Massachusetts Office of Coastal Zone Management
Wetlands Restoration Program

Guidance Document for the Purple Loosestrife Biocontrol Project
November 2006

This guidance document for the Massachusetts Wetlands Restoration Program's Purple Loosestrife Biocontrol Project (the Project) briefly reviews the use of biocontrol measures in Massachusetts to control the invasive wetland plant, purple loosestrife, and provides information to people who may be interested in participating in the Project.

Background

Purple loosestrife (*Lythrum salicaria*) is an invasive wetland plant originally from Europe and Asia. In the United States, there are no natural enemies that control purple loosestrife populations. As a result, the plant spreads rapidly and causes significant negative impacts, including reduced native plant coverage, lower plant diversity, and impaired wildlife habitat. (See the purple loosestrife fact sheet in Appendix 1 for more information.)

Viable options for managing purple loosestrife via conventional means (water level management, burning, herbicides, manual removal, and cutting) have proven extremely difficult and impractical on a large scale. An alternative is the biological control of purple loosestrife via intentional introduction of natural predators.

Extensive studies have identified several beetle species in Europe that feed and breed on purple loosestrife and that control populations there. These beetles have been extensively tested in the United States since 1986 to assess their safety and efficacy as biocontrol agents, leading to a 1992 approval by the United States Department of Agriculture of their use for biocontrol purposes. Published literature indicates that no significant long-term negative impacts on native plant species have been observed. The beetles prefer to eat purple loosestrife and will successfully lay eggs only on that plant.

The beetles used in the WRP project are leaf-feeding *Galerucella sp*. (*G. pusilla* and *G. calmariensis*). The beetles over-winter in a dormant state in the soil, then emerge in the spring to reproduce. The adults feed on purple loosestrife leaves and create a bullet-hole pattern in the leaf. In the summer, adults breed and lay eggs on the leaves and stems of purple loosestrife. Larvae emerge and feed on the leaves of the plant, causing significant damage in the form of a window-pane pattern on the leaves (larval damage does not penetrate the entire leaf, but the upper or lower layers only). The larvae pupate in the soil and the next generation of adults will emerge in the mid-summer. In New England, it is rare for beetles to go through more than one reproductive generation per year. However in warmer climates, this is often possible.

Normally, beetles are purchased from a supplier, transferred from another site where a beetle population has been established, or are reared in a controlled environment and then transferred to the site. Releases occur in the spring or summer at pre-selected and monitored sites of dense purple loosestrife infestation. The goal is to establish a self-sustaining beetle population at each release site that will control purple loosestrife. In general, annual releases over three to four years are needed to cause a significant impact, or decline, in purple loosestrife.

While these natural beetle predators cannot eliminate purple loosestrife entirely, they have been shown to significantly reduce the density of the plant (by up to 90% in some studies) and allow re-establishment of

native wetland vegetation. Beetle populations stay in balance with purple loosestrife availability and will increase or decrease in proportion with the plant's abundance. When the population of purple loosestrife in a wetland is reduced by effective biocontrol measures, beetle populations will decline as well.

Galerucella sp. beetles have been used successfully in the United States to control purple loosestrife infestations since the early 1990s. Treatments have occurred in all of the New England states, including Massachusetts, where beetles were first released on National Wildlife Refuges (Great Meadows NWR and Parker River NWR) in the mid- to late- 1990s.

Massachusetts Wetlands Restoration Program Purple Loosestrife Biocontrol Project

The Wetlands Restoration Program initiated a pilot Purple Loosestrife Biocontrol Project in 2000. The overall goal of the Project is to enhance the health, condition, and diversity of habitats and native species within wetlands that have been degraded by purple loosestrife infestations. As of 2006, WRP has facilitated beetle releases at 17 sites in Massachusetts. Volunteer organizations have participated in beetle rearing, beetle release, and spring and fall site monitoring. Extensive monitoring of treatment sites has occurred to document the effects of the beetles on purple loosestrife growth and the establishment of self-sustaining beetle populations. Several sites in Massachusetts have shown successful reductions in purple loosestrife coverage and vigor after multiple beetle releases over three to four years.

In 2005, funding was allocated by the Federal Aquatic Nuisance Species Taskforce for CZM to hire a half-time purple loosestrife biocontrol project coordinator. Additionally, WRP has received funding through a United States Fish and Wildlife Service Cooperative Agreement to support expansion of the Project. WRP plans to develop additional partnerships and support the expansion of treatment sites throughout the state. The Project will continue to use a volunteer-based model and will partner with schools and conservation organizations to help raise and release beetles and monitor treatment sites. Additionally, WRP is collaborating with government agencies and other partners to develop a long-range strategic plan for the biological control of purple loosestrife throughout Massachusetts.

What WRP Provides

The degree of WRP involvement in purple loosestrife biocontrol varies from site to site. WRP can provide initial technical advice and guidance to all parties interested in purple loosestrife biocontrol in Massachusetts. If organizations are interested in receiving a greater level of assistance for a particular site -- via beetle provision, monitoring assistance, etc. -- then that site should be nominated for WRP assistance using the attached site nomination form (see below and Appendix 2). WRP annually reviews nominations and considers the merits of individual sites for purple loosestrife control, along with available resources, to determine which sites will receive WRP assistance.

Cooperative Agreement

When WRP determines that sufficient resources are available and a site merits more significant involvement, the nominating entity and WRP will enter into a cooperative agreement. See Appendix 3 for the current Cooperative Agreement.

Regulatory Considerations

Familiarity with federal, state and local permits regarding biocontrol agents and wetland activities is important. WRP maintains a permit from the USDA Animal and Plant Health Inspection Service (APHIS) to import exotic *Galerucella* beetles and/or release approved biocontrol organisms into the environment.

The Massachusetts Department of Environmental Protection (MassDEP) supports the partnership efforts described herein and the goal of improving wetland conditions through the introduction of biocontrol beetles to manage purple loosestrife infestations. MassDEP encourages anyone considering purple loosestrife biocontrol activities to collaborate with WRP.

WRP coordinates with the Massachusetts Natural Heritage and Endangered Species Program (NHESP) and local conservation commissions during the review process for proposed release sites. Conservation commissions are provided with information about the Project and data specific to sites in their jurisdiction. WRP and local volunteers continue to monitor selected release sites for at least three years. A summary report of monitoring results is provided to state agencies and the conservation commission in each town where a release has occurred.

Site Selection

WRP considers the following criteria when evaluating a proposal for biocontrol treatment at a new site:

Organizational/Logistical support

- Landowner permission/approval for release, establishment of permanent markers, and long-term monitoring (5-10 years).
- Support from the local Conservation Commission and approval from NHESP.
- Local sponsor/supporting organization willing to oversee the release and commit to long-term monitoring (ideally to rear beetles as well).

Physical criteria

- Sufficient coverage and density of purple loosestrife to support a self-sustaining beetle population (typically 1-2 acres).
- Adequate access for monitoring and release activities.
- Protection of site from major disruptions (e.g., herbicide treatment, other weed control regiment, flooding, or insecticide use). No recent history (during prior two years) of insecticide spraying or plans to spray in future.
- Presence of native wetland species as a seed source to reestablish areas with native vegetation after purple loosestrife declines.
- Consideration of potentially vulnerable native species (e.g., *Lythrum alatum*, *Lysimachia spp*.) or state-listed rare plant species (site records reviewed by NHESP).
- High conservation value (e.g., isolated stands of native plants clearly threatened by purple loosestrife).

Site Nominations

To nominate a site for purple loosestrife biocontrol treatment in collaboration with WRP, fill out the nomination form found in Appendix 2 and submit to Beth Suedmeyer at beth.suedmeyer@state.ma.us. When submitting a nomination, please include your name and contact information (email, address, phone number), the name of the

site, the location of the site (town, nearest street address or intersection, description of location), the name of the landowner, description of the extent and density of purple loosestrife, and a map of the site.

Beetle Orders

WRP assists in procuring beetles for introduction at sites participating in the Project. Beetles may be purchased through a supplier, obtained from one of the beetle rearing sites in Massachusetts, or transferred from sites with established beetle populations. The current source of purchased beetles is the New Jersey Department of Agriculture. As previously mentioned, WRP maintains a USDA - APHIS permit to import *Galerucella sp.* beetles for the purpose of release as biocontrol agents. Organizations that enter into a Cooperative Agreement with WRP and agree to follow the guidance described in this document may be able to use WRP's beetle transport and importation permit.

