until September. Females deposit single, white, oval-shaped eggs into the soil close to the root-crown or into purple loosestrife stems (Figure 15). A female can produce more than 100 eggs annually.

Figure 15. *Hylobius transversovittatus* eggs in a purple loosestrife stem. (UGA1291016)

Young larvae hatch in about eleven days and start to mine the root hairs (if hatched from eggs laid in the soil) or into the stem. They later feed on the outside of the root, and then mine into the center of the root where they continue to feed for one to two years (Figure 16). Mature third instar larvae move to the upper part of the root to pupate.

The new loosestrife root weevil generation emerges from late June to October. Development time from egg to adult is one to two years (see Figure 17).

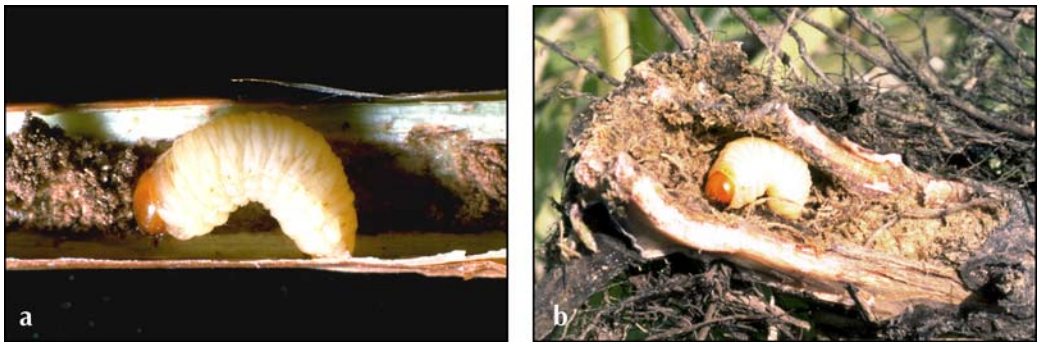


Figure 16. *Hylobius transversovittatus* larvae in a purple loosestrife: a) stem, and b) root.
(Left to right: UGA1291017 and UGA129018)

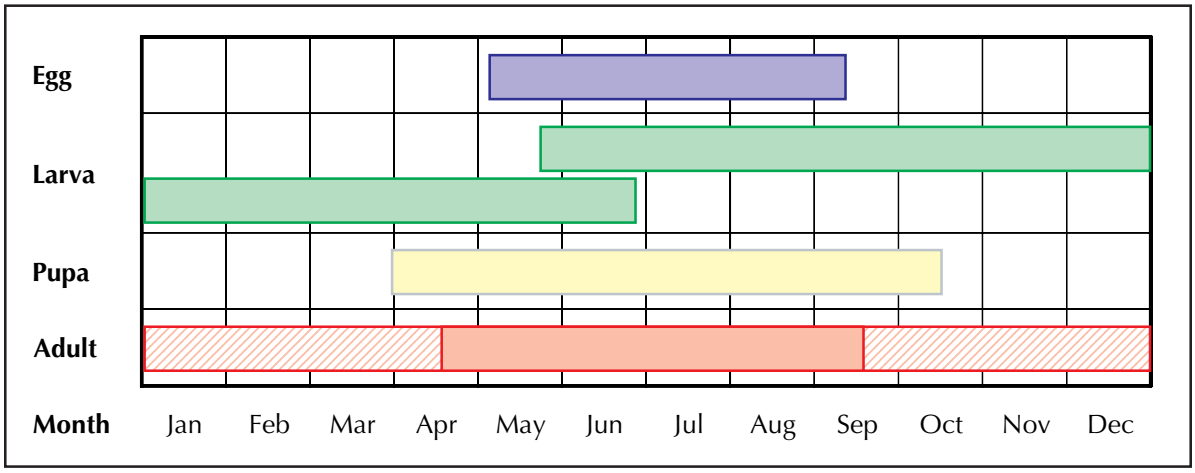


Figure 17. Schematic lifecycle of *Hylobius transversovittatus*. Solid colored bars represent the length of activity for each of the life stages, with adult overwintering presented by the patterned bars. A single generation can take one to two years to mature.

Impact:

The rate of attack (number of larvae feeding in a root) and damage to the plant varies depending on the size and age of the rootstock, the density of purple loosestrife plants, and the number of weevils at the site. Usually, only one or two larvae feed in the same root, but as many as 20 larvae have been found in a single, large root.

Root feeding by loosestrife root weevil larvae can be very destructive to the roots, especially at high larval densities. Small rootstocks can be severely damaged or killed. Large roots can withstand substantial feeding pressure over several years before significant damage becomes noticeable. However, attacked plants are shorter, have reduced root and shoot biomass, and produce fewer seeds.

The loosestrife root weevil is tolerant of a wide range of environmental conditions. However, where water levels fluctuate, females may not be able to lay eggs at the base of the plant. Larvae may drown in roots that are submersed in water for a prolonged period of time (several months). For these reasons, the loosestrife root weevil should not be released in permanently flooded sites.

The loosestrife root weevil can be used effectively with the loosestrife leaf beetles. The combined effects of the three biocontrol agents is expected to be greater than the effects of either insect alone. For example, purple loosestrife regrowth following defoliation by *Galerucella* leaf beetles is greatly reduced when plants are also attacked by the root feeder.

Comments:

A semi-artificial diet has been developed to facilitate mass rearing of the loosestrife root weevil. Refer to the rearing purple loosestrife biocontrol agents section in Chapter 3 of this manual for more information about the semi-artificial diet.

Nanophyes marmoratus Goeze

Order: Coleoptera
Family: Brentidae (see Note below)
Common name: Loosestrife flower weevil

Nanophyes marmoratus is a minute, dark brown weevil, measuring 0.06-0.08 inches (1.4-2.1 mm) in length (Figure 18). It was introduced from Germany and first released in New York and Minnesota in 1994.

Adult loosestrife flower weevils overwinter in the leaf litter, and emerge from sites in late May to early June to feed on young purple loosestrife leaves. As soon as flower buds develop, weevils move to the flower spikes, where they feed on the buds, mate, and begin to lay eggs. Females continue to lay eggs throughout the peak flowering period of purple loosestrife.

Figure 18. Adult *Nanophyes marmoratus* on purple loosestrife flowers. (UGA0021100)

Eggs are laid singly into the tips of flower buds before petals are fully developed (Figure 19). Larvae feed on developing ovaries, hollowing out the bud in the process. As a result, attacked buds never flower. All attacked buds senesce and most drop off the inflorescence before new adult weevils emerge. Mature larvae 1use frass to form pupation chambers at the bottom of the bud (Figure 20).

Figure 19. *Nanophyes marmoratus* egg in a purple loosestrife flower bud. (UGA1291019)

The new generation loosestrife flower weevils emerge from dried, hollowed-out buds in July and August and feed on the remaining green leaves of purple loosestrife before seeking overwintering sites in leaf litter. Holes chewed in the buds when the loosestrife flower weevils emerge from their pupation chambers provide evidence that the weevil is present at the site (Figure 21) Most damaged buds drop from the plant. The loosestrife flower weevil has one generation per year. Complete development from egg to adult takes about one month (Figure 22).

Figure 20. *Nanophyes marmoratus* larva in a purple loosestrife flower bud. (UGA1291020)

Figure 21. *Nanophyes marmoratus* adult exit hole in a dead purple loosestrife bud. (UGA1291021)

Impact:

Bud feeding by adults causes premature flower bud drop; attack by larvae kills the bud, thus preventing flowering and seed production. At high loosestrife flower weevil densities, larval feeding can reduce seed output of a purple loosestrife plant by about 60 percent.

The effectiveness of the loosestrife flower weevil may be reduced where it occurs with the loosestrife beetle (*Galerucella)*. This is because high levels of defoliation by the loosestrife beetle prevents purple loosestrife from flowering, and consequently limits activity of the flower weevil. Initially, the loosestrife flower weevil should only be released where the beetles do not occur or where their densities are low.

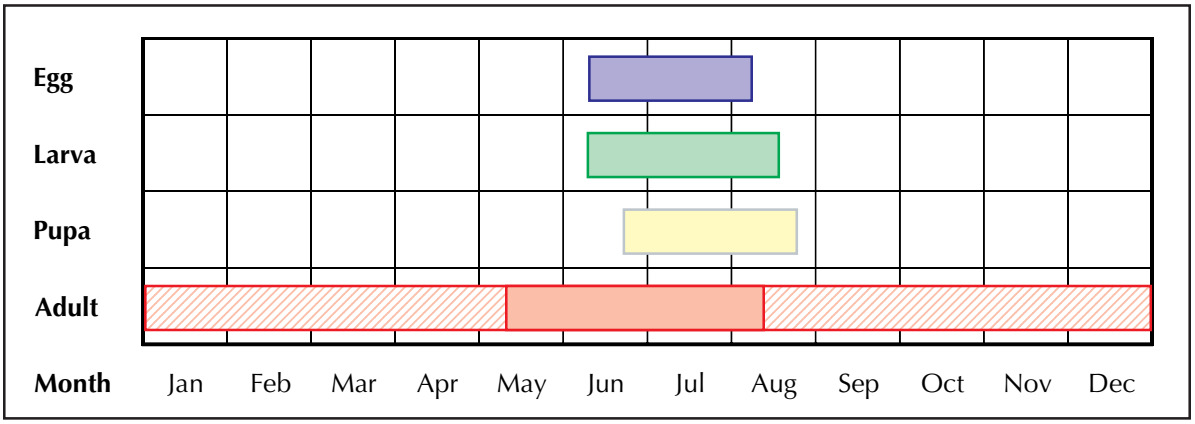


Figure 22. Schematic lifecycle of *Nanophyes marmoratus*. Solid colored bars represent the length of activity for each of the life stages, with adult overwintering represented in the patterned bars. This weevil produces one generation per year.

Note: The taxonomic placement of the genus *Nanophyes* is in transition, and thus you may find it listed under three different family names. The genus was historically in the weevil family Curculionidae, and was reclassified in 1995 into the weevil family Brentidae. It was later moved to the family Apionidae (1999). However, the latest classification (2002) returns *Nanophyes* and all species formerly in the family Apionidae to the family Brentidae. For the purpose of this manual, this classification system is followed. All three families are commonly known as weevils. For more information, key references are provided in the Selected Reference section.

Another species, *N. brevis*, was tested and approved for importation but was never released in North America because of problems with parasitism.

Summary:

Four species of beetles are established in the United States and Canada for the biological control of purple loosestrife. For a quick reference to the beetles, Table 4 compares the adult beetles and their damage, and Table 5 compares the larvae and pupae, and their damage. Figure 23 is an illustration of a purple loosestrife plant showing which parts of the plant are attacked by each of the biocontrol beetles.

Table 4. Comparison of adult purple loosestrife biocontrol beetles and their damage.

