Bureau of Water Quality
Procedural Document

Water Quality Policy Management Team

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Wisconsin Purple Loosestrife Biocontrol Program Overview and Instructions

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Overview and Instructions

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Contents

Contacts: This guide will provide step by step instructions and help you decide if a purple loosestrife biocontrol project is right for you and/or your organization. However, there are several people who can provide further guidance and assistance.

1) Purple Loosestrife Biocontrol Statewide Coordinator
   Wisconsin Department of Natural Resources
   PO Box 7921, WY/4
   Madison, WI 53707-7921
   DNRAISinfo@wisconsin.gov
   608-266-0061

2) Regional DNR and Partner AIS Coordinators, click on the map for your county to get contact information: https://dnr.wi.gov/lakes/invasives/topics.aspx

INTRODUCTION

Welcome to Purple Loosestrife Biocontrol, a practice of aquatic plant management for the wetland invasive plant, purple loosestrife (*Lythrum salicaria*), that does not utilize chemicals. Instead, the program relies on the leaf-eating "Cella" beetles *Galerucella calmariensis* & *Galerucella pusilla*, purple loosestrife predators from Europe where the plant is native. Biocontrol is the use of one species to manage another and has been utilized in agricultural as well as natural systems. Learn about the safeguards and research for the program in Appendix 8.

There are many roles to play depending on your interest level or organizational role. Root stock must be dug and potted. Nets used to cage plants and protect beetles being reared on them need to be sewn. The netted purple loosestrife plants being raised as beetle nurseries need care as they grow. *Galerucella* beetles need to be collected from purple loosestrife in wetlands and added to the rearing plants. Later their offspring will be released into purple loosestrife infestations where they'll feed on the plants to bring them under control.

In Wisconsin, purple loosestrife is a highly invasive wetland plant that is native to Europe and Asia. A healthy purple loosestrife plant can grow to over six feet with multiple flowering stems of hot fuchsia/pink flowers that can release between two and three million seeds per plant. It arrived in North America in the early 1800s and reached Wisconsin sometime after 1900. An excellent resource to learn more is Purple Loosestrife: A Continuing Threat. The brochure includes identification tips and photos of common look-a-like plants. Use the contact information in this guide since the brochure may not have the current contact information.

Wisconsin's Conservation Chapter 23.235 requires the Wisconsin Department of Natural Resources (WDNR) to make control efforts for purple loosestrife and provided the legislative support to conduct research to find the "most environmentally sound manner" of control. Brock Woods (UW Madison-Division of Extension Natural Resources Institute and WDNR) began and nurtured the Wisconsin Purple Loosestrife Biocontrol Program for over 25 years, beginning with extensive research in the mid-1990s. At that time, the plants were overtaking wetlands throughout the state. Their tiny seeds also found their way to roadsides and shorelines.

Although you can release beetles in areas with small infestations, purple loosestrife biocontrol is best in areas with large, dense infestations of 50 plants or more that can sustain a beetle population that reduces the purple loosestrife to manageable levels over time. It is an especially

useful method in areas where access to the plants is difficult, such as an expansive wetland or the riparian areas surrounding a mucky, shallow lake. Once enough beetles are introduced, they can sustain themselves in the area for multiple years and can move to other infested areas nearby. The beetles have been found on untreated purple loosestrife as far as ten miles from a beetle treated site. Because they are not attracted to native species, the beetles will fly to find more purple loosestrife.

Biocontrol will not completely eradicate the plants, but they will become smaller and have fewer flowers. Shorter plants mean more light for the seeds of native plants to sprout which then tend to grow larger. Purple loosestrife's flashy flowers attract pollinators, competing with the native plants for pollinator services. Native plants or seed added to your sites will have a better chance of success once the purple loosestrife is well-managed.

If your goal is to completely remove purple loosestrife, consider digging and chemical treatment. Combining management techniques is known as Integrated Pest Management (IPM). Contact your regional WDNR AIS Coordinator for details.