Beetle Rearing

To date, several schools (middle schools through colleges) have participated in the beetle rearing project by cultivating purple loosestrife, introducing beetles to the plants, and allowing beetles to reproduce, resulting in a 100-fold or greater increase in the beetle population. After the next generation of adult beetles emerges, the population is released to a predetermined treatment site. The rearing process begins in April and extends until late June or July when the beetles are released. The complete protocol used for rearing beetles is found in Appendix 4.

Monitoring

Pre-treatment monitoring is done to determine the composition of plant diversity at the wetland prior to initiation of the biocontrol project. Long term monitoring is required to assess the effectiveness of establishing a self-sustaining beetle population and significantly reducing the purple loosestrife infestation. WRP staff train local land stewards in monitoring methodologies, so that they may continue the long-term, routine monitoring with limited guidance. The protocol in Appendix 5 describes the monitoring to be done each spring and fall. The protocol requires the establishment of permanent quadrats to monitor change over time. Spring monitoring collects data on purple loosestrife plant characteristics and beetle presence and absence in monitoring quadrats. Fall monitoring collects more specific information on purple loosestrife vitality at the time of flowering. Photo documentation of sites from specific vantage points each year visually documents changes in sites over time.

After several years of beetle releases, when a self-sustaining population has been established, it is anticipated that beetles may disperse to other purple loosestrife infested areas up to 10 miles from the original treatment site. In order for WRP to effectively and efficiently plan for future releases and project needs, it is important to know the locations of *Galerucella* beetle populations away from WRP-initiated treatment sites. For this reason, WRP requests the assistance of the public, especially people who spend time in wetlands for work or recreation, in identifying stands of purple loosestrife which have *Galerucella* beetles and/or evidence of *Galerucella* feeding damage. Reports may be submitted using the site nomination form (Appendix 2). An identification card, depicting *Galerucella* and *Galerucella* feeding damage can be found in Appendix 6.

Data Sharing

Collaborating organizations use the data sheets provided with the monitoring protocol (Appendix 5) to record routinely collected data in the field. Data sheets are copied and sent into WRP and entered into WRP's

Statewide Purple Loosestrife Biocontrol Project Database. WRP creates an annual report to share the data and results from the Purple Loosestrife Biocontrol Project.

Appendices

Appendix 1: Purple Loosestrife Fact Sheet from the Plant Conservation Alliance's Alien Plant Working Group

Appendix 2: WRP Purple Loosestrife Inventory and Biocontrol Project Site Nomination Form

Appendix 3: Cooperative Agreement between local supporting organization and WRP

Appendix 4: Purple Loosestrife Biocontrol Beetle Rearing Protocol

Appendix 5: Purple Loosestrife Biocontrol Monitoring Protocol from Cornell University

Appendix 6: Beetle and Beetle Damage Identification Card

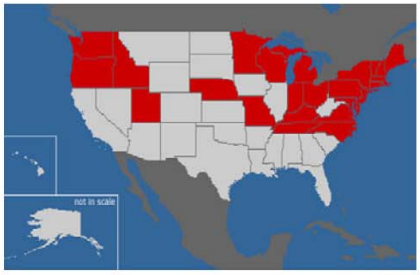
Fact Sheet: Purple Loosestrife

Purple Loosestrife
Lythrum salicaria L.
Loosestrife family (Lythraceae)

NATIVE RANGE
Eurasia; throughout Great Britain, and across central and southern Europe to central Russia, Japan, Manchuria China, southeast Asia and northern India

DESCRIPTION
Purple loosestrife is an erect perennial herb in the loosestrife family, with a square, woody stem and opposite or whorled leaves. Leaves are lance-shaped, stalkless, and heart-shaped or rounded at the base. Plants are usually covered by a downy pubescence. Loosestrife plants grow from four to ten feet high, depending upon conditions, and produce a showy display of magenta-colored flower spikes throughout much of the summer. Flowers have five to seven petals. Mature plants can have from 30 to 50 stems arising from a single rootstock.

ECOLOGICAL THREAT
Purple loosestrife adapts readily to natural and disturbed wetlands. As it establishes and expands, it outcompetes and replaces native grasses, sedges, and other flowering plants that provide a higher quality source of nutrition for wildlife. The highly invasive nature of purple loosestrife allows it to form dense, homogeneous stands that restrict native wetland plant species, including some federally endangered orchids, and reduce habitat for waterfowl.



DISTRIBUTION IN THE UNITED STATES
According to the U.S. Fish and Wildlife Service, purple loosestrife now occurs in every state except Florida.

HABITAT IN THE UNITED STATES
Purple loosestrife is capable of invading many wetland types, including freshwater wet meadows, tidal and non-tidal marshes, river and stream banks, pond edges, reservoirs, and ditches.

BACKGROUND
Purple loosestrife was introduced to the northeastern U.S. and Canada in the 1800s, for ornamental and medicinal uses. It is still widely sold as an ornamental, except in states such as Minnesota, Wisconsin, and Illinois where regulations now prohibit its sale, purchase and distribution.

BIOLOGY & SPREAD
Purple loosestrife enjoys an extended flowering season, generally from June to September, which allows it to produce vast quantities of seed. The flowers require pollination by insects, for which it supplies an abundant source of nectar. A mature plant may have as many as thirty flowering stems capable of producing an estimated two to three million, minute seeds per year.

Purple loosestrife also readily reproduces vegetatively through underground stems at a rate of about one foot per year. Many new stems may emerge vegetatively from a single rootstock of the previous year. "Guaranteed sterile" cultivars of purple loosestrife are actually highly fertile and able to cross freely with purple loosestrife and with other native Lythrum species. Therefore, outside of its native range, purple loosestrife of any form should be avoided.

MANAGEMENT OPTIONS

Small infestations of young purple loosestrife plants may be pulled by hand, preferably before seed set. For older plants, spot treating with a glyphosate type herbicide (e.g., Rodeo® for wetlands, Roundup® for uplands) is recommended. These herbicides may be most effective when applied late in the season when plant are preparing for dormancy. However, it may be best to do a mid-summer and a late season treatment, to reduce the amount of seed produced.

Biological

While herbicides and hand removal may be useful for controlling individual plants or small populations, biological control is seen as the most likely candidate for effective long term control of large infestations of purple loosestrife. As of 1997, three insect species from Europe have been approved by the U.S. Department of Agriculture for use as biological control agents. These plant-eating insects include a root-mining weevil (*Hylobius transversovittatus*), and two leaf-feeding beetles (*Galerucella calmariensis* and *Galerucella pusilla*). Two flower-feeding beetles (*Nanophyes*) that feed on various parts of purple loosestrife plants are still under investigation. Galerucella and Hylobius have been released experimentally in natural areas in 16 northern states, from Oregon to New York. Although these beetles have been observed occasionally feeding on native plant species, their potential impact to non-target species is considered to be low.

USE PESTICIDES WISELY: Always read the entire pesticide label carefully, follow all mixing and application instructions and wear all recommended personal protective gear and clothing. Contact your state department of agriculture for any additional pesticide use requirements, restrictions or recommendations.

NOTICE: mention of pesticide products on this page does not constitute endorsement of any material.

CONTACTS

For more information on the management of purple loosestrife, please contact:

- Cornell University Non-indigenous Plant Species Program, http://www.invasiveplants.net
- Virginia Natural Heritage Program. http://www.dcr.virginia.gov/dnh/invinfo.htm

SUGGESTED ALTERNATIVE PLANTS

Native species of Liatris (blazing star) have showy pink-purple flower spikes and are an important nectar source for many native species of butterflies and other insects.

OTHER LINKS

- http://www.invasive.org/search/action.cfm?q=Lythrum%20salicaria
- http://www.lib.uconn.edu/webapps/ipane/browsing.cfm?descriptionid=72

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REFERENCES

Heidorn, R. and B. Anderson. 1991. Vegetation management guideline: purple loosestrife (*Lythrum salicaria* L.). Natural Areas Journal 11:172-173.

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Virginia Department of Conservation and Natural Resources. 1995. Invasive Alien Plant Species of Virginia: Purple Loosestrife (*Lythrum salicaria*).