Scientific Name	*Galerucella calmariensis* and *G. pusilla*	*Hylobius transversovittatus*	*Nanophyes marmoratus*
Common Name	Loosestrife beetle	Loosestrife root weevil	Loosestrife flower weevil
Adult Description	Light brown leaf beetles	Large reddish-brown weevil with white spots, large snout	Small, shiny, dark brown weevil
Life Cycle	One generation per year, may have a partial second generation	Long lived, often more than two years.	One generation per year
Body Shape/Size	Cylindrical body shape, 0.15 to 0.3 inches (4 -6 mm) in length	Ovoid body shape, 0.4 to 0.6 inches (10 to 14 mm) in length	Round-ovoid body shape, 0.06-0.08 inches (1.4 to 2.1 mm) in length
Eggs	Lays eggs in clusters on leaves and stems, covered with fecal strands	Lays eggs (one or more) in soil or stem near base	Lays eggs singly inside flower bud
Damage	Adults feed on foliage causing a 'shothole' damage pattern in the leaves	Adults feed on edge of leaves	Adults feed on young leaves and flower buds

Table 5. Comparison of larvae and pupae of purple loosestrife biocontrol beetles and their damage.

Species	**Larval Description and Feeding Characteristics**	**Effects**
Galerucella calmariensis and *G. pusilla*	Larva is yellow with brown cross markings and a dark brown head capsule; larva feeds on foliage, window-paning the leaves (top layer of leaf cuticle and epidermis is intact); pupates in soil or aerenchyma (on flooded plants).	Reduces shoot height, root mass, generally stresses plant.
Hylobius transversovittatus	Larva is C-shaped, white with light brown head capsule; larva feeds and pupates inside root.	Reduces shoot height, root mass, root storage reserves, and generally stresses plant.
Nanophyes marmoratus	Larva is C-shaped, white with light brown head capsule; feeds in bud on developing ovary, killing bud; pupates inside hollowed bud.	Reduces flowering and seed production.

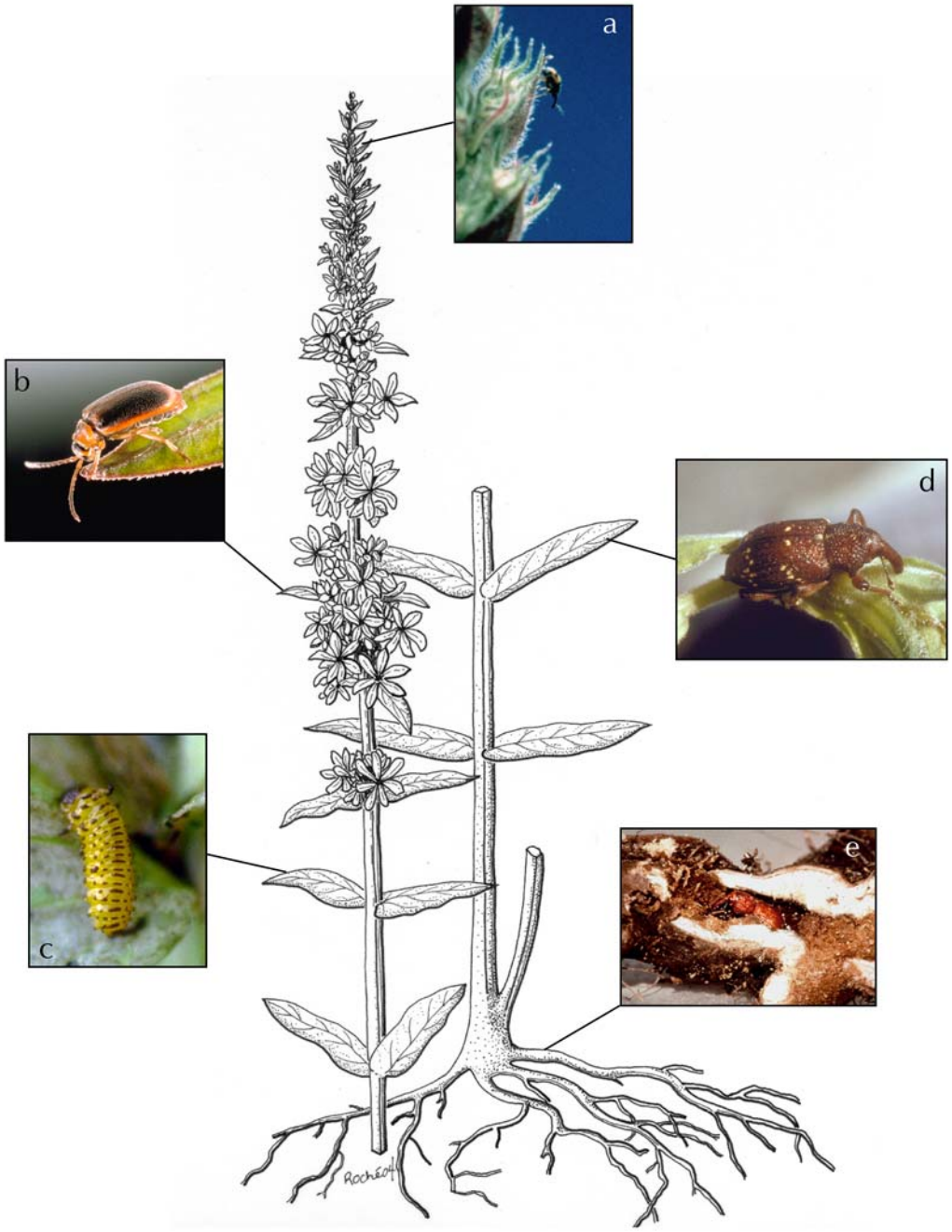


Figure 23. Purple loosestrife biological control agents and the plant parts they attack (drawing adapted from Thompson et al. 1987): a) *Nanophyes* flower weevil adult (UGA0002035); b) and c) *Galerucella* leaf beetle adult (UGA1291023) and larva (UGA0022078); d) *Hylobius* root weevil adult on leaf (UGA0021099), and e) emerging from root (UGA0002034).

CHAPTER 3: DEVELOPING, IMPLEMENTING, AND MANAGING A PURPLE LOOSESTRIFE BIOLOGICAL CONTROL PROGRAM

Implementing a purple loosestrife biocontrol program involves planning the program, selecting release sites, obtaining and releasing insects, and monitoring the success of the program. Monitoring determines (1) if the insects successfully establish at a site, (2) if their populations increase over time, (3) how attack and damage impact the purple loosestrife populations over time, and (4) how other plants in the wetland community respond to release of biological control agents. Depending on the size of your program, monitoring activities may vary among release sites. However, evaluating the success of a purple loosestrife biological control program requires a long-term commitment to regular observations and assessments. Guidelines and protocols to plan, develop, implement, and monitor a purple loosestrife biocontrol program are outlined in this chapter.

This chapter includes six sections:

1. Planning your purple loosestrife biocontrol program
2. Selecting release sites
3. Obtaining purple loosestrife biocontrol agents
4. Handling and releasing purple loosestrife biocontrol agents
5. Monitoring purple loosestrife, the biocontrol agents, and other vegetation
6. Establishing a photo point for monitoring

Familiarity with all aspects of the program before beginning will greatly facilitate its implementation and increase its chances of success.

1. Planning Your Purple Loosestrife Biocontrol Program

Before you begin implementation, careful planning of your purple loosestrife biocontrol program is important. Setting biocontrol program goals, and understanding what is involved in implementing and managing a purple loosestrife biocontrol program increases the probability for success of your program. All of the following are important components of pre-program planning:

a) Assess the current status of purple loosestrife in your management area and adjacent ownerships.
   - Determine how much purple loosestrife occurs in your area and develop a distribution map.
   - Determine what other management strategies have been employed against purple loosestrife in your area. Determine if biocontrol is an appropriate strategy.
   - Discuss your program plans with neighboring landowners/managers, or coordinated weed management areas (CWMAs).
   - Consult with your agency or university biocontrol expert, cooperative weed management area, or county weed superintendent to become familiar with other purple loosestrife activities in your area, especially spraying or mowing programs that may affect a biocontrol program.

b) Define the goals of your program.
   - Determine the weed management objective for your area. If you want to eradicate purple loosestrife from your area, then biocontrol is not the most effective management technique to accomplish this. However, if you simply want to reduce loosestrife cover, flowering, and seed production, and to promote the growth of native plants, then a biocontrol program may be compatible with your management objectives.
   - Determine precisely how you will measure control based on your stated goals.
   - Outline a long-term management strategy.
   - Determine the projected lifespan of the project.

c) Decide which biocontrol agent(s) to release.

d) Establish support logistics.
   - Commit resources for field equipment and supplies (if needed).
   - Recruit and train personnel.
   - Identify sources of biocontrol agents.
   - Outline yearly activities.

2. Selecting Release Sites

The sites that you select to release biocontrol insects should be carefully chosen to meet the primary goal(s) of your program. Consider the following when selecting your site:

- Sites selected to function as future collecting and redistribution sites (field insectaries) should be sufficiently large—ideally greater than 10 acres (4 ha)— safe from disturbance or vandalism, and should be accessible for regular monitoring and collection activities. Also, assess these sites for use as demonstration areas for educational and training purposes.

- Sites intended for long-term insect and vegetation monitoring of control success should be safe from interference by other weed management programs and safe from vandalism. You need a long-term commitment by the landowner to allow your monitoring to proceed for a decade or longer. Consider restricting collection of beetles from these sites in order to track the growth of insect populations.

Visit several prospective release sites. Use the following guidelines and criteria to select a site:

Site factors: An infestation covering a minimum of 1 acre (0.4 ha) in size is recommended, but a larger infestation, such as that shown in Figure 24, is better. Choose a site that is in full sunlight (*Galerucella* beetles, particularly, prefer full sun). Ideally, select a site that has a moisture gradient: this will allow the beetles to select their preferred moisture levels, and large water fluctuations will not jeopardize the release program. Avoid sites that are highly disturbed, where other weed control methods (mowing/cutting, burning) are planned, or where pesticides are used frequently.

Figure 24. Purple loosestrife infestation. (UGA1291024)

Presence of biocontrol agents: Make sure you are aware of and coordinate with other biocontrol activities in your area. If other biocontrol agents are already present at the selected site, move on and choose a different location for release. For example, if you want to establish *Nanophyes* in a field insectary site, first be sure that *Galerucella* is not present.

Ownership: The landowner must be willing to permit release and monitoring over several years. When getting permission to use a site, be sure to secure the following: 1) written permission from the landowner or land manager allowing use of the area as a release site, 2) written agreement by the landowner allowing access to the site for monitoring and collection for a period of at least 10 years, and 3) permission to put a permanent location marker at the site.