OVERVIEW

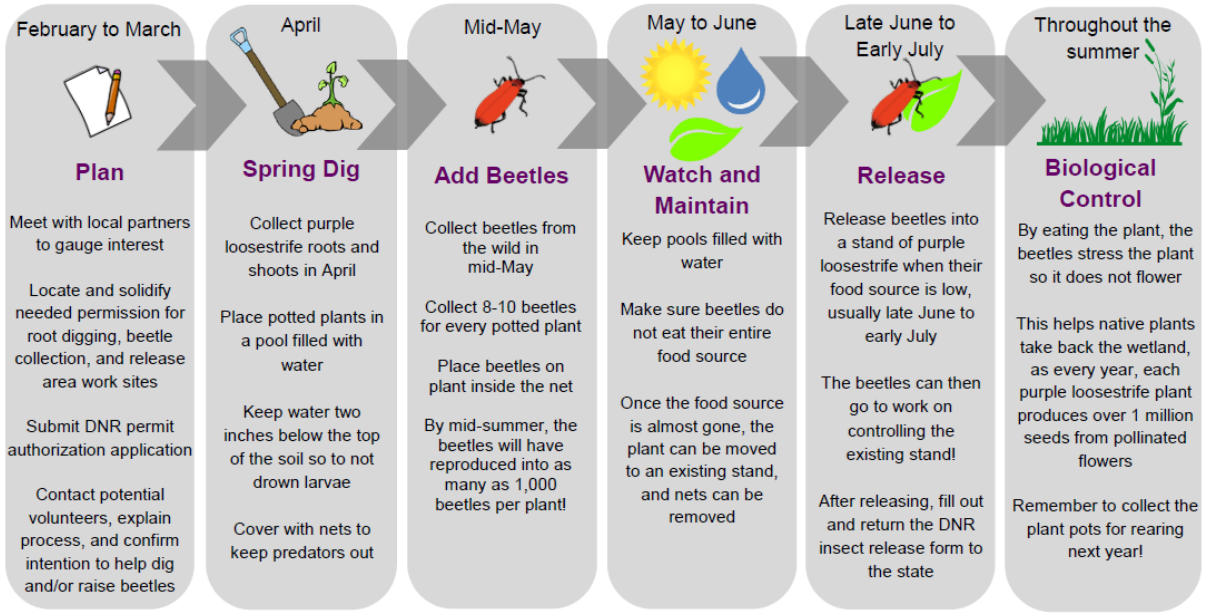


Figure 1 Graphic provided by Golden Sands Resource Conservation &Development Council, Inc., member of the Wisconsin Aquatic Invasive Species Partnership

The above graphic illustrates the common steps for a purple loosestrife biocontrol project, but flexibility is important. Depending on the weather in your region, the dates can vary considerably. The key to digging root stock is to do it as soon as the ground thaws sufficiently. In the southern part of Wisconsin people are often able to dig in late March. Others must wait for late April or early May. Location and weather also dictate the speed of plant growth. Since the program began in the late 1990s, plant growth and timing for the beetle releases has become more varied due to weather fluctuations. Timing beetle collection to the growth of the plants has become more difficult and there is now more need to establish regional insectaries rather than

have the statewide coordinator (located in the Madison area) collect and send beetles all over the state.

Figure 2 Purple loosestrife in bloom, newly net-caged root stock, young adult beetles feeding. Photos: KingCo.gov, S. Boismenue and A. Smith

CHOOSING SITES FOR PURPLE LOOSESTRIFE BIOCONTROL

Beetle rearing is important to the state and a great education project, but before deciding to get involved, you need to find patches in your area that need control. Your DNR AIS Coordinator can provide monitoring guidance and help you find known sites. If you only need a couple plants and beetles to do a very small-scale project for educational purposes, contact the statewide coordinator or the regional/local coordinator for assistance. The book, *See Cella Chow!* is an excellent reference for lesson plans.

You need to know if patches are large enough to support a beetle population. Consider these questions.

1) How tall are the plants on average?
2) How large is the patch?
3) Are there at least 50 plants?
4) Are the plants growing densely or are they widely scattered individuals?
5) If looking at the plants while there are still leaves, do the leaves show feeding damage?
6) Do you have landowner permission? This is required.

SITES OF HIGH NEED: Dense patches of plants over 4-5 feet tall with abundant flowers (seed capsules if observing after flowering, including winter) indicate a good location for biocontrol. They will also provide the best root stock for raising your plants. Late summer is best for monitoring because you can see the flowers, but you can look for purple loosestrife in the winter. See the winter site check tips in Appendix 7.

SITES EXHIBITING GOOD OR LIMITED EXISTING CONTROL: Are there only a few plants? Consider this site for removing plants by digging or cutting and possible chemical control. Is the patch large, but the majority of plants are under 3-4 feet tall with flowers that are sparse on the stalk? If there are leaves present, check for many small, round holes along the veins that indicate beetles feeding. Holes larger than 5mm and oval, especially at leaf edges, indicate a different insect species, not used for biocontrol. Do you see the beetles? Keep in mind that the beetles may have returned to the soil after early August. If it is winter, are most of the flower stems bare or almost bare, no or few seed capsules? All of these traits indicate beetles are at work.

Insectaries: A site indicating beetles at work should be considered for an insectary, a location to gather beetles for plants you are rearing or to share with other cooperators. The program is actively looking for insectaries around the state and wants to map them. If you have found a potential insectary, let the statewide coordinator know the location and if it can be used by others with landowner permission. Appendix 4 provides more information about collecting beetles.

MIXED SITES: Some sites may appear to be a mix of short/weak and tall/hearty plants, with or without obvious beetle damage. It may be that they were mowed at some point, leaving short but robustly flowering plants. Sites that have a mix may be a location where beetles are just becoming established or a population of beetles has been negatively impacted by predators or severe weather. It may be that the beetles have simply moved to a new location. Here, a "booster shot" of beetles could be helpful.

NORTHERN SITES: If you are in the north where ice may linger long into the digging season in spring, consider collecting roots in late summer or fall. See Appendix 6.

REPORTING SITES: The program is continually updating maps of purple loosestrife sites. Even some long-term biocontrol program sites may not be showing up in the WDNR database yet. The Site Revisit form found on the Purple Loosestrife Biocontrol Program webpage can be used to report your existing biocontrol sites if they don't show up on the viewer described below. Include a photo of the plants at the site and send your report to DNRAISinfo@wisconsin.gov.

For new sites, even those outside of your area, fill out an AIS Incident Report and send it with a photo of the patch(es) and plants to the DNR AIS Coordinator for your region for official verification. If you need guidance for intentional, Early Detection monitoring, contact your DNR AIS Coordinator. People using a mapping application that creates GPS points, tracks, and maps, should contact their DNR AIS Coordinator about how to provide the data so that it can be added to the Surface Water Integrated Management System (SWIMS).

Once site data is added to SWIMS, you'll be able to see all verified purple loosestrife locations on the Lakes and AIS Viewer. **To View Data**: Open the Lakes and AIS viewer→Click on show layers→In the layers window, scroll down to 'Invasive Wetland Plants' and click on the plus sign→In the list that opens, check the box for purple loosestrife. Zoom in to your location and right click on the purple icon for more information.

Once you know that you have a good site to work on, check in with the statewide coordinator to see if anyone else is working in your area who you can partner with or assist.