Appendix 2: WRP Purple Loosestrife Inventory and Biocontrol Project Site Nomination Form

To submit a purple loosestrife site inventory and/or site nomination information, please complete this form and attach a site map. Return to Beth Suedmeyer at the address below. (Note: This form is not intended to be used for current WRP biocontrol treatment sites).

○ Check if submitted for Project site nomination.
○ Check if submitted for inventory purposes.

SECTION 1: CONTACT INFORMATION

Submitter's information **Date**
Name
Address Town/Zip
Phone Email

Information on associated organization (if any)
Name
Address Town/Zip
Phone Email

SECTION 2: SITE INFORMATION

Site name
Site Town

Landowner contact information: ○ Check if same as organization above.
Name
Address
Phone
Email

Nearest intersection Geographic coordinates (if known)

Description of location

Approximate Acreage of Infestation (circle one)
Less than ½ acre
½ -1 acre
1-10 acres
More than 10 acres
Unknown

Location type (circle one)
Lake
River
Marsh/Wetland
Roadside ditch
Other _____

Approximate density of purple loosestrife (stems per sq. meter, may indicate a range)?

Purple Loosestrife Condition
Has the site been surveyed for evidence of Galerucella beetle activity (presence of eggs, larvae, adults, or leaf damage)?

Was evidence of purple loosestrife damage by larval or adult beetle feeding (herbivory) found? (*See Galerucella identification cards.)* If yes, describe area of site where found and indicate on map.
Larval? Adult?

Were there any of the following on the purple loosestrife? If yes, describe area of site where found and indicate on map. Galerucella *(See Galerucella identification cards.)*:
Adults? Eggs? Larvae?

Beth Suedmeyer · Wetlands Restoration Program · 251 Causeway Street, Suite 800 · Boston, MA 02114
Fax · 617 626 1240 · beth.suedmeyer@state.ma.us

COOPERATIVE AGREEMENT

for

_____***<site>***_____

PURPLE LOOSESTRIFE BIOCONTROL PROJECT

between

THE MASSACHUSETTS OFFICE OF COSTAL ZONE MANAGEMENT
WETLANDS RESTORATION PROGRAM

and

_____***<Organization>***_____

Through this agreement, the Wetlands Restoration Program (WRP) recognizes a Cooperative Agreement with _____ (the organization) toward improving the aquatic resources and wetland habitat of _____ (the site) through invasive species management activities and ultimately wetlands restoration. WRP agrees to support the organization in pursuing this project and the organization agrees to continue their efforts to implement the Purple Loosestrife Biocontrol Project at this site, as described below.

1. The organization and WRP will implement this biological control project by following the methods and protocols outlined in the WRP Guidance Document for the Purple Loosestrife Biocontrol Project. This includes carrying out the program for monitoring the progress of the project to document results and submitting data to WRP so that other restoration efforts may benefit from information gathered at this site. WRP will provide technical and other assistance in carrying out the restoration project.

2. The parties indicated above will seek opportunities to improve public awareness of wetlands and wetland restoration. This effort includes implementing strategies for involving volunteers in the monitoring and other elements of the project as appropriate.

3. WRP will assist in project development, coordination, and implementation in any way possible. This may include coordination with other agencies and programs, providing technical information, and conducting training and information sessions.

More specifically, the following commitments are required from each party in order for WRP to invest in treatments at a particular site. The collaborators' signatures at the bottom of this form indicate agreement with the following terms.

The organization will:

- Secure landowner permission/agreement for: beetle release (up to 4 years), establishment of permanent plot markers, long-term monitoring (approximately 3 years following last beetle release at the site), and

commitment to protect site from disruptions (e.g., herbicide treatment, other weed control regiment, flooding, or insecticide use).

- Identify local volunteers willing to participate in the release and commit to long-term monitoring (ideally to collect, rear, and release beetles according to WRP guidelines).
- Compile site data and submit to WRP according to a standard monitoring protocol.
- Inform WRP of any disturbance or activity on the site that is likely to impact the purple loosestrife stand or the beetle population.
- Not collect or allow for the collection of *Galerucella* beetles from this site without consulting the WRP.

The Wetlands Restoration Program and its agents will:

- Provide guidance, technical support in beetle rearing (if applicable), beetle release, and beetle population and purple loosestrife monitoring.
- Provide materials and beetles for the project, as determined by WRP to be necessary and subject to available resources.
- Provide guidance for addressing regulatory issues and complying with requirements for biocontrol projects, including assisting in preparation of requests for approvals from state agencies and notices to Conservation Commissions
- Participate in release and monitoring activities as allowed by staff schedules.
- Help promote the collaborative project and address any project concerns.
- Compile data and annually report on the status of the project.

Nothing in this agreement shall obligate the Commonwealth of Massachusetts, the Executive Office of Environmental Affairs, or the Office of Coastal Zone Management to expend any funds or provide technical assistance in excess of current appropriations or otherwise prohibited by law.

The Wetlands Restoration Program reserves the right to terminate this cooperative agreement, at any time in its sole discretion.

This agreement is entered into this _____ day of _____ in the year _____.

Representatives:

_____
Signature, Representative Supporting Organization

_____
Signature, Representative Wetlands Restoration Program

Please complete form and return a copy to -
Beth Suedmeyer
Wetlands Restoration Program
Massachusetts Office of Coastal Zone Management,
251 Causeway Street, Suite 800
Boston, MA 02114-2136
Fax: 617-626-1240
Email: beth.suedmeyer@state.ma.us

Biological Control of Purple Loosestrife: A Guide for Rearing Leaf-feeding Beetles

Alyson Loos, Jr. Scientist
David Ragsdale, Professor Entomology

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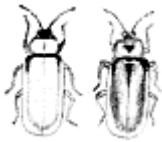
Introduction



Biological control (biocontrol) is using a living organism to control a pest. The goal is to reduce the numbers of the target pest organism, not to eradicate the pest. Biocontrol has been used to effectively control exotic weed and insect pests by introducing natural enemies to an infested area. Two species of beetles in the genus *Galerucella* are used for biocontrol of the exotic wetland weed purple loosestrife (*Lythrum salicaria*).

Purple loosestrife is an aggressive perennial plant of European origin found throughout Canada and the United States. Minnesota currently has over 1,800 known sites infested with purple loosestrife that collectively cover approximately 38,000 acres. Purple loosestrife is a serious concern because it displaces native wetland plants and can become the dominant plant, thereby reducing species diversity and changing the ecosystem of a wetland. A single purple loosestrife plant with multiple stems can produce between one and two million seeds that are easily dispersed along rivers and waterways. Even a few purple loosestrife plants pose a serious threat to an entire wetland.

The leaf-feeding beetles (*Galerucella* spp) reduce the growth and reproduction of purple loosestrife. The adult beetles feed on the leaves of purple loosestrife and lay their eggs. Once the eggs have hatched, the larvae feed on the leaves and stems as they move down into the soil. The larvae cause the most damage to the plant and reduce the number of seeds produced. The leaf-feeding beetles released in Minnesota originated in Germany, and years of host-range screening were conducted to determine host specificity before approval was granted by the United States Department of Agriculture to release these beetles as biological control agents.

The beetles feed primarily upon purple loosestrife and have a low preference for a few native *Decodon* and *Lythrum* species. The risk to these native species was determined to be far greater if we did nothing, because their habitat would be overrun by purple loosestrife.

This publication is a guide to rearing leaf-feeding beetles for biological control of purple loosestrife. Successful establishment of the beetles will reduce the impact of purple loosestrife on native wetland plants.

Purple Loosestrife

Identifying

Purple loosestrife stems end with a spike of many individual flowers. Each flower has five to six pink-purple petals (Figure 1). Other key characteristics are: 1) a four-to-six sided stem that can be two-to-six feet tall and woody with several stems arising from a perennial crown root, 2) leaves usually opposite or whorled at the base of the stem, becoming alternate at the top, and 3) a prominent leaf venation with pinnate veins ending in a common vein parallel to, and extending along the entire leaf margin (Figure 2). Don't be confused by purple loosestrife look-a-likes. Information on look-a-likes and replacement alternatives can be found in the *Replacing Loosestrife* section of this publication.

Figure 1

Figure 2

Controlling Biologically

The following is a step-by-step guide for growing purple loosestrife, rearing the beetles, and releasing the beetles into a purple loosestrife infested wetland. Because purple loosestrife is a noxious weed, you must obtain permission from the Minnesota Department of Agriculture and Department of Natural Resources to grow these plants.