Establish a permanent location marker: After selecting a site, place a brightly colored marker (wood or metal stake) in the infestation. The stake must be tall—about 6 ft (2 m)—and clearly visible to mark the exact location of the release site. If possible, record the global positioning system (GPS) coordinates of the marker.

Establish a photo point: Photographs taken annually from the same location can effectively illustrate the success of a control program. Refer to Section 6 for detailed instructions on establishing a photo point.

Prepare a map: A map of the infestation is helpful to visually assess the size and distribution of purple loosestrife at the site before biocontrol agents are released. A detailed map and written directions to the release site are essential for others to locate the site: note permanent roads, creeks, rivers, mile markers, etc. on the map and in the directions. If possible, include a legal description and GPS coordinates so that the site can be easily re-located.

Monitor baseline vegetation: It is highly recommended that you assess the amount of purple loosestrife and other vegetation at the release site before releasing the biocontrol agents. Pre-release monitoring provides baseline data, allows meaningful before-and-after comparisons, and will provides important information on the effectiveness of the biocontrol program (see Section 5.2 for information on vegetation monitoring).

3. Obtaining Purple Loosestrife Biocontrol Agents

Biocontrol insects are obtained either by collecting them (yourself or by a cooperator), rearing them, or purchasing them from a commercial supplier. Consider the following when planning your purple loosestrife biocontrol program:

- Field collecting the biocontrol agents provides robust and healthy insects, and is the preferred method for obtaining the *Galerucella* species and *Nanophyes*. If a cooperator is field collecting the insects for you, be sure they collect the agents at the right time of year and under the best conditions, and send you clean, sorted insects as soon as possible. Be sure cooperators know well in advance when you need the biocontrol agents for release.

- Opportunities for collecting the root feeder, *Hylobius,* are at present limited because it is not widely established in the field and population levels are low where it is established. This weevil is best obtained from cooperators who maintain field insectaries, or purchased from suppliers who rear them.

- Commercial suppliers provide clean, healthy insects. However, purchasing the insects can be expensive. While some suppliers charge as little as $0.10 per beetle, others charge as much as $0.70 per beetle for the *Galerucella* leaf beetles. Please remember that different prices may also mean different quality and make sure you know under what conditions the insects you purchase were

raised or collected. Field-collected beetles are of superior quality when compared to lab-reared individuals, and obtaining adults immediately after they reappear from overwintering will allow you to release individuals with maximum reproductive potential. *Nanophyes* and *Hylobius* are even more expensive. Make sure you know the origin of your insects when making purchasing decisions.

3.1 Collecting purple loosestrife biocontrol agents

General Collecting Guidelines

This section provides information on collecting, rearing and purchasing purple loosestrife biocontrol agents with emphasis on the two *Galerucella* species and *Nanophyes*. *Hylobius* is more difficult to collect.

All purple loosestrife beetles insects are most efficiently collected as adults. Planning your collecting activities is important: know how to identify the biocontrol agents, time the collection, prepare equipment and supplies, and train personnel in identification and collection techniques. Follow these general guidelines when planning your collection activities:

Containers. Containers must provide safe environments for the insects while still preventing escape. Use sturdy, breathable containers that allow air flow and will not collect condensation. An example of good containers are pint-sized, nonwaxed ice cream cartons (Figure 25). Plastic containers can also work well if a large hole has been cut in the lid and the hole is covered with mesh or organdy to allow airflow into the container and minimize the buildup of condensation. Glass or metal containers are not suitable.

Figure 25. Non-waxed paper insect containers. (UGA1291025)

To prepare the containers, place cut purple loosestrife stems with leaves loosely in the container, or fit a moist (but not wet) piece of florist foam 1 inch (2.5cm) thick and the diameter of the container snugly at the bottom of each container, and push cut stems with leaves into the florist foam. This provides shelter and food to keep your beetles healthy. Do not put flowers or seeds in the container. Do not put water in the container.

After insects are collected in the field, transfer them as soon as possible into your breathable, durable containers. Keep containers shaded and cool at all times while collecting, sorting, counting, and transporting. Bring a large cooler with pre-frozen ice packs to the field, and tape the ice packs to the interior side of the cooler so that they do not roll around and crush the insect containers.

Collecting Methods. Three commonly used methods to collect purple loosestrife biocontrol agents are tap & funnel, sweep net, and aspiration methods. The most appropriate method will depend on the density of the insect population, available time, and available personnel.

- **Tap-and-Funnel.** The tap-and-funnel method is an efficient and productive way to collect *Galerucella* and *Nanophyes* when their populations are high. It is used to gather large numbers of beetles in a short amount of time. Using a stick (a shortened length of broom handle works well) or your hand, knock the beetles from the plants into a funnel taped to a plastic bottle or container (see Figure 26). Then brush the insects down the sides of the funnel and into the plastic container. Knocking insects into a plastic tray also works, but adults are very active and, in warm weather, will quickly fly away.

Figure 26. Collecting *Galerucella calmariensis* from purple loosestrife using the tap-and-funnel method. (Background: UGA1291026; inset: UGA1291027)

  Tapping is not selective, so other insects and spiders will be collected along with the biocontrol agents. These insects must be separated from the biocontrol agents before shipping or otherwise transporting the biocontrol agents for release to a new site. Cooling the insects (for example, for 30 minutes in a refrigerator or cooler) before insects are sorted greatly reduces the beetles' activity, and makes sorting much easier. Use an aspirator (described below) to sort the beetles.

- **Sweep net.** A sweep net is made of a cotton or muslin bag on a hoop, 15 inches (38 cm) in diameter, attached to a handle 3 feet (0.9 m) long (Figure 27). As its name implies, it is generally used to "sweep" adult insects off a plant.

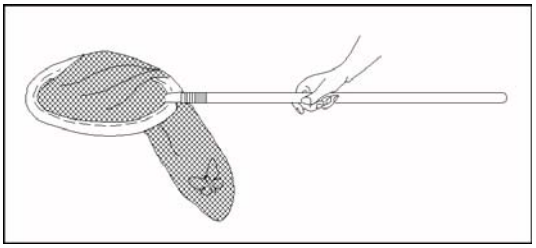


Figure 27. Sweep net for collecting purple loosestrife beetles.

Using a sweep net produces a 'cleaner' catch, reducing the need for elaborate sorting. However, sweeping purple loosestrife with a net is difficult and inefficient because woody stems from previous growing seasons interfere with sweeping the current year's growth, where insects are active.

A more effective way to use a sweep net is to bend the purple loosestrife flower heads into the net and shake the plants to dislodge adults into the net. This method works especially well for *Nanopyhes*, and when *Galerucella* populations are low. The 'catch' must still be sorted before placing the biocontrol agents into containers.

- **Aspirator:** An aspirator is a device used to suck insects from a surface into a collection vial. An aspirator (Figures 28 and 29) is used to collect insects out of a sweep net or off a sorting tray—though it can also be used to take insects directly from the plants. Aspirating is easy and selective, and is the preferred method to sort insects.

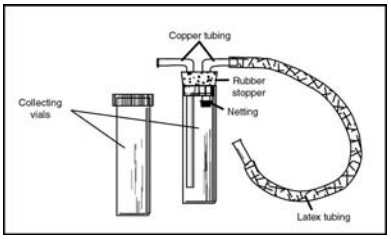


Figure 28. Aspirator used to collect purple loosestrife beetles.

Sorting separates the biocontrol agents from unwanted insects, other organisms and debris, such as weed seeds, collected along with the beetles. Empty the contents of the net or collecting container onto a tray and aspirate the purple loosestrife beetles out of the debris. If the collected material is first chilled, the insects will move slower and will be easier to catch.

Figure 29. *Galerucella calmariensis* collected in aspirators. (UGA1291029)

KEEP INSECTS SHADED AND COOL WHILE SORTING, COUNTING, AND TRANSPORTING THEM

Specific Collecting Guidelines

Collect biocontrol agents from field insectaries or from open field sites having an abundance of insects. Collect adults at the beginning of their peak emergence times, when female beetles have their maximum oviposition potential, and are most likely to establish thriving populations at the site where they are later released. Emergence (the optimal time for collection) will vary from region to region and year to year. For example, *Galerucella* can be collected as early as mid-April in warm, western locations, and as late as early June in cooler, eastern locations. You may need to contact your state biocontrol expert, county weed superintendent, or university extension educator to identify the best collection sites and times. Approximate recommended collection times are provided in Table 6.

Table 6. Methods and timetable for collecting purple loosestrife biocontrol agents.

Agent	What to Collect	When to Collect	How to Collect
Galerucella calmariensis and *G. pusilla*	Adults	Mid-May to early June and again from early to late July.	Tap & funnel, sweep net, aspirating.
Hylobius transversovittatus	Adults	Late June to late August.	Use sweep net to knock individually beetles into net, or catch beetles dropping off plants; handpick under leaves at ground level.
Nanophyes marmoratus	Adults	Late June to early July and again mid-August.	Sweep net, tap & funnel.

Wait for a day with good weather. Do not collect in the rain: insects will hide and become difficult to find in rainy weather, and excess moisture causes health problems—beetles may also drown in wet collection containers. The only exception to this rule is the root feeder *Hylobius*, for which overcast and rainy days are optimal for collecting the generally night-active adults.

Leaf beetles (*Galerucella*): Adults are easily collected with a funnel or sweep net during the warm part of the day when they are most active. Aspirating is also a suitable, although slower, method for collecting adults. The best time to collect adult beetles is in mid-May to early June, when they have emerged from overwintering sites and are actively feeding, congregating, and mating. The second period of activity occurs from early July to August, when the next generation of adults emerge. Larvae or eggs are not generally collected because of extremely high mortality rates during transportation.

Flower weevil (*Nanophyes*): *Nanophyes* adults are easily collected by bending and shaking the developing purple loosestrife inflorescences over a sweep net, dislodging the beetles into the net. Aspirating individual adults or mating pairs from plants is possible but slow and laborious. Collect *Nanopyhes* during the heat of the day—most appropriate collection times are when inflorescences begin to form but before plants begin to flower—mating pairs congregate at the top of these plants, and are easily seen. Depending on the location of the site, the collecting period is generally about late June to mid-August.

Root weevil (*Hylobius*): *Hylobius* is the most difficult of the purple loosestrife biocontrol agents to collect because it is generally nocturnal or only active on rainy or overcast days. Also, population numbers are currently still low, so field collecting is limited. Where it is possible to collect, do so in the early evening or at night with a flashlight. Weevils are most easily collected by locating an adult on a plant, carefully approaching the plant, placing a sweep net under the stem, and allowing the beetle to drop into the net (they quickly drop from the plant when they sense your presence). Adults can also be collected at the base of the plant by removing the leaf litter and handpicking individual beetles, but this activity is very time-consuming, and will only yield good results at high weevil densities. Adults can be collected from late June to late August. (Note: because

Hylobius is not widely established at present, it will be necessary to purchase the weevils from rearing operations until field populations have built up sufficiently to allow collection.)