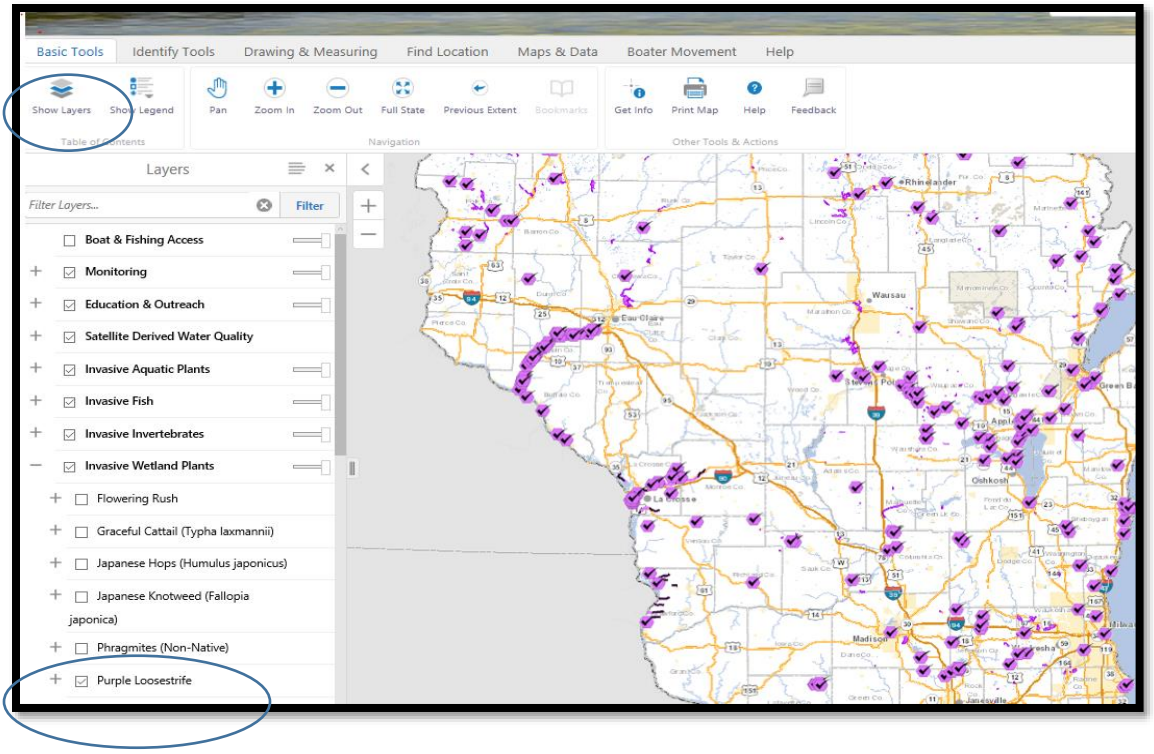


Figure 3 WI Lakes and AIS Viewer

APPLICATION AND PERMIT – WHO SHOULD APPLY AND HOW?

Purple loosestrife is a Restricted species in Wisconsin, so anyone moving purple loosestrife and raising it must complete an application for program participation and to receive an NR40 permit issued by the Statewide Monitoring Coordinator. People who are helping during a dig, release or with rearing may not need one. See Appendix 2 to better understand who needs to fill out an application. A copy of the application can be found on the Purple Loosestrife Biocontrol Program webpage.

Your application tells the statewide coordinator if you need netting or beetles provided, helps to track where control is happening, and serves as the request to receive the WDNR required permit to move purple loosestrife from one location to another. Once the application is signed and sent to the statewide coordinator, you will be sent a permit letter. There is no cost. If you previously donated funds to the program, please consider using those funds for your supplies or donating to one of Wisconsin's many fine organizations working every day to protect our environment, such as the Natural Resources Foundation or a local non-profit.

PROGRAM INSTRUCTIONS

TOOLS NEEDED – * NO COST TO COOPERATORS: BEETLES, NETTING, ASPIRATOR, VIALS

- Decontamination tools for footwear and tools, used when leaving wetlands

    - Hand held brushes
    - Jug(s) of water
- Digging
    - Waders or high-topped boots for walking in wetlands (used for all wetland visits)
    - Shovels and/or pitchforks-pitchforks are excellent for getting intact roots
    - Hand shears for clipping when you collect roots
    - Extra-large garbage bags or large tubs for hauling roots
    - Optional: native seed to drop into the holes left by your dug root stock
- Potting and growing
    - A sunny area near a water source for refilling pools
    - 2 to 5-gallon plastic pots at least 12" across - often free from local garden centers or landscape companies
    - Wading pools – each should hold 4-6 pots without crowding the growing plants; a limited number may be available through the statewide and local coordinators
    - Potting soil with a fertilizer – about 2 cubic feet per 6 pots
    - Fertilizer if not in the soil or to add when plants not growing well
    - A sturdy frame: clothes line, fence posts or other method to keep the tops of the nets raised to at least 5-6 feet
    - Netting (1 net/pot)-provided by the statewide coordinator, but the long side is sewn by volunteer(s). *
    - Duct tape, bungee cords or double-sided Velcro to secure net around the bottom of the pot. We have partners who have been using the same 7/8th inch Velcro for multiple years.
    - Cord, such as twine or fabric clothesline rope to tie the top of net closed and secure it to supports
    - For holes in netting, fabric glue or needle and thread can be used for repairs. Tape risks catching beetles and larvae in the netting.
- Collecting beetles– see Appendix 4 to learn how to build beetle traps and how to collect beetles
    - Aspirators and vials - available from statewide coordinator, if needed*
    - 2-liter bottles
    - Electrical tape
    - Cotton balls
    - Zippered bags
- Release
    - Large garbage bags – for hauling out any flowers you cut, netting and pots
    - Flagging for pots left at release sites

JANUARY – EARLY APRIL

Monitoring and Site Revisits: As needed

Application: Send in your application anytime from January through April. Early is recommended if you need netting or plan to dig in March. A copy of the application can be found on the Purple Loosestrife Biocontrol Program webpage.