Step 1. Field collection of root crowns

- Equipment
  - Long-handled round-pointed shovel
  - Extra heavy garbage bags
  - Pruning shears
  - Personal gear (hip or chest waders, gloves & protective eyewear)

Root crowns of purple loosestrife are collected from wetlands and grown in pots to provide a food source for the beetle adults and larvae. Contact your county agriculture inspector for permission to transport root crowns as part of this biological control project before you do any collecting.

Root crown harvesting

Purple loosestrife root crowns need to be harvested in early spring. Crowns should be collected as soon as wetlands have thawed in late April to early May (before loosestrife buds begin to appear). Shoot growth from purple loosestrife crowns is dependent upon weather conditions. Therefore, it is important to collect and pot root crowns *as soon as possible* in the spring, because it takes between three-to-five weeks before plants are large enough to begin rearing beetles. Northern Minnesotans may want to travel south to find a wetland that has thawed by late April. Another option (if you have access to a cold room facility), is to collect root crowns in the fall and store them over the winter. Dig root crowns after the first hard frost in early October, when flowers have senesced. Root crowns must be moist and can be kept in garbage bags when stored in a cold room facility (approximately 40°F). The keys to storing root crowns over the winter is making sure they are moist and that they receive no light.

Root crown digging

Choose a wetland that has easy access for hauling root crowns back to your vehicle. They are heavy! Use the shovel to cut around the outer base of a multi-stemmed loosestrife plant to dig up crowns. Large crowns can be cut (using your shovel) or pulled apart. A plant with six-to-eight stems is the appropriate size for beetle rearing when potted. Clip the old stems at the base and leave them in the wetland. Collect the number of root crowns needed for your project size, and haul them in garbage bags out of the wetland. Use the strongest garbage bags you can find. Wetlands are muddy and wet, so wear appropriate boots and clothes.

Step 2. Culturing and maintaining host plants

To produce plants of sufficient size for beetle rearing, root crowns should be bigger than the size of a softball, and can be trimmed to fit into a 3-gallon pot.

Large potting projects:

- Equipment
  - 22 pots (3-gallon)
  - One bale of potting soil (e.g., Pro-Mix™ 3.8 ft^3. compressed soil)
  - Fertilizer (e.g., 1 cup Osmocote™ controlled-release fertilizer or equivalent per Pro-Mix bale)
  - Two 5-6 ft. diameter plastic wading-pools

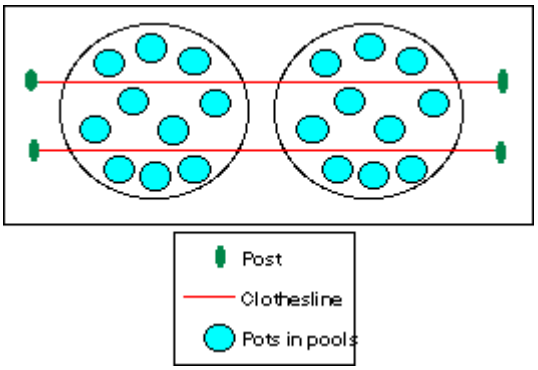
Dump the entire bale of compressed potting soil into a wading pool and dampen completely with water. Thoroughly mix in the fertilizer. Fill a pot half-full with the fertilized soil, add a root crown, then finish filling with potting soil. After all of the crowns have been potted, water the pots again and rinse out the pools. Find a location in full sunlight where you plan to do your rearing and set the pools side by side. If there is a risk of freezing temperatures, place the pools against a south-facing building where a plastic drape can be used as a cold-frame until the risk of frost is over. Place the pots into the pools and fill the pools half-full with water. Water the pots again, too. **Important:** make three to four holes in the sides of the pools just above the half-full waterline. This will keep the pools from flooding during heavy rains. Once plants begin to grow, keep water in the pool, but do not water the pot directly. Watering the pots washes out the fertilizer and causes algal growth in the pools. Remember that vegetation underneath the pools (e.g., lawn), will be killed at the site (see Figure 3).

Small potting projects:

- Equipment
    - Three-gallon pots (your desired number)
    - Dishpan (Rubbermaid™) for each pot
    - Potting soil for each pot
    - Fertilizer (2 tsp. Osmocote controlled- release per pot)

Follow the general instructions for large scale potting, only fertilize as recommended and replace the pools with individual dishpans for each pot.

Figure 3. Top view of the beetle rearing set up



Growth of purple loosestrife

Plants will need between three and five weeks to grow to the desired height before beetles can be introduced. Crowns sprout two-to-three weeks after they have been potted in early spring and then grow rather fast. When stems are approximately 12 inches tall, carefully pinch off the tip of each stem with your fingers. This stimulates the growth of lateral buds which the young beetle larvae use as food. When stems are at least 18 inches tall, beetles can be introduced. Placing beetles on plants that are too small, have too few stems, or have stems that are too old (stems with flower buds), reduce the number of insects produced.

Step 3. Beetle rearing preparation

Set up the rearing structure necessary for your project size (large or small). You should assemble the structures and the screen cages *before* you get the beetles.

Assembling large structures:

- Equipment
    - Four steel T-sign posts (7 ft.)
    - Wire (flexible for twisting) or plastic-coated clothesline

Construct two "clotheslines" which will later be used to support the screen cages for beetle rearing. At the site you have chosen for your pools (in full sunlight), drive sign posts in at opposite ends of the pools, and string a wire tightly between each of the two posts about 4 feet above ground (Figure 3).

Assembling small structures:
Insert a 3 foot tomato cage into each pot when buds begin to sprout. The tomato cages will be used later to support the screen cages for beetle rearing.

Assembling screen cages:
Start with a 60 X 54 inch piece of no-see-um cloth or bridal veil material for each screen cage. Sew a 1 inch seam along the 60 inch length of the material for threading a cord through. Fold the 60 inch length in half and sew up the one side to make a 54 inch long cylinder. Thread a sturdy 65 inch cord through the 1 inch seam (tape a pencil to the cord for easy threading) and attach a cord stop. Leave the top end open.

Step 4. Beetle rearing

This section describes a simple beetle-rearing procedure. It also tells you where to get beetles and what to expect once you have set up the rearing cages and added the beetles.

Beetle supply

Contact the DNR for a site where you can hand-collect the beetles to begin your rearing project. In subsequent years, you may hand- collect in the spring from a wetland site where you have released beetles to start artificial rearing. The beetles are easiest to collect in early May, when they have just emerged and loosestrife is about 12 to 18 inches tall in the wetland. Beetles begin to emerge about the same time crab apple trees and lilacs begin to bloom.

Introducing the beetles

- Equipment
    - Screen cage for each pot
    - Cable ties
    - Clothes pins/binder clips

Your potted loosestrife plants should be between 12 and 18 inches tall before you introduce the beetles. First, hand pick off any predatory insects and spiders (e.g., ladybugs). **Do not** use insecticides. Cover the plants with the screen cages and cinch the draw cord at the bottom of the screen cage tightly around the upper lip of the pots. **Reminder:** check the screen cages occasionally to make sure they remain tightly cinched and they have not slipped down or blown off. If cages are frequently slipping, duct tape may be used to seal the screen cages around the lip of the pots. Through the open top of the screen cage, add 10 beetles per cage by lightly grabbing the beetles with your fingers. Do not use tweezers to handle the insects. Adult beetles are harmless and docile. Close the cage by twisting the top a few times, folding it over and securing it with a cable tie. For large-scale projects, attach the cage to the "clothesline" with a clothes pin. For small- scale projects, the cages are supported by the tomato cage.

Beetle Life Cycle

The four life stages found in beetles (egg, larva, pupa, and adult) are described here. These descriptions and Figure 4 below will help you identify the various life stages for future monitoring in the field. Refer to Figure 5 for the approximate amount of time each life stage takes and the relative amount of overlap among

the various life stages. *Temperature and weather conditions* will be important factors in the amount of activity you see in the beetles, and the number of days each life stage will take.