3.2 Rearing purple loosestrife biocontrol agents

Programs to rear purple loosestrife biocontrol agents have focused primarily on the *Galerucella* leaf beetles because they are relatively simple to rear. Rearing enables large numbers of beetles to be rapidly produced and distributed. In many parts of the United States and Canada, communities, agencies, youth groups, and schools have developed mass rearing programs to help distribute the beetles throughout their states and regions and to provide opportunities for public awareness and education.

You can find many examples of state programs on the internet. For example, the Illinois Natural History Survey (http://www.inhs.uiuc.edu/cee/loosestrife/bcpl.html) manages a very successful rearing program with elementary and high schools. The Vermont Purple Loosestrife Biocontrol Program has hosted a volunteer community rearing program of *Galerucella* beetles for several years (http://www.anr.state.vt.us/dec/waterq/ans/plpage.htm). Other active programs are found in Michigan (http://www.miseagrant.org/pp), and Minnesota (http://www.seagrant.umn.edu/exotics/purple.html). For additional information about rearing purple loosestrife biocontrol beetles, visit the internet web sites listed in the Selected References section.

General *Galerucella* Rearing Guidelines

We focus in this guide on rearing the *Galerucella* leaf beetles. The methods outlined here also work well for *Nanophyes*, except that adult weevils are introduced when plants begin to form flower buds (by mid-summer). Rearing *Hylobius* is not recommended because it is comparatively slow and usually requires highly specialized facilities or large numbers of plants.

- **Obtain plants:** In early spring before plants sprout, dig about 100 mature purple loosestrife roots, each with at least five or six stems. Take the roots to your rearing location and pot them into plastic 4-gallon (3.8 l) pots filled with standard commercial potting mix and a slow-release fertilizer. Place up to 12 pots in a plastic wading pool about 6 feet (2 m) in diameter. Fill the pool with 4 inches of water to simulate a wetland environment (Figure 30). Check the water daily and refill as needed throughout the growing period. If wading pools of the proper size aren't available, construct a pool using pond liner or thick plastic sheeting that can hold water to keep plants moist and healthy.

- **Cover plants:** Place a 4- to 5-foot (1.2 – 1.5 m) tall tomato cage over the pot (if you intend to rear *Nanophyes*, these cages will need to be taller, to allow the plants room to develop inflorescences), and cover the cages with fine mesh netting (no-see-um netting works well). Secure the top and bottom of the netting with twine, tape or heavy rubber bands: this will keep the beetles in the cage and prevent entry of aphids or other pests and predators that might interfere with plant or beetle growth.

- **Introduce beetles:** When plants are about 1 foot (30cm) tall, place 10 to 15 beetles in each cage (use only plants that produce an abundance of healthy shoots). Be sure to put only one beetle species in each cage. If you rear *Nanophyes*, place six to ten individuals in each cage. Adults will feed for a few days then begin mating and laying eggs. *Galerucella*, while small at first, will be obvious in a few weeks. Development (from egg to adult) takes approximately 30-40 days, and new adult beetles will begin to appear and congregate near the top of the plants. *Nanophyes* larvae will be concealed inside the bud. Each large plant yields between 500 and 2,000 *Galerucella* beetles or 300 *Nanophyes* adults. Make sure the newly-emerged adult beetles have sufficient foliage to feed.

Figure 30. Wading pools used to grow purple loosestrife and to rear *Galerucella calmariensis*. (UGA1291030)

- **Transport beetles after rearing:** Transport the newly reared beetles in the covered, potted plants to the release site and place them next to healthy, wild purple loosestrife plants. Remove the net bag and the wire cage; gently shake the net bag to dislodge any beetles remaining in the bag. Remove the plant from the pot and rest it on the ground in the infestation; this allows the beetles to disperse on their own. Record the location of the site, the weather conditions at the time of release and, if possible, record the GPS coordinates or legal description of the release site. Use the Purple Loosestrife Biocontrol Release Form to record the release.

- **Overwintering potted plants and beetles:** It is quite easy to overwinter beetles outdoors to have a large supply for the next year. Retain a portion of the reared beetles on large, caged plants removed from the wading pool. Make sure they have sufficient high-quality food; beetles will feed for a while and disappear into the duff at the base of the plant, where they will remain until spring. To maximize plant and beetle survival, thoroughly mulch and shade the plants throughout the fall and winter. Other elaborate and specialized rearing programs use large cages (Figure 31) or greenhouses (Figure 32) to mass-produce adult beetles, but such programs are expensive and labor-intensive.

Figure 31. Large mesh cages of potted purple loosestrife for rearing biocontrol agents. (UGA1291031)

Figure 32. Mesh-covered purple loosestrife plants in a greenhouse used to rear biocontrol agents. (UGA1291032)

Rearing *Hylobius* on semi-artificial diet

An alternative mass rearing method using a semi-artificial diet has been developed for *Hylobius*. While not widely used in weed biocontrol, rearing insects on artificial diet is a well-established practice in agricultural pest management. The semi-artificial diet, which has the consistency of stiff jelly, is comprised of multiple ingredients, but most importantly contains ground purple loosestrife roots. Root weevil larvae are reared individually on diet in small containers. They complete development in three months instead of one-to-two years under natural conditions in the field.

Advantages of artificial diet:

- Development time is reduced from one-to-two years to three months
- Eliminates slow, tedious field collection
- Large numbers of beetles can be reared
- Enables year-round beetle rearing

Disadvantages of artificial diet:

- Expense
- Requires specialized equipment and skill

See the article by Blossey et al. (2000), cited in the Selected References section, for information on use of a semi-artificial diet to rear *Hylobius*.

Questions to ask suppliers

- Does the supplier have a valid USDA-APHIS permit?
- Are the agents field-collected?
- How will the agents be shipped?
- How long will the agents be held in transport?

3.3 Purchasing purple loosestrife biocontrol agents

When purchasing biocontrol agents from a commercial supplier or when obtaining insects from a cooperator, it is important to ensure that the insects are healthy and received in good condition. It is also important to obtain the biocontrol agents at the correct time of year. Be sure also to have the correct permits for interstate transportation of insects. For more information or to find a commercial supplier, contact your cooperative weed management area, your county weed control superintendent, or your state biocontrol of weeds expert.

4. Handling and Releasing Purple Loosestrife Biocontrol Agents

How the insects are handled and transported after they are reared or collected will affect whether they survive at the new site. This section includes guidelines for handling, shipping, and releasing biocontrol agents.

4.1 Handling purple loosestrife biocontrol agents

Containers. Refer to Section 3.1 for a description of suitable containers. It is important to use breathable containers to have air flow to the insects and to prevent condensation. Seal the container with tape and label the container with the name of the biocontrol agent, the quantity of weevils, the collection site, and the date.

Supplies for transportation

- Sturdy, breathable containers
- Masking tape
- Paper towel or styrofoam
- Cooler
- Frozen ice packs
- Cardboard box (for shipping)

Transportation. To minimize stress and harm to beetles, transport and release them as soon as possible (preferably within 24 hours). Ensure insects have plenty of fresh food at all times. Keep containers cool and dry during transportation. Put the containers in a cooler with ice packs secured to the bottom or sides of the cooler. Cover the ice packs with newspaper to prevent direct cold and condensation from contacting the containers. Adult beetles can survive a few days in a refrigerator if necessary, but keep storage time to a minimum.

4.2 Shipping purple loosestrife biocontrol agents

To ship biocontrol agents, plan the route and timing of shipments to prevent undue delays and stress on the insects. Collect the insects early in the week and ship right away by overnight courier (for example, FedEx, UPS, or DHL) to be sure they arrive before the weekend. Instruct your cooperator(s) to release the insects immediately upon receipt. Observe the following general guidelines:

- **Know the regulations:** It is important to know the regulations pertaining to shipping biocontrol agents across county or state borders. Interstate transportation permits may be required. To learn the current regulations, contact your local cooperative weed management area, state department of agriculture, or the USDA Animal and Plant Health Inspection Service–Plant Protection and Quarantine office (USDA-APHIS-PPQ) (http://www.aphis.usda.gov/ppq).

- **Prepare the insects:** Prepare the insects right before the scheduled shipment to minimize stress. Follow these steps:

  - Separate beetles from all other unwanted material, such as other insects, spiders, weed seeds, etc.
  - Put the beetles in breathable or vented shipping containers (described above).
  - Pack the containers in an insulated shipping box (Figure 33) with a frozen ice pack well secured to the inside. Seal each frozen ice pack in a plastic freezer bag to prevent condensation inside the box as the ice pack melts.

Figure 33. Shipping box for purple loosestrife beetles. (UGA1291033)

Common mistakes in transportation

- **Excess heat.** Do not expose packaged or containerized biocontrol agents to direct heat or sunlight.
- **Excess moisture.** Do not put water into the containers.
- **Lack of air.** Use only breathable or vented containers.
- **Mortality.** Provide insects with sufficient food. Do not store insects more than three days.

4.3 Releasing purple loosestrife biocontrol agents

There are many factors that can determine whether insects establish and flourish at a release site. Few of these are under control by humans (weather, wildfire, etc.), but follow these general steps for releasing biocontrol agents to give the insects the best start possible:

- **Timing the release:** Release the beetles at the appropriate growth stage of purple loosestrife. For example, when releasing *Nanophyes*, purple loosestrife should be at or near the flowering stage. If most plants are beyond the flowering stage, it is too late to release at that site. Locate a different infestation, perhaps at a higher elevation, that is at the flowering stage.

- **Number of biocontrol agents to release per site:** The number of insects to release per site depends on; 1) the insect species, 2) resources available to collect, 3) the time of year.

  - For *Galerucella*: it is possible to collect large numbers of beetles (several thousand), thus a release of 1,000 beetles is possible. But even releases of 200 adults in the spring have yielded good results. When collecting from the summer generation, a minimum release of 2,000 *Galerucella* is recommended.
  - For *Nanophyes*: at high densities, it is possible to collect several hundred beetles in an hour. A minimum release of 200 adults per site is recommended.
  - For *Hylobius*: it is more difficult and labor intensive (or expensive) to obtain *Hylobius*, but a release of at least 100 beetles is recommended.

- **Release of biocontrol agents:** Release insects as soon as possible after you receive them; do not wait for good weather (but avoid heavy downpours). Release in the early morning hours between 6 and 10 a.m. or in the cooler evening hours between 6 and 10 p.m.; biocontrol agents are less likely to scatter if released at cooler times of the day. In general, you can release insects either in cages or make open releases. Caged releases prevent immediate dispersal of insects but require you to put up and take down equipment.