Volunteer recruitment: Of course, you can start looking for volunteers at any point, but depending on the scope of your project, early is best. If you usually work alone, but you would like to find out if there is a local group to work with, contact the statewide coordinator to help you find them.

Check old supplies, buy new, request and prep nets: Free netting is provided from the statewide coordinator and requested on the application. However, you can make netting requests anytime after November 1 until spring.

Netting-check your old netting or order new. If there are holes or there is general damage making them unusable, request new netting. If your old, yet usable nets were sewn shut on one short end, it makes it difficult to add your beetles to the net cage. It's ok to cut off the stitched end or request new netting if that makes them too short.

- Nets should be sewn. Stapling and hot gluing do not make a sufficient seal to keep beetles in or predators out. If you do not sew, consider asking for volunteers from local sewing groups/quilters, high school Home Ec. type classes, Master Naturalists or Master Gardeners, etc. or any local volunteer group that might have a sewer as a member.
- The netting will be approximately 6.5' long. Fold the sheet of netting in half lengthwise and sew a straight seam about a ½" from the edge to make it into a long sleeve. Then sew a zigzag seam next to the first seam. It's ok if the ends do not come out totally even. The netting is cut off large rolls and most pieces won't be perfect. DO NOT sew the top or bottom of the nets.

Pots-check in with local garden centers and landscapers at any time of year to see if they have used plastic pots available. Older pots that have been used a few times, might crack and need to be replaced. The pots must have drainage holes. Some people have also used 5-gallon plastic paint buckets and drilled holes into the sides near the bottom.

Pools-pools can become brittle and cracked, so check them to see if new are needed. Individuals and groups generally buy their own pools. If help is needed, contact the statewide coordinator to learn if assistance is available. Once you have your pools, drill a few holes in the sides about 4-5" from the bottom. This will let them drain to a safe level once beetle larvae go into the soil in your pots. The water in the pools should be 2" below the surface of the soil in the pots to prevent drowning pupating larvae when they move into the soil.

Frames- Make a plan for what you will use to hold the netting up over the plants. Nets should be tied tall enough to give the plants 5-6' of room to grow. Examples are shown in Appendix 3.

Mass rearing cages-A few people have mass rearing cages. The cages are 12'x12'x6' heavy duty frames and entirely netted. See Appendix 5 for more information. If you have a mass rearing cage, check both the netting and framing early for needed repairs. If there is damage that is too

extensive for a simple repair, contact the statewide AIS Coordinator to find out how to get a new net or send one in for repair.

MARCH THROUGH EARLY MAY

Preparing: Whether working with a group or as an individual, start checking your digging areas as soon as there is a chance they have thawed enough to get a shovel or pitchfork in the ground. Why dig so early?

1) Digging when there is little to no growth, prevents damage to young shoots
2) While the weather is cold, it is less likely beetle predators will be established
3) Getting the plants into pots and growing as soon as possible makes it more likely they'll be ready for beetles in time; the beetle collection window is short

** Make sure all landowner permissions have been granted.**

Group leaders- As one long time coordinator says, "As AIS Coordinators, we try to plan and schedule events, so we can be as efficient as possible. Purple loosestrife doesn't work that way." Let those involved in other projects know that you might have to reschedule if the purple loosestrife is suddenly ready to dig. The same issue may come up when it is time to collect or add the beetles to the plants. Give your volunteers a heads up when you start checking the digging sites and let them know that you will try to give them as much notice as possible. Keep them in the loop by giving them a list of possible work dates.

Have your planting supplies/frames ready in the location where you'll do potting and growing.

The Dig

1) As soon as the ground is thawed enough, gather the tools, boots or waders and head out to your digging site. Take large garbage bags or tubs to hold your roots.
2) Make sure you have brushes and clean water to clean off your tools and footwear when you leave the dig site. See #9 below.
3) Plan to dig 12-15 plants for every 10 you intend to pot. Small plants can be combined. The goal is to have 6-8 stems per pot. Extra plants can be raised for feeding the larvae/beetles if they start to destroy their plants before you can get them back to the wetland. Later, any plants that are not needed can be bagged, labeled 'Invasive plant-DNR approved for landfilling' and put in the trash.
4) The best plants for digging are tall, hearty plants still standing after last year, that have **6-8 stems** covered in dry seed capsules. Break off the stems, leaving about 8-10" to act as a handle. Ideally, shoots shouldn't be up, yet, but you may find the buds if you look closely at the base of any old stems.
5) Pitchforks are preferable over shovels if the ground is wet and loose because they will do the least damage to roots. Shove the pitchfork around and under the plant to loosen the ground further. Then you should be able to rock the fork under that plant to pop it out.
6) Shovels will work if the ground is too solid for a pitchfork. Dig as far out from the main stem as possible to protect the roots. If the root mass is too large for the pots that you will

use, you can use the shovel to split the plant in two or more. Often, a large clump is actually 2 or more plants and can be pulled apart.