Figure 4. The life stages of beetles

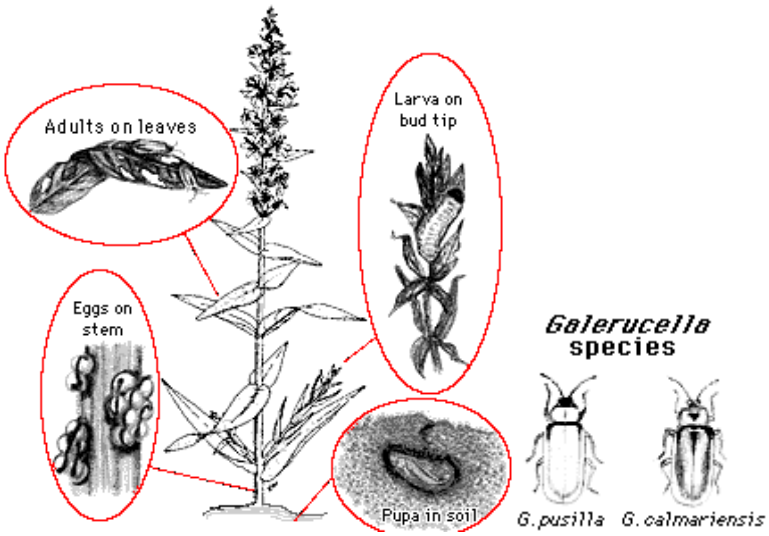
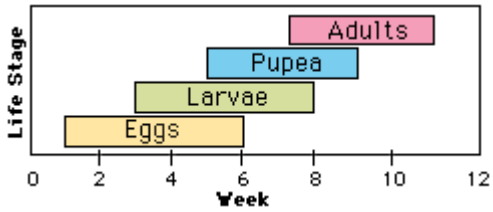


Figure 5. Developmental time periods for the four life stages of *Galerucella* spp



Eggs

Adults aggregate near the top of the plant where feeding damage of small holes in newly expanded leaves is most obvious on each stem (Week 0). Adults begin feeding soon after they have been released into the cages and will live for up to 40 days. Tiny egg masses will be evident on leaves and stems throughout the plant seven-to-ten days after adults have begun feeding. The egg mass (clutch) size will average seven eggs per mass. Females lay an average of 10.5 eggs per day for 30 days or more. Eggs are round and white with frass (beetle excrement) laid over the top of the eggs. As *humidity* is important for egg hatching, make sure pools or dish pans remain half-full with water so the humidity remains as high as possible. Once plants have grown another one-to-two feet, adults are hard to see, but their leaf-feeding damage is easy to spot. If it seems like no beetles are present (indicated by a lack of leaf damage) after the first week, look around the cage, in the lower parts of the plant, and along the soil for live adults. If there are no adults present, then check the screen cage for holes or other possible means of escape. You may need to recollect adults from the field in order to ensure a successful rearing project.

Larvae

Eggs hatch two-to-three weeks after they are laid. Although newly hatched larvae are very hard to see, the larval damage is quite evident because they crawl into buds and destroy this tissue. We call this damage "tip-feeding." Tip feeding is easy to spot and is often accompanied by frass which indicates larval presence. Larvae are yellow with a dark head capsule and molt three times, each time increasing in size. Over 80% of the larval growth and damage occurs in the 3rd larval instar. Their feeding damage is described as "window" feeding because the leaf tissue is left brown, thin and translucent. It is unlike adult feeding damage which is described as "skeletonized," where complete holes are made in the leaves, but leaf veins are left intact.

Pupae

Larvae complete development after two-to-three weeks of feeding. Large, yellow 3rd instar larvae (ca. ¼ inch) wander down the stems of the plant and bury themselves into the soil. When the stems and leaves have relatively few larvae remaining on them and there is little or no green tissue left, then most larvae have formed pupae which are found in the top ½ inch of soil. Excessive water and saturated soil during pupation is detrimental. Once 3rd instar larvae are seen, pools should be no more than half-full of water and allowed to dry up when most leaf tissue is gone from the plants. Never water the pots themselves; only water the pools to sub-irrigate the pots. This allows the top few inches of soil to stay dry, providing a more favorable habitat for pupation.

Adults

Adults emerge two-to-three weeks after larvae have entered the soil to pupate. They will be light colored (no dark coloration on either their front or back sides) and will tend to aggregate at the top of the cage. Each pot that began with 10 adults will produce between 1,000 and 2,000 beetles. As soon as you start seeing the first new adults emerge, promptly take the pots to the field for release. If a prompt release is not possible (i.e., impermissible weather, weekend, limited time and/or workers), then it is critical to maintain a fresh supply of foliage for the emerging adults until they can be released. Newly emerging adults will *not survive* if larvae have completely defoliated your plant, and especially not if the days are hot. To feed adults, use freshly clipped loosestrife stems collected at a nearby wetland (these can be collected ahead of time and stored in a garbage bag in a refrigerator for several days). Recut the stems (about 12 inch long) with a sharp blade at a 45° angle while submerged in water. Insert the stems into a 1 quart canning jar filled with water. A full bouquet (10-to-12 stems) will keep the beetles from crawling down into the jar, and provide enough food for one-to-two days depending on the number of adults. Put this bouquet into the screen cage by carefully propping it up against dead stems. Since beetles will be emerging from the soil, avoid placing the jar directly on the soil surface.

Step 5. Releasing beetles into the wetland

Once the first new adults have emerged, it is time to take the pots to the wetland. Newly emerged beetles are rather delicate and handling them at this stage is not recommended. Newly emerged adults cannot fly until 24-to-36 h after emergence.

Choosing a site

The DNR will provide a list of sites that are approved for insect release. An ideal location for releasing beetles is a site that is moderately to heavily infested with purple loosestrife, easily accessed, less prone to spring flooding, and preferably does not have standing water throughout the summer. These criteria will help ensure a good site for subsequent beetle reproduction and monitoring. If your city or township does *adult* mosquito control, check with the appropriate personnel to prevent any fogging or spraying for the remainder of the summer in the wetland you are planning to make your release. In fact, avoiding such areas would be preferable.

Releasing beetles

- Equipment
    - 7 ft. PVC (3/4 in.) pole (spray-painted orange at the top) or colored surveyor flags

When transporting the pots and screen cages to the site, keep in mind the conditions in which you will be traveling (i.e., distance to site, vehicle's climate conditions, etc.). To make sure beetles arrive in the best condition, avoid jarring, high temperatures and especially avoid tipping the pots over. *Important:* prolonged exposure (over an hour) to intense sunlight and heat is detrimental to the beetles. The simplest way to release adults is to take the entire pot with the screen cage into the field. Place two-to-four pots adjacent to purple loosestrife plants. Break off some nearby loosestrife stems and lay them in the pot so that newly emerged beetles can walk onto fresh foliage. Remove the screen cages and shake out any adults onto nearby foliage. Leave the pots at the site for the remaining beetles to emerge on their own, and mark the site with a PVC pole or flags.

Step 6. Reporting

An appendix is attached to this publication that must be filled out to report each site that you release beetles on, and where you released them on that site. Copy the form and fill out a separate report for *every* release you make and mail the information to Luke Skinner at the DNR.

Step 7. Monitoring in the field

Released adults feed on leaves for a few weeks, but disappear around mid-August to overwinter in the leaf litter and soil near their host plant. High overwintering survival for initial releases will translate into establishing a beetle population capable of flourishing for years.

Fall (year of release):

Wait at least 4 weeks after the release before recovering your pots to use for rearing next spring. Look for beetle establishment at the site by looking for evidence of adult feeding. *Reminder:* do not expect to see much activity from the beetles the first year. They are not expected to lay eggs and may have already disappeared into the leaf litter and soil to overwinter. Take a photograph when the site where you released the beetles is in full bloom. Choose a photo point that you can easily return to at the *same time* and *place* to take annual photos for monitoring purposes over the years.

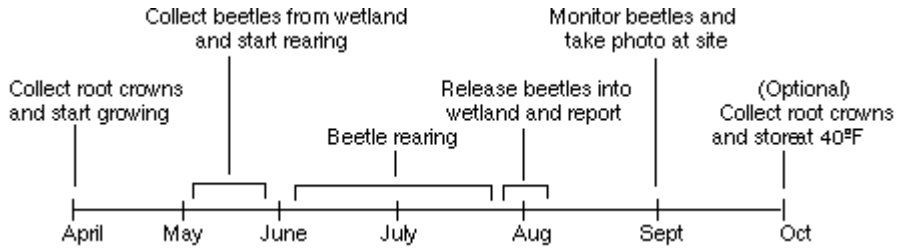
Spring (following year):

When purple loosestrife stems are 12-to-18 inch tall in the wetlands, monitor your release site(s) for signs of adult feeding. Later, return to the site to observe egg laying and larval feeding. Refer to the descriptions of the life stages discussed earlier. Do not collect beetles from the wetland this first year. These insects need to reproduce two-to-three years before populations are large enough to permit harvesting adults for additional artificial rearing. If you want to continue rearing a second year, contact the University of Minnesota-Department of Entomology, or the Minnesota DNR for where to obtain insects.