  For *open releases*, get to the desired release location, open shipment containers, and gently shake all insects, purple loosestrife stems, and florist foam at the base of a vigorously growing purple loosestrife plant. Do not release over water, but at the shore or on dry land if possible. Release all the insects at once at a single location; do not disperse them at the site, as insects will naturally disperse on their own.

  For *caged releases,* place a mesh or organdy bag over a plant (Figure 34), release insects into the caged plant, and tie the bottom of the bag to the stem. Cages confine the insects for a period of time so they adjust to the site and easily find one another, but cages need to be removed within a few days.

- **Frequency of release:** Often, a single release will be sufficient to establish an insect population, but more than one release may be necessary if previous releases failed. Do not get discouraged, and bear in mind that it could take two or more years to determine if the agent released (especially *Hylobius*) successfully established.

- **Releasing multiple species of agents:** As a rule, release only one biocontrol agent if you plan to establish a field collection site. Locate release sites at least 2 miles (3 km) apart. At other sites, you can freely mix species to assess how control is influenced by different insect combinations. Be aware that insects will migrate and establish new populations over time.

- **Place the permanent location marker:** Place the marker at the location of the insect release. This location will be later used to relocate the site and for monitoring activities.

Figure 34. Releasing *Nanophyes marmoratus* on caged purple loosestrife. (UGA1291034)

- **Take photos:** Take a series of photographs to record the release (see Section 6).

- **Fill out and submit a release form:** Complete the Purple Loosestrife Biocontrol Release Form (see Appendix). Submit the form to your county weed superintendent, cooperative weed management area, or university or state biocontrol expert. Keep a copy for your records.

Summary: Handling and Releasing Purple Loosestrife Biocontrol Agents

- Use sturdy, breathable containers with food according to instructions.
- Sort insects before packaging them to ensure that predators (e.g., spiders) or weed seeds are not trapped inside the containers.
- Avoid physical damage to the biocontrol agents by taping down ice packs.
- Keep insects cool during transportation.
- If release or shipping is not immediate, store the insects in refrigerators no colder than 40 to 50° F (4 to 10°C) for no longer than three days, or keep them in an ice chest until they are ready to be shipped or transported.
- Release during the cool times of the day; do not wait for good weather.
- Take photos; fill out the Purple Loosestrife Biocontrol Release Form (see Appendix).

5. Monitoring

The purpose of monitoring is to evaluate the success of your purple loosestrife biocontrol program and to determine if you are meeting your purple loosestrife biocontrol goals. Monitoring activities use standardized procedures over time to assess changes in populations of the biocontrol agents, purple loosestrife, and the wetland communities.

Monitoring can answer questions about whether a) the beetles have become established at the release site, b) the beetle populations are increasing or declining, c) the beetles have spread from the initial release site, and d) the beetles are having an impact on purple loosestrife in the wetland plant community.

Monitoring methods can be simple or complex. Basic assessments can be done with a minimum of time and effort each year. The duration of monitoring can also vary, from a single year to demonstrate the biocontrol agents established, to multiple years to follow the population of the biocontrol agent(s), the decline of purple loosestrife, and changes in the overall plant community.

Qualitative monitoring

Qualitative monitoring is based on subjective assessments. It can include recording the presence or absence of insects, estimating the amount of plant damage, estimating the distribution or density of purple loosestrife at the site, or making observations about wildlife at the release site over time. Another qualitative assessment is before-and-after photographs (Figure 35), which is a photo record of the site before or at the time beetles are released that is repeated at one- or two-year intervals. Because of its descriptive nature, qualitative monitoring tends to be less time-, labor-, and supply-intensive than quantitative monitoring. The descriptive nature of qualitative monitoring does not readily allow for detailed statistical analysis; however, if sufficiently detailed qualitative data is collected, useful information can still be derived. Data obtained from qualitative monitoring may guide more intensive monitoring in the future.

Figure 35. Purple loosestrife infestation: a) before (1998) and b) after (2003) control by *Galerucella* beetles. (Left to right: UGA1291035 and UGA1291036)

Questions to Ask in a Monitoring Program

- Have the biocontrol agents successfully established populations at the site?
- Are the beetle populations increasing or declining?
- How far have biocontrol agents spread from the initial release site(s)?
- Are the biocontrol agents found in sufficient number to be collected and distributed?
- Are the biocontrol agents causing visible damage to the target weed?
- Is the purple loosestrife declining at the site?
- Are changes occurring within the plant community?

Answers to these questions will allow land managers to do the following:

- Establish that biocontrol agents are impacting the target weed.
- Determine if supplemental biocontrol agent releases or other weed management activities are needed.
- Document changes in the plant community.

Quantitative monitoring

Quantitative monitoring is based on measurements, and is used to record and measure changes in a specific population over time. Quantitative monitoring can be as simple as counting the number of insects per unit area, counting the number of flowering purple loosestrife stems in a given area, counting insects and buds, estimating degree of leaf feeding damage by adults and larvae, counting and measuring plants, or even quantifying bird, mammal, and amphibian populations over time. More detailed quantitative monitoring of purple loosestrife can include measuring height, seed production, biomass per unit area, plant community diversity, or even a record of birds, amphibians, or mammals in the study area to determine the faunal diversity associated with the plant community. Although quantitative monitoring may take more time to plan and implement, and also requires more specialized skills and training than qualitative monitoring, the data obtained from a properly designed quantitative monitoring system can be analyzed statistically.

5.1 Monitoring Purple Loosestrife and its Biocontrol Agents

Before you begin your monitoring program, it is essential to outline your long-term monitoring objectives. It is important to know, before you begin, what and how much data will be collected. Be sure you have the necessary resources, trained personnel, and equipment to conduct the monitoring. Follow these additional steps:

- **Choose sites to monitor:** Select sites that are accessible and have a dense infestation of purple loosestrife (Figure 36). Be sure the site will be available to you to monitor for at least three to five years after release. Begin monitoring where the insects were first released, as this is where the highest density of biocontrol agents are likely to occur and where changes to the purple loosestrife are more likely first to be detected.

Figure 36. Purple loosestrife monitoring site. (UGA0021091)

- **Schedule monitoring activities:** Schedule monitoring activities at the same time each year to enable comparison of year-to-year conditions. The recommended quantitative monitoring protocol outlined below requires monitoring twice each year: in the spring to monitor insect activity, and in the late summer to monitor distribution and density of purple loosestrife and other vegetation.

- **Choose a monitoring method:** Select a monitoring method and plan based on your monitoring goals, what information you want to gather, and the number of sites you intend to monitor. Consider the *Questions to Ask* (in box below) and the Monitoring Plan Questionnaire (see Appendix) to help determine the goals of your monitoring program.

  Consider that:

  - The better you define your biocontrol program goals and monitoring strategy, the better you will be able to evaluate your success.
  - It is important to standardize monitoring procedures.
  - Monitoring only becomes useful if methods are used consistently year to year.
  - Even a full-scale quantitative monitoring protocol can be implemented with little effort. Once sites are established, time commitments usually require a few hours in the spring/early summer and a few hours in the fall for each monitoring site.

The recommended monitoring protocol described below is intended for select sites. If you manage a number of release sites, or if you do not have the time or expertise to complete the entire monitoring protocol as outlined, a more qualitative monitoring is recommended. A less intensive monitoring scheme is provided in Table 7. The categories involve "quick" scoring schemes of the number of biocontrol insects you observe, the amount of purple loosestrife defoliation, and photos taken at one- to two-year intervals. Included is timing for each activity.

Table 7. A rapid assessment scheme to monitor purple loosestrife and its biocontrol agents.

Monitoring Subject	Time to Observe	Objective/Method
Galerucella pusilla and *G. calmariensis*	Mid-May to mid-June	Are eggs, adults, larvae present on the plant? Observe and count.
Nanophyes marmoratus	Late May to mid-July	Are adults present? Dissect seedheads and determine if larvae are present.
Hylobius transversovittatus	Late June to August	Are adults present? Excavate and dissect roots.
Defoliation	Mid-July	Estimate how much purple loosestrife foliage has been removed or is dead.
Other plants	August	Record other plants and their abundance.

- **Decide when to begin monitoring biocontrol agents:** Monitoring should ideally begin the season before you release biocontrol agents (this provides baseline data) or, at a minimum, the year of your release. *Galerucella* easily colonizes a new site, especially if a large number (1,000 to 1,500 beetles) was initially released. *Nanophyes*, too, easily establishes itself at sites not colonized by *Galerucella*, although the population of *Nanophyes* is slower to build up than *Galerucella*. *Hylobius* may take two to three years after release to be detected (thus, if *Hylobius* is not found a year after the release, do not assume that it failed to establish: revisit the site for a few more years).

5.2 Detailed Purple Loosestrife Quantitative Monitoring

The detailed protocol outlined below is used for quantitative assessments of purple loosestrife and its biocontrol agents. It is a standardized monitoring protocol developed by the Ecology and Management of Invasive Plant Species Program at Cornell University (www.invasiveplants.net). The advantage of a standardized monitoring protocol is that it allows meaningful comparisons to be made among similar programs in different locations and regions, enables researchers and managers to evaluate the relative success of specific purple loosestrife biocontrol programs, and perhaps helps to understand why certain programs fail.

In this protocol, assessment locations are selected, a quadrat frame is put into place and marked with stakes, quantitative data are taken, and then the frame (but not the

stakes) are taken to the next assessment location. Leaving the marking stakes in place allows the monitoring team to perform the same assessment at the same locations in subsequent years.

Follow these steps to begin a quantitative monitoring program:

1. Collect materials.

For each quadrat frame:

- Two 10-foot lengths of ¾-inch PVC or CPVC pipe, cut into two 1-m lengths and four 0.5-m lengths
- Four right-angle elbows and two straight-line connectors for the PVC or CPVC pipe
- PVC glue (a half-pint is enough for several frames)
- One can of spray foam insulation—optional (one can is enough for several frames)

Other materials, supplies, and tools:

- 1-meter measuring sticks
- 50-m measuring tape
- Four plastic or galvanized metal stakes (galvanized metal electric conduit or PVC pipe are inexpensive and readily available at hardware stores)
- Hammer
- Permanent marker
- Compass
- Handheld GPS receiver (if available)
- Camera
- Stopwatch
- Clipboard, pencils, and pen
- Forms 1 to 4 (see Appendix)

Figure 37 shows many of the materials needed for this monitoring method.

2. Construct a sampling quadrat.

We recommend using a two-piece quadrat frame composed of two short U-shaped halves that slide together to form a square. The frame can be filled with foam insulation to create a floating quadrat for use in flooded sites.