7) Pull the stems and roots of any other plants off your root mass. Dead loosestrife roots are black and brittle. Cut these off. Brush off the top of the root ball to remove any dead organic material and loose dirt. You do not need to remove soil from the roots. It will contain the nutrients the plant prefers. Leave all the removed material in the wetland.
8) If you brought native wetland seed, drop it into the remaining hole. The seed can be uncovered or covered with a very light layer of soil. Buried wetland seed will not grow.
9) Clean up: Because the project is a WDNR permitted activity and purple loosestrife seeds can be hiding in the soil, contain the plants and soils to the best of your ability, thoroughly brush and rinse footwear and gear before leaving the site. When available, handheld steamers help kill seeds and organisms. Refer to the DNR disinfection website for further information: https://dnr.wi.gov/topic/Invasives/disinfection.html

Potting

1) In a pool, spray wash just the top of the root ball with a garden hose to remove any eggs of plant or insect predators; bag the waste for the landfill labeling the bag "Invasive Species-WDNR Approved for Landfilling"
2) In second pool, mix water and potting soil so it is thoroughly wet.
3) Add some soil to the bottom of the pot and place your roots on top, trimming the roots as necessary to fit; you want them to "just fit".
4) Fill each pot with enough soil to allow the root crown to sit 2" below the top of the pot; if combining small roots, they should total 6-8 stems.
5) Pack the soil into any air pockets, but not too firmly; the larvae will need to penetrate the soil surface later.
6) If the soil does not have fertilizer, sprinkle slow release fertilizer (amount as shown for pot size) onto the soil and mix in about 1".
7) Cage the plants with netting.
    a. Use duct tape, double-sided Velcro or very snug bungee cords around the pot and bottom of the net; pots often have a bit of a lip that will help keep the net from slipping off; if using duct tape, keep it out of the water and be prepared to replace it if it gets loose.
    b. Close the top of the net with heavy twine or clothesline rope 5-6" from the top; wire can also work; you will open this end later to add beetles, but it needs to be tight enough to keep out predators like spiders.
8) The pools should be in an area with full sun and under the structure used to support the netting. Avoid areas subject to strong winds.
9) Place your pots in the pools; one pot (2 if the pool is large) can go in the middle and the rest around the pool side, but don't crowd them; airflow and sunlight need to reach the plants.
10) Tie the top of each net to the support, whether it's a clothes line or rope strung across another structure; the goal is to have the net tall enough (5-6' minimum) to give your plants maximum growing room and the beetles easy access around the plants.

11) Add water to the pools so that it comes to just under the drilled holes; remember the water level should stay 2" below the soil height for healthier pupating beetles.
12) Expect the plants to take at least 4-6 weeks to grow and rear beetles before the release.

LATE MARCH – JULY (STEPS AFTER POTTING)

Plant Care

1) Regularly check your pools to make sure there is water.
2) To avoid mosquito larvae living in the pools, occasionally fill the pools to just above the drain holes to flush out any larvae.
3) Crowns take a week or two to begin growth and then grow quickly.
4) When stems are 12-15" tall, spread the small leaves at the ends of each stem and remove the growing point (meristem) with a scissors or tweezers; Breaking off the plant tip with your fingers is more likely to cause a tear which will take energy from the plant to heal. This step will give you bushy plants with lots of foliage for the beetles and also keep the plants from getting too tall for their cages.
5) If plants are not growing well after two weeks, add a bit of fertilizer.

Adding and raising your beetles

1) Plants are ready for beetles when they reach 1.5' tall
2) There are three options for getting beetles
    a. Collect yourself, if you know where to find them. See the How to Collect document in Appendix 4.
    b. Work with your local coordinator to find beetles, whether or not you personally help collect them
    c. Contact the statewide coordinator for beetles or to help you find a nearby coordinator to provide them
3) Whether you receive beetles by hand or by overnight shipment, you must get them into the cages as soon as possible; until they go into the cages, keep them cool and out of direct sunlight. If they are in a zippered bag and have eaten the leaves provided, you can open a corner, carefully and slip in a few leaves for them to eat until they are put on the plants. You can also blow in some air but be careful. They are escape artists!
4) If you receive your beetles in small vials or small bags with about 10 beetles/vial or bag, open the top of each net and either shake the beetles on to the plants or just drop the open vial or bag into the net cage. Make sure the vial did not land upside down, trapping the beetles. Retie the net.
5) If you have large bags of beetles, you will also be provided an aspirator and vials. You can use the aspirator to 'suck' beetles out of the zippered bag either directly into the cage, or to be sure they don't escape, into an attached vial. Aspirator instructions are in Appendix 4.
6) The advantage of having an aspirator is that you can often catch the escapees, since they will gravitate to the netting because their favorite food is inside. Local coordinators are provided

aspirators to help those in their areas, and one can be sent directly to groups, but those should be returned to the statewide coordinator.

Figure 4 On a shoreline with a wetland and consistent water levels, Cathy Higley (Vilas County) raised her beetles with a unique twist.

Releasing your beetles or larvae

Permission to access the site should already be in place at this point. You may need to do a release at the larval stage, the pupating stage or the young adult stage, dependent on how much damage there is to the plants. It is not uncommon to make a plant check and discover a plant looks dead or nearly dead overnight because of heavy larvae production or the pupating beetles hatching. Or you may find the top of your beetle cage crawling with hundreds of new beetles. In either case, you need to get the plants to the release site immediately to prevent starvation. More details about each stage are in Appendix 1.

1) Release areas should have abundant plants. Remember that one of your cages could provide up to 1000 new adults.
2) Transport your pots with cages on! Avoid jarring and high temperatures. If carrying in a pickup or trailered boat, lay them down.
3) Release where access and footing are safe.
4) Where there are 50 or more plants, you want to cluster 2-4 pots worth of beetles in large patches. If the area covers several acres, you will probably want to find multiple locations to cluster pots. The adults will fly to new plants as needed.
5) If you are releasing along shorelines, choose areas with several plants close and others nearby. Use your best judgement for the number of pots per location.
6) Uncover your plant next to a free growing plant and as much as possible, entwine the branches of the two plants. This is vital, if there are still larvae which could be hiding on leaves or in the soil, so they can crawl over to fresh leaves.