Beetle maintenance

Figure 6 is an overview of the year-round activities needed to maintain a beetle population. The key steps and dates for beetle rearing have also been summarized below for quick reference.

Figure 6. Year-round activity for rearing *Galerucella* beetles



Month	Steps for beetle rearing
January-March	Contact county agriculture inspector for permission to collect root crowns.
April	Step 1. Field collection of root crowns Step 2. Culturing and maintaining host plants Step 3. Beetle rearing preparation Contact DNR for site to collect beetles
May	Collect beetles from wetland site
June-July	Step 4. Beetle rearing
July-August	Step 5. Releasing beetles into the wetland Step 6. Reporting.
September	Step 7. Monitoring in the field and photograph.

Removing Loosestrife

If you currently have purple loosestrife or a cultivar growing in your garden, it could contribute to the loss of native wetland vegetation. To remove purple loosestrife properly, dig up the entire plant (roots and all), place in a plastic bag and dispose of it in a landfill. Composting is not advised, as the seeds may not be destroyed and the thick woody stem and roots decompose slowly.

Replacing Loosestrife

As part of restoration ecology, you can replace your purple loosestrife with an alternative selection of environmentally-friendly perennials.

Loosestrife look-a-likes
Blazing star (*Liatris spicata*) Blue Vervain (*Verbena hastata*) Fireweed (*Epilobium angustifolium*) Swamp loosestrife (*Decodon verticillatus*) Winged loosestrife (*Lythrum alatum*)
Alternate plantings
Blazing star, Gay feather (*Liatris* spp.) Delphinium (*Delphinium* spp.) False spirea (*Astilbe arendsii*) Foxglove (*Digitalis purpurea*) Lupine (*Lupinus*) Lobelia (*Lobelia cardinalis*) Obedient plant (*Physostegia virginiana*) Salvia (*Salvia superba*) Siberian iris (*Iris*) Spike speedwell (*Veronica spicata*)

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- Follow this link for a form for recording information on Purple Loosestrife Biocontrol Insect Releases that you can print out and send in.

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This publication may serve as a companion piece to the slide set SS7081, *Biological Control of Loosestrife*.

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Purple Loosestrife Monitoring Protocol
June 2003

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122E Fernow Hall, Cornell University, Ithaca, New York 14853 USA
homepage: http://www.invasiveplants.net

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Introduction
Site Selection and Quadrat Setup
Data Collection
Form 1 (site location information)
Form 2 (spring sampling)
Form 3 (fall sampling)
Form 4 (plant species list)
Quick Reference Guide
Form 2
Form 3

Introduction:

Purple loosestrife (*Lythrum salicaria*) is a perennial European herb that invades wetland communities in North America. Since 1992, several insect species have been released in North America as biological control agents against purple loosestrife. To evaluate the success of the control program it is paramount to document changes in target weed populations, control agent abundance, and changes in plant communities. The following guidelines are intended to help assess progress of the biocontrol program by monitoring the abundance of both purple loosestrife and the biocontrol insects. Monitoring should be initiated before, or at a minimum, at the same time, biocontrol organisms are released. The resultant 'preimpact' data provide a baseline to document 'post-release' changes. Due to the long-term nature of these investigations (5-10/20 years) it will be of overriding importance that changes in personnel do not put the continuation of the monitoring program at risk. The standardization of data collection should enable easier transitions and will also facilitate comparison of data obtained by different people/agencies and in different regions across North America.

This monitoring protocol is designed to detect establishment and spread of the biocontrol insects, and their impact on purple loosestrife. The protocol can also be used to detect change in herbaceous vegetation relative to change in purple loosestrife. For best results, monitoring should be conducted twice a year; in late May-early June to assess presence and abundance of the biocontrol insects, and between late August and early October to assess abundance and reproductive activity of purple loosestrife. The suggested procedures represent a minimum effort, and more detailed investigations (especially on ecosystem effects) are encouraged. The final goal is to establish a database where results from different regions can be collected, stored, and made available through our web site (http://www.invasiveplants.net). In addition, written reports on the status of the program should be published in a peer-refereed journal. All collaborators submitting data will be cited on manuscripts.

Site Selection and Quadrat Setup:

Initial site selection criteria recommended the use of small sites of less than 2 acres for insect releases and avoidance of permanently flooded sites. Experience over the past years has indicated that these restrictions are **NOT** warranted. Control of purple loosestrife will be achieved in small and large sites, and flooded sites have been controlled as well or even better than sites without flooding. To determine response of associated vegetation to the reduction in purple loosestrife, it would be beneficial to locate the study site in an area with native vegetation. Also, control can be faster in mixed plant communities but near monocultures have been controlled as well. Prescribed burning of wetlands is also tolerated, as long as there is sufficient unburned habitat where overwintering beetles can survive. The study site should be sufficiently distant from a trail or road to limit vandalism.

We recommend using a two-piece quadrat frame composed of two open-ended "U" halves that snap together to form a square. Construct the quadrat frame from two 10' lengths of 3/4" diameter PVC or CPVC pipe, 4 right-angle elbows and 2 connectors of

the same diameter, and PVC or CPVC glue. The inside dimensions of the finished frame should measure 1.0m by 1.0m. After cutting the conduit to the correct lengths, glue two elbows to each 1m long piece (make sure the elbows are perfectly aligned to 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. Using a permanent marker, mark 1 dm intervals on each side to assist with estimating percent cover. The frame can be filled with foam insulation to create a floating quadrat for use in flooded sites. In the field, slide one of the half-frames into position, and then attach the other to it.

Materials needed: 1.0m2 quadrat frame, permanent marker, GPS unit (if available), 50m tape, conduit and hammer, Form 1, pencils and clipboard.

A minimum of 5 1.0m2 permanent quadrats should be established at each site, and **10** if possible (more are even better). This allows statistical analysis of the expected decline in purple loosestrife density and performance, the abundance of biocontrol insects, and the change in associated vegetation. Experience shows that control agents reduce the number of purple loosestrife stems and the number of plants per area, to as little as 5% of the original abundance. Quadrats smaller than 1 square meter in size have a high probability of losing all purple loosestrife plants, which in turn would require a change in sample unit size. To avoid this, the recommended quadrat size is 1 square meter. Quadrats should be placed at random **into the purple loosestrife nfestation. ALL quadrats must contain purple loosestrife; if necessary, shift the location of the quadrat so that purple loosestrife covers at least 30% of the quadrat.** Various methods are available to randomize the quadrat placement. The easiest is a transect running through the vegetation with quadrats placed at predetermined intervals (e.g. every 5, 10, or 20 meters). This method works well in most sites and facilitates relocation of the permanent quadrats.
Start at least 5m from an edge (road, stream, upland etc.) Record the position and numbers of the quadrats on the vegetation map on Form 1. Use GPS coordinates for easy relocation in dense vegetation. To establish the permanent quadrats, first locate the position of each quadrat, then place the 1m2 frame into the vegetation, carefully inserting the arms of the first U-shaped frame through the vegetation and as close to the ground as possible. Then, attach the second half of the frame. Avoid trampling vegetation in and near the quadrat. At each of the four corners drive a 1-2m (5-8') long plastic or galvanized steel pipe into the ground (galvanized metal electric conduit or PVC pipe are inexpensive and readily available at hardware stores). This will allow exact placement of the quadrat in future years. Write the quadrat number on each conduit with a permanent marker or other means. Allow the pipe to stick up high enough to facilitate relocation, and low enough to minimize vandalism. Quadrats can also be marked with fence poles, and flagging tape can be attached to help relocation. Be aware that too obvious markings can attract vandalism - poles are used for target practice by hunters (personal experience). We have had good success using GPS data to relocate our permanent quadrats even in dense and tall vegetation.