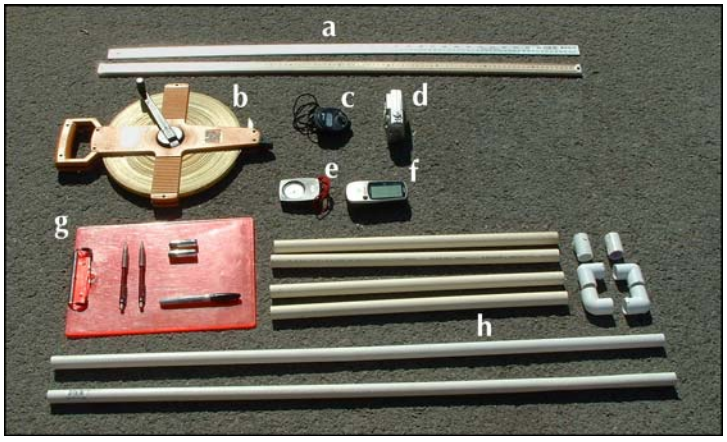


Figure 37. Supplies needed to monitor purple loosestrife: a) meter-sticks, b) measuring tape, c) stopwatch, d) camera, e) compass, f) GPS, g) clipboard and writing instruments, and h) frame components. (UGA1291037)

The inside dimensions of the finished frame should measure 1m by 1m. After cutting the pipe to the correct lengths, glue two elbows to each 1m-long piece (make sure the elbows are perfectly aligned with each other). Then, glue each elbow to a 0.5m-long piece to form two open U-shaped half-frames. Glue the connectors to the short sides of ONE of these half-frames (the connectors will hold the frame together while being used). Using a permanent marker, mark 10 cm intervals on each side of the frame pipe to assist with estimating percent cover.

3. Record site location.

Using Form 1: Purple Loosestrife Biocontrol Monitoring–Site Location (see Appendix), describe the monitoring site, and include a sketched map and a vegetation diagram. Record the position and numbers of the quadrats on the vegetation map.

4. Determine quadrat locations and set up the frame at field sites.

Quadrats should be placed at random into the purple loosestrife infestation. ALL quadrats must initially contain purple loosestrife; if necessary, shift the location of the quadrat so that purple loosestrife covers at least 30 percent of the quadrat. Various methods are available to randomize the quadrat placement. The easiest is a *transect*, a straight line running through the vegetation. Quadrats are placed at predetermined intervals (e.g., every 5, 10, or 20 meters) along the transect line. To do so:

- Locate the beginning of the transect at least 15 ft (5m) from an edge (i.e., roadside, streambank, field).
- Using the 50m measuring tape, stretch the tape in a straight line through the vegetation, being careful not to overly trample the vegetation.

- Secure the two ends of the transect with a stake to hold it in place while the quadrats are established.

At each site, have 5-10 quadrats. Keep in mind that more quadrats are better. Knowing how many quadrats you will have at a site, determine the distance between quadrats (e.g. 5, 10m) there will be along the transect.

- Starting from one end of the transect, locate the position of the first quadrat on the transect.
- Place the quadrat frame into the vegetation by sliding the arms of the first U-shaped frame through the vegetation and as close to the ground as possible, moving carefully to avoid disturbing any insects on the vegetation. Then, gently slide the second half of the frame into the vegetation from the opposite direction and attach it to the first half, completing the $1m^2$ frame. Avoid trampling vegetation in and near the quadrat.
- At one corner of the quadrat drive a 5-8 ft (1-2m) long plastic pipe or galvanized steel stake into the ground (galvanized metal electric conduit or PVC pipe are inexpensive and readily available at hardware stores). Mark each quadrat the same and note which corner is staked. This will allow exact placement of the quadrat in future years. Allow the pipe or stake to stick up high enough to be seen, but low enough to minimize vandalism. Write the quadrat number on each marker, and, if possible, record the GPS coordinates of each quadrat. Flagging tape will also help to relocate the quadrat in the future. Figure 38 shows a typical quadrat placement.

Figure 38. Quadrat set within a purple loosestrife infestation. (UGA1291038)

When you complete monitoring and recording data for the first quadrat, disassemble the frame and set it up at the next quadrat location along the transect. Repeat this until all the quadrats have been placed, marked with a stake, and the monitoring information is recorded. When the monitoring is complete for the

site, remove the transect measuring tape, leaving only the quadrat markers behind. The same set of quadrats can be used year after year. This allows statistical analysis to be conducted on purple loosestrife density, the abundance of biocontrol insects, and the changes in purple loosestrife and other vegetation.

5. Monitor biocontrol agents.

Using Form 2: Purple Loosestrife Biocontrol Monitoring–Insect Monitoring and Instructions (see Appendix), evaluate the biocontrol agents on plants within each quadrat. The protocol involves the following:

- **Visually count biocontrol agents:** For each agent, count for one minute the number of beetles you see on plants in each quadrat. If more that one species is at the site, count one minute for each species. For *Galerucella*, where multiple life stages are present at a time, count for one minute, each, the number of adults, larvae, and eggs.

- **Estimate feeding damage:** In addition to counting insects, it is useful to look for signs of feeding damage. Estimate the amount of foliage that has been damaged or eaten by adults (and *Galerucella* larvae) feeding on foliage.

- **Estimate cover of purple loosestrife:** Looking over the quadrat, estimate the percent of the quadrat that is covered by purple loosestrife.

- **Make observations:** Observe the insects feeding and note their activity.

6. Purple loosestrife and other vegetation.

Purple loosestrife should be monitored before biocontrol agents are released. It is important to know the size and density of the purple loosestrife infestation before biocontrol is implemented as it gives managers baseline data against which future monitoring data will be compared. Monitor sites once a year or every other year. Purple loosestrife, and other vegetation, is evaluated within a quadrat set up as described above. Using Form 3: Purple Loosestrife Biocontrol Monitoring–Vegetation Monitoring and Instructions (see Appendix), do the following:

- **Visual estimates:** Visually estimate how much of the quadrat is covered with purple loosestrife and other vegetation such as cattails and shrubs, expressed as a percentage (Figure 39). Personnel may have to be trained in estimating cover percentage.
- **Stem counts:** Count the number of purple loosestrife and cattail (or other dominant vegetation) stems in the quadrat. Count the total number of inflorescences in the quadrat.

Figure 39. Estimating purple loosestrife coverage.
(UGA1291039)

- **Measure a sample of stems:** Select the five tallest stems in the quadrat to measure. For each stem, measure height, number of inflorescences (on main stem and branches), and length of longest inflorescence. To count the number of flowers, remove the central 2-inch (5-cm) portion of the inflorescence and count the number of flowers and buds. These data enable you to evaluate how individual plants in the infestation are being affected by biological control.
- **Make observations:** Note any special features about the site or any changes at the site since the last examination, such as disturbance, fire, flooding, grazing, and bird nesting activity (or lack thereof).
- **Monitor other vegetation:** Use Form 4: Purple Loosestrife Biocontrol Monitoring–Associated Vegetation (see Appendix) to record other vegetation within the quadrat in the plant community. This form is used to track changes in the composition of the plant community as purple loosestrife declines.

6. Establishing a Photo Point for Monitoring

Photographs of the release site are a valuable qualitative assessment tool. Photos taken annually from a designated photo point can provide a visual record of trends or changes in the vegetation at the site over time, though it does not necessarily show their causes. Thus, photographs are best used in conjunction with other monitoring techniques.

Supplies for taking photos

- Camera (digital or 35 mm with color film)
- Notebook and forms
- Previous year's photo (for reference)
- Metal post
- Bright-colored spray paint

When setting up a photo point, consider the following:

- Always take photographs at the same time of year. Choose the time of year to take the first set of photographs; flowering stages are ideal because of the contrast between flowers and the surrounding vegetation. Taking photographs once a year is sufficient, but it may prove useful to take pictures of the site more frequently.
- Always take photographs from the same direction and at approximately the same time of day.
- Determine the location of the photo point when you establish the release site. If possible, chose a location from which to take photographs that overlooks a large portion of the purple loosestrife infestation.
- Set a colorful metal stake in the infestation as a photo point marker. Include the marker in the photographs. Note and document the location of the photo point marker to find it if it is obscured by vegetation.
- Label images (slides, prints, or digital filenames) clearly, with the year that the photograph was taken.

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Purple Loosestrife Internet Resources

Plant Identification

http://www.lakeheadca.com/lsstrife.htm

http://www.fs.fed.us/database/feis/plants/forb/lytsal/references.html

http://www.mobilebaynep.com/AMRAT/AMRAT%20Species.htm

http://www.entomology.wisc.edu/mbcn/fea501.html

http://www.conservation.state.mo.us/conmag/1998/08/5.html

http://www.cwma.org/prpl_losestrif.html

http://www.seagrant.umn.edu/exotics/purple.html

http://www.ext.nodak.edu/extpubs/plantsci/weeds/w1132w.htm

http://asuwlink.uwyo.edu/~caps/purple/purplels.html

http://www.colostate.edu/Depts/CoopExt/Adams/weed/loosestrife_id.htm

http://www.co.weber.ut.us/weeds/types/p_loosestrife.asp

http://www.ag.unr.edu/wsj/purpleloose.html

http://www.unce.unr.edu/publications/FS02/FS0258.pdf

http://www.minidoka.id.us/weed/noxious/purple_loosestrife.htm

http://oregonweeds.org/weeds/weed_loosestrife.html

http://www.nwcb.wa.gov/weed_info/ploosestrife.html

http://cru.cahe.wsu.edu/CEPublications/pnw0380/pnw0380.pdf

http://dnr.metrokc.gov/wlr/pi/loosestf.htm

http://www.ecy.wa.gov/programs/wq/plants/weeds/purple_loosestrife.html

http://paipm.cas.psu.edu/Ploosestrife.htm

http://members.efn.org/~ipmpa/Noxploos.html

http://plants.usda.gov

http://www.ducks.ca/purple/

http://www.dnr.state.mn.us/ecological_services/exotics/plprog.html

http://www.miseagrant.org/pp/

http://www.npwrc.usgs.gov/resource/1999/loosstrf/loosstrf.htm

http://www.hort.uconn.edu/ipm/ipmbio.htm

http://www.inhs.uiuc.edu/cee/loosestrife/bcpl.html

http://www.invasiveplants.net/InvasivePlants/PurpleLoosestrife/PurpleLoosestrife.asp