7) You should plan to mark where you leave the pots and retrieve them later. This is especially important for pots that still have larvae or pupates.
8) Complete the beetle release form found on the Purple Loosestrife Biocontrol Program webpage for each site. Enter the data into SWIMS (See Appendix 8) or return the form to your local or the statewide coordinator for entry. Pictures of the plants at your site are appreciated—only 1-2 that clearly show purple loosestrife plants. If your site hasn't been verified, yet, by WDNR, the photos will complete that step.
9) Plan to return after two or more weeks to collect pots.

LATE SUMMER AND FALL

Site Revisits

Conducting a site revisit can be done when you retrieve pots, in late summer or any time you want to assess the status of your project. The Site Revisit Form, found on the Purple Loosestrife Biocontrol Program webpage, is valuable for any of these visits. Checking your sites after releases each year will help you determine if biocontrol is making the desired impact. These visits can indicate if you will need to do more concentrated releases in the future or that your site will make a good insectary.

1) During visits to retrieve pots or later in the summer/early fall, note the plant conditions you find. Is there significant damage to the plants from the beetles? Are they discolored, do they appear to be dying or dead? Note that it's unlikely they are completely dead because the root crowns of purple loosestrife contain a tremendous amount of energy, but the stress to the plants will make an impact.
2) During a winter or early spring visit, check the remains of the flowering stems. Healthy plants will have stems heavily covered with dried seed capsules. Impacted plants will have sparse to no seed capsules.

Like the beetle release data, the site revisit data should be entered into the SWIMS database. SWIMS users can enter the data themselves. Others can provide their form to their local coordinator or the statewide coordinator.

Thank you!

APPENDIX 1: *GALERUCELLA* SP. BEETLE LIFE CYCLE

1) Old adults from previous year: dark brown, often with a black stripe along the edge of each wing cover; 4-6 mm long and half as wide. They overwintered in the surface soil layers of the wetland or surrounding uplands and have emerged to feed and mate. They live about 40 days and each female will lay about 10 eggs a day for 30 days. They feed between the leaf veins by chewing small, roundish holes in the tissue. This is called 'skeletonization.' When rearing beetles, if you don't see the beetles or signs of the leaf damage within a week of adding them to the cages, check the cage for holes or other possible means of escape. Fix any

damage and check the outside of your nets for escapees to be put back in the net. Also, look for predators, such as spiders that may have eaten them. Remove any predators. You may be able to use an aspirator to catch a few beetles from another trap. If not, you can save the plant to use as food if beetles destroy another plant before you're ready to release them.

2) Eggs: tiny, less than 1mm and cream colored with an uneven black line of frass (black insect excrement) deposited on them. They are usually laid in bunches, often along the edge of adult feeding damage on stems and leaves. Humidity provided by keeping water in your pools is very important for hatching, which will occur 2-3 weeks after laying.

3) Larvae: very small and hard to see at first. Larvae damage in the shoot tips, called "tip-feeding" is usually quite obvious, especially due to visible frass. Larvae are yellow with a dark head capsule and molt five times, each time increasing in size. Over 80% of the larval growth occurs in the 4th and 5th larval instars. Their feeding damage is described as "window paning" because the leaf tissue is left brown, thin and translucent, unlike the holes left by adults. If your plant appears to be dying due to extreme window paning, get it out to a release site. Take off the net but leave the plant in the pot so that larvae that have moved into the soil don't fall into the water and die. Make sure to entwine the branches of the potted plants with one on site so the larvae can crawl to new leaves.

4) Pupae: Larvae become pupae after 2-3 weeks when the 5th instar larvae move to the soil after intensive feeding. This is often the best time to take your plants out for release. It will seem as if most of your larvae have disappeared, but they are actually in the top couple inches of the soil as pupae. At this point, you do not want the upper layer of the soil in your pots to be saturated. If the leaves of your plant are severally damaged, not leaving enough food for young adults, you can take the plants out for release. Take off the net but leave the plant in the pot so that larvae that have moved into the soil don't fall into the water and die.

5) New adults: if you placed 10 beetles in your net a few weeks earlier, you can have as many as 1000 young adults emerge. They will be light tan with no dark lines on the wing covers. They'll turn dark brown in a few days. They often collect at the top of the net, ready to disperse and eat. As soon as you see a few, get your pot out to the release site. Adding fresh stems from other plants can help them live in the net longer, but you risk having them escape when you add it. If you find hundreds of beetles at the top of the net, get them to a release site ASAP.

Amanda Smith

Mike Alaimo

APPENDIX 2: WHO NEEDS AN APPLICATION AND PERMIT

It is illegal to transport, transfer (sell), transport, or introduce an NR40 restricted invasive species. If authorized by a permit issued by the Wisconsin Department of Natural Resources under this chapter, a person may transport, possess, transfer or introduce a restricted invasive species for research, public display, or for other purposes specified by the department in the permit.

The current Purple Loosestrife Biocontrol Application is available on the program webpage: https://dnr.wi.gov/topic/Invasives/loosestrife.html

In general, anyone who is transporting, possessing while growing the plants, and releasing the plants with beetles back to a management site should complete an application for a permit.