Data Collection

Assessment of insects and plants will occur twice each growing season. Four data forms are provided and described in detail on the following pages: Site location (Form 1); Spring monitoring (Form 2), Fall monitoring (Form 3), and Associated plant species (Form 4; optional). In addition, 'Quick Reference' sheets are provided for use in the field. Because different data will be collected at the two sampling times, make sure you have the correct form when sampling. To assess the abundance of biocontrol insects, and the growth of purple loosestrife and other species, a series of estimates are used. All estimates reflect the growth within each quadrat and NOT of the site as a whole, or plants near but not in the quadrat.

Instructions for Form 1: Site location, background information

Site Location:
Enter name of the site (for example: Fillmore Glen State Park, north unit: be as specific as possible); and the location (town, county, state, etc.). If Global Positioning System (GPS) coordinates are available, enter this information in the spaces provided.

Contact Person and Legal Landowner:
Provide the name, address, telephone number and email address of a contact person. This person can be the releaser or a local contact. If the contact person is not the legal landowner, please provide this information in addition.

Site Characteristics:
Check one of the options or provide specifics if none of the options are applicable.

Road Map:

Photocopy a road map (preferably a county road map) to the site from a Road Atlas or MapQuest and paste it into the space provided. Mark the location of the site. An arrow should indicate North on the map. If a written description of directions is needed, attach the description to this page. Be specific: assume the reader has never been to the locale. Attach additional pages if needed.

Site and Vegetation Map:
Provide a map of the area, or copy of an aerial photo, with access roads, approximation of purple loosestrife infestation outlined, other vegetation types, trails, creek etc. Paste map into space provided. If insects have been released, indicate with Arabic numerals (corresponding to numbers under Insect Release) points of single or multiple control agent releases. An arrow should indicate North on the map.

Photographs of changes in vegetation over time are a powerful tool for presentations or to reinforce quantitative data. One or several permanent photo-points should be marked in the area of insect release(s) using flagging tape or stakes driven into the ground. The position of these photo-points should be indicated on the vegetation map. The direction in which the picture was taken should also be indicated with an arrow. Take pictures once a year at the same time of the year. The showy flowers of purple loosestrife suggest taking pictures at the peak of the flowering period. Make sure to record which photos were taken from which location and when.

Insect Release History:
Document date, control agent species, life stage (adults, eggs or larvae), the number of individuals released, and how individuals were released, as well as time of day and weather conditions. Use additional sheets if necessary. Code each release with an Arabic numeral and insert number at the release point on the vegetation map (see above). Update this information as needed (for example, if additional releases of insects occur).

Instructions for Form 2: Purple Loosestrife Biocontrol Monitoring (Spring)

Materials needed: 1.0m2 quadrat frame, data sheets (Forms 2 and 4), stopwatch, pencils, clipboard, permanent marker to refresh quadrat numbers.

The first counts should be made in late spring/early summer, 2-3 weeks after *Galerucella* adults appear after overwintering at your site (the average height of purple loosestrife shoots should be at least 20-30 cm). This will vary depending on latitude and local annual weather**, and therefore no specific dates are provided**. This first site visit is intended to estimate the abundance of biocontrol insects (adults of *Galerucella* and *Nanophyes* are easily spotted feeding on shoot tips, eggs and larvae of *Galerucella* can be counted). *Hylobius* adults are largely night active but you will encounter them on overcast days, early in the morning or late in the evening (no need to adjust site visits to their activity periods but record time of observation on data sheets). Begin with quadrat 1 and fill out Form 2, then move to the next quadrat. Use new data sheets each year. Monitoring is easier with two people, one to make the observations and the other to record data.
1) Before collecting data, please record in spaces provided: site name, date (year, month, day), and the names of the observers (last name, first name), as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. This information needs to be collected at each visit. It will allow for later corrections of observed insect abundances due to prevailing weather patterns. *Hylobius* adults, for example, are more likely to be observed in the morning or on overcast or rainy days. The opposite is true for the two *Galerucella* species and *Nanophyes marmoratus*.
2) The first task is to assess insect abundance using counts. The most useful approach to standardize among observers is using counts in fixed time intervals. Use a 1 minute total search time for each insect species released and for each life stage that can be observed. For example, at a site where only *Galerucella* (one or both species) 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* was released, 1 minute total search time will be spent looking for adults. Where *Galerucella* and *Hylobius* have both been released, 3 minutes will be spent searching for *Galerucella* and 1 minute for *Hylobius*, etc. It is recommended to do the field work with 2 people observing the sample quadrat from different sides. Total search time then has to be divided by the number of observers (i.e.; 1 minute total for all observers to search for a life stage of *Galerucella*). Do not attempt to count *Galerucella*, *Hylobius*, and *Nanophyes* at the same time, this will get too confusing; rather, look through the quadrat several times. First look for adults, which will most likely be spotted sitting on shoot tips; look for *Galerucella* eggs and larvae last. Eggs and larvae of the leaf-feeders can be found on any plant part (including the base of the stem and under leaves).
3) Now, 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). The two *Galerucella* species can **NOT** be separated in the field, therefore, they are counted together. It is impossible to observe eggs or larvae of root feeders or of flower feeders, but it is possible to count eggs and larvae of *Galerucella*. The very first instars of the

Galerucella species feed internally in shoot tips. You can note their presence but should not spend search time opening each tip (larvae are very small!). This may interfere with their development.
4) Next, count or estimate the number of each insect species present. As long as you are able to count the exact number of adults, eggs, or larvae please provide that number. If the allowed search time does not enable you to count all present individuals, use estimates in Chart A. For example if you were able to count 35 insects in the entire quadrat, enter 35. If you were unable to count all insects in one minute, but you had already counted 35 insects in ¼ of the quadrat, then estimate the total number of insects in the entire quadrat (35 * 4 ~ 140) and enter the Roman Numeral IV (between 100 and 500 insects present: Chart A) under G*alerucella* abundance. During the first years, you will be lucky to find more than just a few adults, if any, so be patient. Over time (months and years), insects will travel quite large distances (several miles). If you monitor field sites within the travel distance of these insects, you need to spend time searching for insects, **even if they have not been released at your site**. We now frequently encounter insects far beyond their initial release sites.
5) Next, scan the purple loosestrife for any damage to the leaves or shoots. After insect release, look especially for the 'shotgun' feeding pattern of the *Galerucella* beetles. Estimate the percent leaf area of purple loosestrife removed by insect feeding integrated over the entire quadrat, using Chart B. Initially, this will be very low or non-existent. Estimating the amount of leaf area removed by insect feeding will initially be difficult because you need to scan through the vegetation, and leaves and plants will show different amounts of feeding damage, but you will get better over time. Experienced observers should introduce new personnel to the methods and to their assessments to increase the accuracy of reported results. We expect to observe large differences over time, especially following high abundance of *Galerucella* larvae and adults.
6) After you have completed the insect counts, stand near the frame, and looking straight down, estimate how much of the quadrat is covered by purple loosestrife and, independently, how much is covered by cattail (Use cover estimates in Chart B, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc). If both loosestrife and cattail are abundant, these estimates may total >100%, due to layering. That is okey, as we are interested in monitoring how much of each is present. We use cattail (*Typha* sp.) as the most common associate in wetlands across North America. If you do not have cattail, leave this category blank or substitute with the most common species at your site.
7) Then, count the number of purple loosestrife 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 5cm 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.
8) Count the number of cattail stems following the same procedure.
9) Record information about other insects using purple loosestrife, if any. With the increase in control agent abundance we might see the number of other herbivores increase, and potentially, the number of predators using an abundant food source. We would like to evaluate some of these potential changes. If you frequently observe species, take pictures and collect samples for identification. Freeze insects or store individuals in alcohol. Record whether the particular species is present, abundant, or very abundant. Outbreaks of other species on other wetland plants can be recorded on either the spring or fall sampling form.
10) Other Observations: Record any general observations or useful information; disturbances, flooding, fire, bird nests etc., for the sample quadrat or the site in general. Most of this information will be difficult to evaluate, therefore do not spend too much time on this.
11) If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat using Form 4. Use cover estimates included on the form, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc).

Instructions for Form 3: Purple Loosestrife Biocontrol Monitoring (Fall)

Materials needed: 1 meter stick; 1.0m2 quadrat frame; data sheets (Forms 3 and 4), pencils, clipboard, camera, GPS unit to relocate quadrats.