Control and Eradication

http://www.lakeheadca.com/lsstrife.htm

http://www.entomology.wisc.edu/mbcn/fea501.html

http://tncweeds.ucdavis.edu/esadocs/lythsali.html

http://www.seagrant.umn.edu/exotics/purple.html

http://asuwlink.uwyo.edu/~caps/purple/purplels.html

http://www.colostate.edu/Depts/CoopExt/Adams/weed/loosestrife_id.htm

http://www.unce.unr.edu/publications/FS02/FS0258.pdf

http://invasives.fws.gov/Index.PT.Loosestrife.html

http://members.efn.org/~ipmpa/Noxploos.html

http://www.dnr.state.mn.us/ecological_services/exotics/plprog.html

http://www.miseagrant.org/pp/

http://www.ceris.purdue.edu/napis/states/ct/ctpls/pls1999.html

http://www.inhs.uiuc.edu/cee/loosestrife/bcpl.html

Biological Control Identification

http://www.hort.uconn.edu/ipm/general/biocntrl/looseposters1.pdf

http://www.entomology.wisc.edu/mbcn/fea501.html

http://www.wes.army.mil/el/aqua/apis/biocontrol/html/nanophye.html

http://www.ext.nodak.edu/extpubs/plantsci/weeds/w1132w.htm

http://mtwow.org/bio-control-purple-loosestrife-links.htm

http://asuwlink.uwyo.edu/~caps/purple/purplels.html

http://www.unce.unr.edu/publications/FS02/FS0258.pdf

http://www.nwcb.wa.gov/weed_info/ploosestrife.html

http://paipm.cas.psu.edu/Ploosestrife.htm

http://members.efn.org/~ipmpa/Noxploos.html

http://www.four-h.purdue.edu/download/purple/pdf/pl_promo.pdf

http://pi.cdfa.ca.gov/purpleloosestrife/BioControl.htm

http://www.ducks.ca/purple/

http://www.dnr.state.mn.us/ecological_services/exotics/plprog.html

http://www.miseagrant.org/pp/

http://www.hort.uconn.edu/ipm/ipmbio.htm

http://www.invasiveplants.net/InvasivePlants/PurpleLoosestrife/PurpleLoosestrife.asp

http://www.inhs.uiuc.edu/cee/loosestrife/bcpl.html

http://www.forestryimages.org

http://www.bugwood.org

Biological Control Rearing

http://www.entomology.wisc.edu/mbcn/fea501.html

http://www.ianr.unl.edu/pubs/weeds/g1436.htm

http://mtwow.org/bio-control-purple-loosestrife-links.htm

http://www.dccl.org/information/Purple_Loosestrife/REARGUI2.doc

http://www.miseagrant.org/pp/

http://www.extension.umn.edu/distribution/horticulture/DG7080.html

http://www.hort.uconn.edu/ipm/ipmbio.htm

http://www.inhs.uiuc.edu/cee/loosestrife/bcpl.html

Research

http://www.entomology.wisc.edu/mbcn/fea501.html

http://www.ag.unr.edu/wsj/purpleloose.html

http://www.unce.unr.edu/publications/FS02/FS0258.pdf

http://www.ducks.ca/purple/abstracts/abstrct1.html

http://www.accesskansas.org/kda/Plantpest/PestManagement/plant-purpleloosestrife.htm

http://www.miseagrant.org/pp/

http://www.npwrc.usgs.gov/resource/1999/loosstrf/loosstrf.htm

http://www.ceris.purdue.edu/napis/states/ct/ctpls/pls1999.html

Impact

http://www.entomology.wisc.edu/mbcn/fea501.html

http://www.conservation.state.mo.us/conmag/1998/08/5.html

http://asuwlink.uwyo.edu/~caps/purple/purplels.html

http://www.ag.unr.edu/wsj/purpleloose.html

http://www.unce.unr.edu/publications/FS02/FS0258.pdf

http://www.nwcb.wa.gov/weed_info/ploosestrife.html

http://dnr.metrokc.gov/wlr/pi/loosestf.htm

http://www.npwrc.usgs.gov/resource/1999/loosstrf/loosstrf.htm

Education

http://www.lakeheadca.com/lsstrife.htm

http://www.dnr.state.wi.us/org/caer/ce/eek/veg/plants/purpleloosestrife.htm

http://paipm.cas.psu.edu/Ploosestrife.htm

http://members.efn.org/~ipmpa/Noxploos.html

http://www.four-h.purdue.edu/download/purple/pdf/pl_promo.pdf

http://www.miseagrant.org/pp/

GLOSSARY

alternate	Leaves that are arranged singly along a stem; one leaf or bud at each node on alternate sides of the stem.
aerenchyma	A spongy tissue with large air spaces found between the cells of the stems and leaves of aquatic plants, providing buoyancy and allowing the circulation of gases.
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).
basal	At the base of a plant or plant part.
biological control	The intentional use of a weed's natural enemy for control purposes; also referred to as biocontrol.
bolting	Plant stage at which the flower stalk begins to grow.
capsule	A pod or seed vessel made of two or more cells, which becomes dry and splits open when mature to release its seeds.
cuticle	The wax layer lining the epidermis of plant leaves, preventing dehydration.
density	Number of individuals per unit area.
diapause	Period of dormancy in insects.
duff	Partially decayed organic matter on the forest floor.
elytron (pl. elytra)	Hardened forewing of a beetle.
epidermis	The outer layer of cells of plant tissue.

emergence	Act of adult insect leaving the pupal case or re-appearing after overwintering.
exoskeleton	Hard, outer frame of an insect that provides structure.
frass	Plant fragments, usually mixed with excrement, deposited by feeding insects.
host specificity	The dietary restriction of an organism to a single or limited food (for herbivores: the number of plant species accepted as food), highly-evolved, often obligatory association between an insect and its host(s).
inflorescence	The flowering structure of a plant.
instar	The period or stage between molts in a insect larva.
larva (pl. larvae)	Immature insect stage between the egg and pupa.
leaf beetles	Small leaf-eating beetles.
membranous	Thin and transparent.
metamorphosis	The change from one life stage to another in insects, such as from larva to pupa.
molt	The process of shedding the exoskeleton in insects during metamorphosis.
monoculture	An area vegetated by a single plant species.
nocturnal	To be active at night time.
node	Points on a stem from which leaves, shoots, or flowers grow—also known as a "joint."
organdy	A fine, transparent cloth or fabric.
oviposit	To lay or deposit eggs.
perennial	A plant living more than two years.
phenology	Chronological sequence (influenced by climate) of an organism's life cycle.
photosynthetic tissue	The production of carbohydrates in plant cells from carbon dioxide and water in the presence of light energy.

pubescence	The short, fine hairs covering a leaf, stem, or flower.
pupa (pl. pupae) (v. pupate)	Nonfeeding, inactive stage between larva and adult in insects.
quadrat	A specific area used to sample vegetation (e.g.,1 square meter).
qualitative	Measurement of descriptive elements (e.g., age class, distribution).
quantitative	Measurement of number or amount (e.g., number of seeds per capsule).
senescence	Decline and death of an organism due to age.
snout	The prolongation of the head of a weevil.
synchrony	Occurring at the same time.
thorax	Body region of an insect behind the head bearing the legs and wings.
transect	A straight line or path through an area.
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 roots.
viability	The proportion of seeds propagules (for example seeds) that are alive.
weevil	A type of plant eating beetle, the adults having distinct snouts of variable lengths.

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SOURCES OF FIGURES

Cover	E. Coombs, Oregon Department of Agriculture (purple loosestrife), S. Schooler, Oregon State University (purple loosestrife beetle), and M. Schwarzlaender, University of Idaho (purple loosestrife root weevil)
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Figures 27, 28	K. Loeffleman, University of Idaho

Figures 31, 32	David Voegtlin, Illinois Natural History Survey http://www.inhs.uiuc.edu/cee/loosestrife/mrear.html
Table 1	
Lythrum alatum, L. lineare, L. hyssopifolia, and *L. virgatum*	Adapted from Illustrated Companion to Gleason and Cronquist's manual: Illustrations of the Vascular Plants of Northeastern United States and Adjacent Canada, Holmgren (1998)
L. flagellare	New York Botanical Garden (http://www.nybg.org/bsci/hcol/vasc/Lythraceae.html)
L. curtissii	Georgia Department of Natural Resources (http://www.georgiawildlife.com/assets/documents/lythcu.pdf)
L. ovalifolium	Adapted from Aquatic and Wetland Plants of the Southwestern United States, Correll and Correll (1995)
L. maritimum	Adapted from Manual of the Flowering Plants of Hawai'i, Wagner et al. (1999)
L. californicum, L. tribracteatum	Adapted from Intermountain Flora, Cronquist et al. (1997)
Table 2	
Spirea douglasii	L. Wilson, University of Idaho
Epilobium angustifolium	L. Wilson, University of Idaho
Verbena hastata	Michigan Department of Natural Resources (http://www.michigan.gov/dnr/0,1607,7-153-10370_12146_12213-36285—,00.html)
Lythrum salicaria	Eric Coombs, Oregon State University
Table 3	D. Landis, Michigan State University (*Galerucella*) M. Schwarzlaender, University of Idaho (*Hylobius*) Bioimages (http://www.bioimages.org.uk) (*Nanophyes*)

Photographs used in this publication can be accessed on line through reference codes (UGA0000000) in the captions for the figures. Point your browser at http://www.forestryimages.org, and enter the reference code at the search prompt.

APPENDIX: TROUBLESHOOTING GUIDE AND FORMS

Troubleshooting Guide: When Things Go Wrong

Purple Loosestrife Biocontrol Release Form

Monitoring Plan Questionnaire

Form 1: Purple Loosestrife Biocontrol Monitoring – Site Location

Form 2: Purple Loosestrife Biocontrol Monitoring – Biocontrol Agent Monitoring

Form 3: Purple Loosestrife Biocontrol Monitoring – Vegetation Monitoring

Form 4: Purple Loosestrife Biocontrol Monitoring – Associated Vegetation

Note: Please make photocopies of these appendices and use them as worksheets.

Troubleshooting Guide: When Things Go Wrong

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

Problem	Probable Cause	Solution
Biocontrol agents unhealthy	Physical damage to insects	Prevent collection containers from colliding; use crush-resistant containers. When aspirating, change vials often, avoid long exposure and crowding in vials.
	Drowning	Do not put excess water in the collection containers but keep the foam moist. Prevent accumulation of excess moisture in the 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 purple loosestrife stems with foliage (no flowers, seeds, or roots) in container; minimize time insects are in containers.
	Release delay	Transport or ship agents immediately after collection; release agents at new site immediately upon arrival or receipt of beetles.
	Parasitism and/or disease	Check source of agents when obtained from a supplier; ensure insect population is disease-free when collecting or receiving shipment.
Number of eggs low	Agents past reproductive stage	Collect adults at times of early peak activity (i.e., when insects are abundant and mating).
Few beetles collected	Wrong collection method used	Refer to Table 4 for recommended collection time and technique.
	Collection done at wrong time	Refer to Table 4 for recommended collection time and technique.
	Collection technique	Beetles can be killed during sweeping or aspirating; use vacuum aspirator if aspirating by mouth is not working; practice aspirating and sweeping.
	Conditions at time of collection	Collect in suitable weather.
Biocontrol agents not found after release	Site is unsuitable	Refer to "Releasing Biocontrol Agents" section.
	Site too small	Select a larger site and dense stand of purple loosestrife.
	Monitoring method	Monitoring method is inappropriate or monitoring was done at the wrong time.
	Not enough agents released	Repeat releases with a larger number; don't give up.
	Bad luck	Bad weather event, flooding, spraying, or other event may have eradicated beetles. Initiate another release.