IF you are a Coordinator for a project with multiple volunteers participating in these actions, you may apply for a permit under your name and give them copies of the permit which will then cover everyone. The application should include:

- address where the plants will be possessed (beetles reared),
- number of plants
- location where plants/beetles will be introduced (specific areas to the best of your knowledge at application time and the county)

If you are only moving beetles (no plants) from a collection site to a site needing management, a permit is not needed. You should still complete the Beetle Release form for program integrity. This type of release may be effective if you capture spring beetles before egg laying or new beetles prior to mid to late August when they go underground for winter.

Examples:

Person	Role(s)	Application needed?
Beaver Creek Reserve AIS Coordinator	Overall Coordination: training, advising, assisting, site selection as well as digging, transporting, rearing and/or releasing	**Yes**
Beaver Creek Reserve AIS Coordinator	Training/advising/site selection; not taking part in digs, growing, release	**No**, but update Statewide Coordinator on local activities
Beaver Creek Reserve volunteer or staff person other than coordinator	Providing the digging and plant moving muscle	**No**
Beaver Creek Reserve volunteer or staff person	Will raise plants at a site other than the Reserve, regardless of if they help dig or release	**No**-Covered by the Coordinator's permit
Beaver Creek Reserve partner or someone seeking assistance	Leading their OWN rearing project and volunteers with the support of Beaver Creek Reserve AIS Coordinator for training, site selection, etc.; Not part of BCR project	**Yes**
Biocontrol volunteer, no affiliation with a group	Superhero doing it all on their own!	**Yes**

APPENDIX 3: FRAMING FOR NETTING

It is important to provide a support for your nets to give the purple loosestrife plenty of light and room to grow. The plants need 5-6' of available height. Putting them under a home clothesline works fine. One group put their pools into a corn crib and ran pipes above them, using the open wire of the crib to support the poles. Then they tied the nets to the poles. They did well in spite of some shade, probably due to fertilizing.

Placing a post directly into the pot usually does not work well. This method makes the pot unstable. Even before the plants start to fill the nets, wind can easily blow the entire pot out of the pool.

WDNR photos

Amanda Smith

APPENDIX 4: HOW TO COLLECT BEETLES

Beetle Traps

When collecting a large number of beetles to be divided among partners, you can make beetles traps like this one. Other people will collect beetles directly into zippered plastic bags or vials (using an aspirator), especially when doing smaller projects. If you'll get the beetles to the host plants promptly, you can collect 10 per bag/vial. If you need to hold the beetles for a period of several hours or overnight, either use the bottle trap steps or collect them directly into 1-gallon size zippered plastic bags, so you can more easily blow in some air and add some leaves for food as needed.

Brock Woods

Bottle trap directions

- 20 oz and/or 2 liter bottles
- Scissors or X-acto knife
- Electrical tape
- Cotton balls
- Gallon-sized zippered plastic bags for field use
- Cooler for field use

1. Discard the cap. Wash and dry the bottle.
2. Cut the bottle off just <u>below</u> the point where it starts to become straight instead of curved. If you flip the cut-off top over and it just falls into the bottle, you've cut too high and will need to start with a new bottle.
3. The inverted top should fit snugly. Use the electrical tape to secure the inverted top to the bottle. Electrical tape works best because it is easy to remove when you're ready to dump the beetles into a zippered plastic bag. Carefully smooth the tape so there are no gaps for beetles to escape.
4. The cotton balls will be used as a stopper to keep the beetles in the trap.

When and where to look for beetles:

1. In southern Wisconsin, start checking sites for beetles in early May, once purple loosestrife shoots are appearing and easily found. Depending on location, the beetles may not show up until mid-late May or early June. Large numbers may collect on stems even when the plants are only a foot tall.
2. Keep in mind that the plants being reared should be at least 1.5 feet tall before adding beetles. If your plants are small, a small amount of fertilizer may help growth.
3. Check often! REMEMBER: To maximize egg production, your goal is to find early beetles that haven't yet laid eggs and may not have had a chance to mate, yet.
4. Sunny days are best. Collect after dew has dried. Dew or rain can mean water in the trap causing the beetles to stick inside.
5. Start with sites where beetles have been released before or where past surveys indicated beetle damage. You may need to expand the search to new areas. Since they often

overwinter in uplands, some beetles may have moved to nearby sites. Heavy winds can also relocate them.

What to look for:

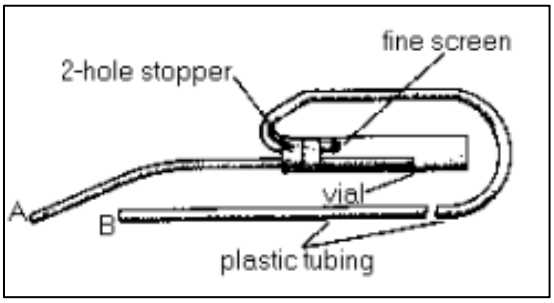
1. Look for feeding damage, small round holes among the veins. Holes that are 5mm and oblong, often on leaf edges are more likely from a particular native moth larva.
2. If you see damage, but few to no beetles, check back after a few days. If you still can't find many beetles, the site may still be developing a sufficient population. Widen your search.
3. The beetles tend to congregate at stem tips.