The second site visit should be from late August to early October to measure performance of purple loosestrife (height and reproductive effort). We do not record insect presence during this visit. Monitoring is easier with two people; one to make the observations and the other to record data.
1) Before collecting data, please record in spaces provided: site name, date (year, month, day), and the names of the observers (last name, first name), as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. This information needs to be collected at each visit. Locate permanent photo points and take photographs of the study site.
2) Slide the 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 B, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc). If both species are abundant, these estimates may total >100%, due to layering. That is okey, as we are interested in monitoring how much of each is present.
3) Count the number of loosestrife 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 5cm 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.
4) Count the number of cattail stems following the same procedure.
5) Next, count the total number of purple loosestrife 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) Count the number of cattail inflorescences. The number of fertile cattail stems often increases as purple loosestrife declines.
7) 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 5cm length of inflorescence. If the plant did not produce any inflorescences or if they are shorter than 5cm please record this in the appropriate form. Repeat this process for the remaining 4 loosestrife stems.
Note: The attack of all biocontrol insects, but especially the flower-feeder, changes the number of flower buds producing seeds. This measurement allows us to assess the impact of these insects.
8) Select the five tallest cattail stems in each quadrat. Measure height of each stem (to the nearest cm) and indicate if sterile or fertile.
9) Record information about other insects using purple loosestrife, if any. With the increase in control agent abundance we might see the number of other herbivores increase, and potentially, the number of predators using an abundant food source. We would like to evaluate some of these potential changes. If you frequently observe species, take pictures and collect samples for identification. Freeze insects or store individuals in alcohol. Record whether the particular species is present, abundant, or very abundant. Outbreaks of other species on other wetland plants can be recorded on either the spring or fall sampling form.
10) Other Observations: Record any general observations or useful information; disturbances, flooding, fire, bird nests etc., for the sample quadrat or the site in general. Most of this information will be difficult to evaluate, therefore do not spend too much time on this.

Instructions for Form 4: Purple Loosestrife biocontrol monitoring (Associated Vegetation)

If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat on Form 4.
1) Before collecting data, please record in spaces provided: site name, date (year, month, day), and the names of the observers (last name, first name), as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. This information needs to be collected at each visit.
2) Estimate what percent of the quadrat is unvegetated (i.e., soil, water, litter, etc.), and what percent is vegetated; these estimates should total 100%. To make cover estimates more accurate, mentally estimate the unvegetated portion of the quadrat, and compare it to your estimate of the vegetated portion.
3) Next, estimate total percent cover of purple loosestrife and of cattail (copy from Form 2 or 3), and of all other vegetation (i.e.; not purple loosestrife or cattail). Use Chart B for cover categories. If possible, estimate percent cover by life form groups (grasses and sedges; herbs; woody). Estimates may exceed 100% due to overlapping of vegetation.
4) If you are familiar with vegetation, also record which species are present and estimate percent cover of each species. While it is acceptable to estimate only the most abundant species, these may change over time and it is best to record all species if possible. If any of the plant species are difficult to identify, collect a sample from outside the sampling quadrat for later identification. Consult a botanist before making identification that may be inaccurate.
Rather specify life form over taxonomic identification if uncertain!

PURPLE LOOSESTRIFE

Spring Monitoring Quick Reference (Form 2)

Materials: 1.0m2 quadrat frame; data sheets (Forms 2 and 4), stopwatch, pencils and a clipboard, permanent marker.

1) Write Site name, state, date, names of investigators, time, weather, and GPS coordinates if known at the top of Form 2.
2) Walk to quadrat 1. Slide quadrat frame into location. Observe adults of all three biocontrol species. Count or estimate the number and life stage of each insect species present. Use a 1 minute total search time for each insect species released and for each life stage that can be observed. As long as you are able to count the exact number of adults, eggs, or larvae please provide that number. If the allowed search time does not enable you to count all present individuals, use estimates in Chart A.
3) Look for evidence of leaf attack. Estimate percent of purple loosestrife leaf area removed by insect feeding, estimated over the entire quadrat (use Chart B).
4) Estimate cover of purple loosestrife and, independently, cattail (Use cover estimates in Chart B, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc.).
5) Count the total number of loosestrife stems rooted in the quadrat.
6) Count the total number of cattail stems rooted in the quadrat.
7) Record any additional information; other insects using purple loosestrife, disturbances, flooding, fire, bird nests etc., for the sample quadrat or the site in general.
8) Optional: Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat using Form 4.
9) After completing Form 2 for quadrat 1, proceed to quadrat 2, and repeat the process (steps 2-8). Continue until all quadrats have been located and recorded.

PURPLE LOOSESTRIFE

Fall Monitoring Quick Reference (Form 3)

Materials: 1 meter stick; 1.0m2 quadrat frame; data sheets (Forms 3 and 4); pencils, clipboard, camera, GPS unit

1. Write Site name, state, date, names of investigators, time, weather, and GPS coordinates if known, at the top of Form 3. Take photographs at permanent photo points.
2. Walk to quadrat 1 and slide quadrat frame into location. Estimate cover of purple loosestrife and, independently, of cattail.
3. Count the total number of stems of purple loosestrife and of cattail.
4. Count the total number of inflorescences of purple loosestrife and of cattail.
5. Select the five tallest loosestrife stems. For each stem:
a. Measure stem height
b. Count number of inflorescences
c. Measure length of longest inflorescence
d. Count number of flowers in the center 5 cm of the inflorescence.
6. Select the five tallest cattail stems. For each stem, measure height and indicate if fertile or sterile.
7. Optional: Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Form 4.
8. After completing Form 3 for quadrat 1, proceed to quadrat 2, and repeat the process (steps 2-7).
Continue until all quadrats have been located and recorded.

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

N

Site and Vegetation Map

N

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 (Spring)

SITE: _____ STATE: _____

DATE: _____ _____ _____
year month day

GPS: N ____° _____'
W ____° _____'

TIME: _____

TEMPERATURE: _____

WEATHER: _____

INVESTIGATORS:

Last name	*First name*

Chart A: Insect Abundance

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

Chart B: Damage Class, % Cover

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											

Please send a copy of the completed form to:
Dr. Bernd Blossey, Fernow Hall,
Cornell Univ., Ithaca, NY 14853

Notes:

FORM 3: PURPLE LOOSESTRIFE biocontrol monitoring (Fall)

SITE: _____ **STATE:** _____

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

INVESTIGATORS:

Last name *First name* **TIME:**_____

_____ _____ **TEMPERATURE:** _____

_____ _____ **WEATHER:** _____

_____ _____

Chart B:

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

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

Please send a copy of the completed form to:
Dr. Bernd Blossey, Fernow Hall,
Cornell Univ., Ithaca, NY 14853

FORM 4: PURPLE LOOSESTRIFE biocontrol monitoring (Associated Vegetation)

SITE: _____ **STATE:** _____

DATE: _____ _____ _____ **GPS:** N ____°_____'

year *month* *day* W ____°_____'

INVESTIGATORS:

Last name *First name* **TIME:**_____

_____ _____ **TEMPERATURE:** _____

_____ _____ **WEATHER:** _____

_____ _____

Percent Cover	
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 or other scale to indicate percent cover)									

Purple Loosestrife Beetle (*Galerucella* sp.) Identification

Please help WRP monitor beetle progress!

Let us know if you see the beetles or beetle evidence shown below. *Galerucella* sp. beetles are biocontrol agents released to feed specifically on the invasive wetland plant, Purple Loosestrife.

Above from left to right: *Galerucella* sp. eggs, larvae, and adults (3-5 mm long and half as wide). Adult and larval feeding patterns can be distinguished from each other. Adult feeding leaves a bullet-hole like pattern with the leaf tissue completely penetrated. Larval feeding does not penetrate the entire leaf; a thin layer of leaf tissue remains visible. Larva prefer to feed on the newest leaves/shoot tips.

Massachusetts Office of Coastal Zone Management
Wetlands Restoration Program

Photo credits - www.forestryimages.org:
Bernd Blossey, Cornell University
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Linda Wilson, University of Idaho
Eric Coombs, Oregon Department of Ag

Purple Loosestrife Beetle (*Galerucella* sp.) Identification Card

PURPLE LOOSESTRIFE BIOCONTROL IN MASSACHUSETTS

Surveillance of purple loosestrife infested wetlands outside of beetle release sites is an important component of biocontrol project monitoring. WRP requests that individuals who spend time in wetlands report observations of *Galerucella* beetles and/or evidence of *Galerucella* feeding on purple loosestrife.

For more information on the
Purple Loosestrife Biocontrol Project
or to submint observations, contact –

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