Problem	Probable Cause	Solution
Beetles do not build up populations in years after release	Existing populations may be too small	Release additional beetles.
	Predation	Investigate and release large numbers to "swamp" site to avoid specific predators.
	Site unsuitable	Don't give up: repeat steps outlined above or try to assess which factors are critical and can be changed.
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.

Purple Loosestrife Biocontrol Release Form

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

County: _____ **State:**_____ **# Released:** _____

Biocontrol Agent: _____ **Date Collected:** ___/___/___
(mm dd yy)

Source of Agents: _____

Life Stage (circle)**:** Larvae Pupae Adults Eggs Other (specify) _____

Land Ownership (circle)**:** Private County State USFS BLM COE BOR BIA/Tribe TNC Other (specify) _____

Legal: T_____ R_____ Sec_____ Q_____ QQ_____ **Lat**: Deg____Min____Sec_____ **Long**: Deg___Min_____Sec_____

ENVIRONMENT

Temperature (°F):_____ **Wind:** Calm Light Moderate Strong Gusty **Wind Direction:** N S E W

Weather (circle)**:** Clear Ptly Cloudy Cloudy Rain **Release Time**:_____am/pm

Site Elevation:_____

Disturbance: (check all that apply, circle most prevalent) Cultivation ___ Fire ___ Flood ___ Grazing ___
Roads ____ Recreation ____

Directions to Site (include a map to the site on the back of this form):

_____

_____

SITE CHARACTERISTICS

Site Name:_____ **Size of Infestation** (acres)**:**_____ **Weed Cover %:**_____

Weed Height:_____ **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):

- River/Stream bank ☐
- Wetland ☐
- Ditch/Canal ☐
- Lakeshore ☐
- Reservoir ☐
- Wet Meadow ☐
- Dry Meadow ☐
- Other (specify): _____

Comments (continue on reverse if necessary):

Monitoring Plan 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 montiroing plan.

What is the management objective of the biocontrol release site?

a) Establish a study site for long-term monitoring.

b) Establish nursery site to increase numbers of beetles for future collection and redistribution.

c) Create an open release site; no additional monitoring is intended.

Notes: _____

_____

_____

What will be measured?

a) Biocontrol agent presence/absence.

b) Biocontrol 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? _____

Form 1: Purple Loosestrife Biocontrol Monitoring–Site Location

Site Name:_____ State:_____GPS: N _____° _____'

Town: _____ County: _____ W _____° _____'

Date: _____ _____ _____
year *month* *day*

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

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

SITE CHARACTERISTICS:
Habitat type: ___River ___Wetland ___Lake ___Meadow ___Irrigation Ditch ___Other

Road Map to Site

Site and Vegetation Map

INSECT RELEASE HISTORY:

Date (year-month-day)	Species	Number and Stage (egg/larvae/adult)	Position of Release On Map (1,2,3,4…)

Form 2: Purple Loosestrife Biocontrol Monitoring–Insect Monitoring

SITE: _____ **STATE:** _____

DATE: _____ _____ _____
year *month* *day*

GPS: N _____º_____'

W _____º_____'

INVESTIGATORS:
Last name *First name*

_____ _____

_____ _____

_____ _____

TIME: _____

TEMPERATURE: _____

WEATHER: _____

Chart A
Insect Abundance (#/stem)

I	1-10
II	11-25
III	26-100
IV	100-500
V	>500

Chart B:
Damage or Percent Cover Class

A	<1%
B	1-5%
C	6-25%
D	26-50%
E	51-75%
F	76-95%
G	>95%

A = Adults
L = Larvae
E= Eggs

Hyl = Hylobius
Nano = Nanophyes

1 = present
2 = abundant
3 = very abundant

Quad	Galerucella			Hyl	Nano	Purple Loosestrife			Cattail		Other Insects seen:
#	A	L	E	A	A	%damage	%cover	#stems	%cover	#stems	
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

Notes:

Instructions for FORM 2: Purple Loosestrife Biocontrol Monitoring – Insect Monitoring

Materials needed: 1.0 m^2 quadrat frame, data sheets, stopwatch, pencils, clipboard.

General: The purpose of this activity is to estimate the abundance of biocontrol insects at the site. Conduct the monitoring in late spring/early summer, about a month after *Galerucella* adults appear after overwintering at your site. 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) Position the quadrat: Carefully approach the quadrat and watch for adults of all three species when you slide the quadrat frame into position. *Hylobius* and *Nanophyes* adults often drop from the vegetation once you touch stems (or even as you approach the quadrat).

3) Beetle counting: Use a 1 minute total search time for each insect species released and for each life stage that can be observed. At a site where only *Galerucella* was released, 3 minutes total will be spent searching for eggs, larvae, and adults (one minute for eggs, one for larvae, and one for adults). At a site where only *Hylobius* or *Nanophyes* were released, count only the adults for one minute. At sites with two or three species, count each species separately. Use Chart A to record the category of abundance (I-V).

4) Estimate feeding damage: Examine the purple loosestrife for any damage to the leaves or shoots, such as the 'shothole' feeding pattern of the *Galerucella* beetles. Estimate the percent leaf area removed by insect feeding over the entire quadrat, using Chart B to determine the category (A-G) of damage. Practice estimating the amount of leaf damage with an experienced observer. Initially after release, the amount of leaf damage will be low or non-existent. As *Galerucella* density increases, so to will the amount of leaf damage, which can be very high (> 50%) at high beetle density.

5) Measuring vegetation: After you have completed the insect counts, stand over the quadrat and visually estimate how much of the quadrat is covered by purple loosestrife and how much is covered by cattail (use cover estimates in Chart B). Cattail (*Typha* sp.) is the most common plant associated with wetlands across North America. If you do not have cattail, leave this category blank or substitute with the most common species at your site. Then, count the number of purple loosestrife (> 20 cm tall) and cattail stems. Be careful to distinguish between a stem and a branch; only stems are counted.

6) Other observations: Record any general observations or useful information; disturbances, flooding, fire, bird nests, etc., for the sample quadrat or the site in general.

Form 3: Purple Loosestrife Biocontrol Monitoring–Vegetation Monitoring

SITE: _____ **STATE:** _____

DATE: _____ _____ _____ **GPS:** **N** _____**o**_____'
year *month* *day* **W** _____**o**_____'

INVESTIGATORS:
Last name *First name* **TIME:**_____
_____ _____ **TEMPERATURE:** _____
_____ _____ **WEATHER:** _____
_____ _____

Chart A: Percent Cover

A	<1%
B	1-5%
C	6-25%
D	26-50%
E	51-75%
F	76-95%
G	>95%

S= sterile
F = fertile

PL = Purple Loosestrife

Quad #	Percent Cover (Chart B)		Number of stems		Number of inflorescences		Purple Loosestrife (5 tallest stems)				Cattail (5 tallest stems)	
	PL	Cattail	PL	Cattail	PL	Cattail	Height (cm)	# of inflores-cences	Length (cm) terminal inflorescence	# Flower buds center 5cm of inflorescence	Height (cm)	S/F
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												

Instructions for Form 3 - Purple Loosestrife Biocontrol Monitoring – Vegetation Monitoring

Materials needed: 1 meter stick, 1.0 m^2 quadrat frame, data sheets, pencils, clipboard, camera, and GPS unit to relocate quadrats. The site visit should be from late August to early October.

G**eneral:** The purpose of this activity is to estimate the abundance of purple loosestrife and other vegetation in the wetland community, and to record measurements of purple loosestrife plant attributes. Conduct monitoring in late summer to mid autumn. 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) Position the quadrat: Carefully position the quadrat on the ground in the purple loosestrife infestation, being sure not to damage the plants, i.e., break stems, etc.

3) Estimate percent cover: Slide the quadrat frame into position, as close to the ground as possible; move stems in or out of the frame so that all stems originating in the quadrat are included. Standing near the frame, estimate how much of the quadrat is covered by purple loosestrife and, independently, how much is covered by cattail. Use cover estimates in Chart A to estimate percent cover.

4) Count stems: Count the number of loosestrife and cattail stems, beginning at one corner of the quadrat and working systematically across the quadrat. To be counted, a stem must be >20cm tall and originate within the quadrat; if it originates under the frame, or outside the frame and leans over the quadrat, then it is not recorded. Be careful to distinguish between a stem and a branch; only stems are counted. A stem originates from the ground or within 5 cm of the ground, while a branch originates from a stem at least 5 cm above the ground. In dense stands, it is helpful to look beneath the loosestrife canopy, and to move stems with your hands while counting; this is the easiest way to separate stems from branches.

5) Count inflorescences: Next, count the total number of purple loosestrife and cattail inflorescences in your quadrat. Make sure to count only those inflorescences that originate on stems rooted in your quadrat. An inflorescence is the portion of stem above and including the lowest flower bud. Even if only one flower bud is present, it is counted as an inflorescence. Be careful to only count flower buds, and not the small bundles of reddish leaves in the inflorescence axils.

6) Measure plants: Select the 5 tallest purple loosestrife stems in each quadrat (if there are fewer than 5 stems/quadrat, measure all that are present); four measures will be made on each stem; a) Measure the stem height (to the closest cm); b) Count the number of inflorescences on that stem (including all side branches), c) Measure the length (to the closest cm) of the longest inflorescence on this stem (this will generally be the terminal inflorescence); d) Remove the central 5cm portion of this inflorescence. Count the number of flower buds in this 5 cm length of inflorescence. If the plant did not produce any inflorescences or if they are shorter than 5 cm please record this in the appropriate form. Repeat this process for the remaining 4 loosestrife stems. Repeat with 5 tallest cattail stems.

7) Other observations: Record any general observations or useful information, such as disturbances, flooding, fire, bird nests, etc.

Form 4: Purple Loosestrife Biocontrol Monitoring–Associated Vegetation

SITE: _____ **STATE:** _____

DATE: _____ _____ _____
year month day

GPS: **N** _____º_____
W _____º_____

INVESTIGATORS:

Last name *First name*

_____ _____

_____ _____

_____ _____

TIME:_____

TEMPERATURE: _____

WEATHER: _____

Chart A: PercentCover

A	**<1%**
B	**1-5%**
C	**6-25%**
D	**26-50%**
E	**51-75%**
F	**76-95%**
G	**>95%**

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
Percent of Quadrat (Use Chart B or actual estimates that total 100%)										
Vegetated										
Unvegetated (soil, water, litter, etc)										
Vegetation Cover (Use Chart B; total may exceed 100% due to overlapping of vegetation)										
Purple Loosestrife										
Cattail										
All other vegetation:										
Forbs										
Grasses and Sedges										
Woody plants										
Individual Species (names) (Check if present or use Chart B to indicate percent cover)										