Trapping your beetles:

1. *Galerucella* beetles escape predation by simply dropping off the leaves to the ground to hide when disturbed. You'll use this to your advantage.
2. Break off a short stem (2-3") with a few leaves and place in the bottle for food and shelter.
3. When you find a beetle or group of them, open the bottle or zippered plastic bag, and hold the bottle below the beetles. Just tap or blow on the leaves or tip the stem over your trap or bag and give the stem a gentle shake to send the beetles falling into it. Close the trap or bag between collections.
4. If using a bottle, keep an eye on the tape to make sure it doesn't loosen, allowing beetles to escape. They will crawl up or fly to the top of the bottle. The inverted top slows them down, so you can easily get them to drop to the bottom again with a gentle tap or two.
5. Once you have so many beetles in a bottle that they can start to fly out, it's time to put them into a zippered plastic bag. Remove the tape and set to side. Quickly cover the bottle with the bag and dump the beetles in. Leaving a small corner of the bag open, gently blow into the bag to fill it with air before closing.
6. Remove/squish any possible predators like weevils and spiders that have gotten into the bag.
7. Keep the filled bags in a cooler and out of the sun.
8. If your beetles need to be stored for travel and shipped to others, bring some stems of purple loosestrife back to your sorting site for them.
9. Don't worry about collecting too many beetles. You can always "re-stock" the site with some of the beetles you raise.

Counting and sorting beetles

1. If you are going to use an aspirator to put the beetles into vials or smaller bags, you'll only put 10 beetles per vial/bag, so just count the vials/bags when you're done. People raising beetles in mass rearing cages can use an aspirator to collect on the cage walls. The aspirator is also great for collecting escapees indoors or in vehicles! Aspirators provided for free by WDNR are similar the image below from the University of Kentucky. You place the end of the metal tubing (A) close to the beetle. With the plastic/rubber tubing (B) in your mouth, give a quick suck and the beetle will be drawn into the vial. The fine screen protects you from pulling the beetle into your mouth instead.



2. Counting a large bag of moving beetles is fairly easy. In a cool place (reduces movement), let some of the air out of the bag. Use a pencil, ruler or some other lightweight straight edge to hold over the bag to visually mark off areas. Careful, not to lay the tool down and crush the beetles! Count each area as you move the straight edge around over the bag.
3. Beetles kept in large bags need to be placed on plants again within 2-3 days, but sooner is better! You will need to add purple loostestrife leaves and blow air into the bag as needed, which can be often depending on the number of beetles in the bag. If they need to be shipped, use an overnight service and include some leaves on a stem in the bag(s).
4. Beetles in small bags or vials should be put into the net cages with the plants ASAP.

APPENDIX 5: MASS REARING CAGES

There are a limited number of cages available for program participants from the WDNR. Mass rearing cages are 12'x12'x6' and allow you to raise up to 100,000 beetles in one space. Cages can be set up on a prepared wetlands site over in-ground purple loosestrife plants or on a flat, dryland site using up to nine kiddie pools or lined with plastic sheeting to create a single large pool as shown in the diagram that follows.

When not in use, a tub large enough to store the netting is recommended. Rodents will eat their way through folded netting stored in the open. Scented dryer sheets may help.


S. Boismenue

Wetland sites

Start with a level site with dense stands of purple loosestrife. Choosing a safe site with consistent water levels is vital. If the area is prone to flooding, beetle pupae or overwintering adults can drown. Steps one and two will reduce the risk of beetle predators.

Site preparation:

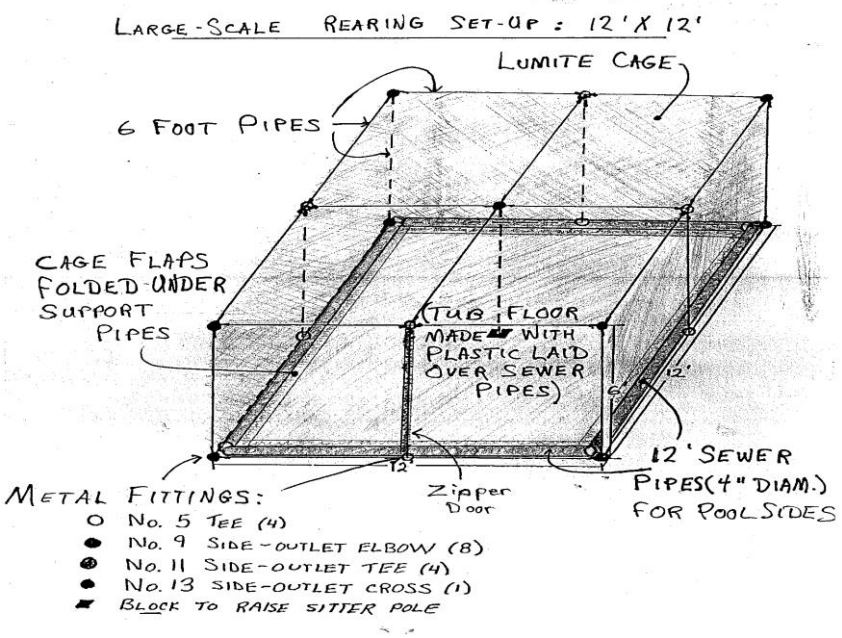
1. Note how tall the dead stems on the purple loosestrife plants are to estimate how many beetles you'll need later. The goal is to add 10 beetles for every 10 stems that are 5-6 feet tall.
2. Remove all above ground vegetation including the purple loosestrife in winter or early spring (before growth) by cutting. Rake away all remaining ground litter.
3. Set up the cage immediately after site prep

4. Additional purple loosestrife can be dug and replanted in the cage until you have up to 80-85 plants total.
5. Add beetles when plants are 1.5-2’ tall.
6. When the young beetles begin to emerge (4-8 weeks), damaging the nursery plants and collecting on the top of the cage, open the cage to release them. Beetles can also be collected for other sites by entering the cage and collecting off the plants and cage walls.

Dry sites

Start with a level site in full sun. The cage can sit on any surface. The directions below assume you will use 8-9 kiddie pools depending on size.

1. Set up the cage with kiddie pools.
2. Dig and pot purple loosestrife as you would for rearing in pools normally.
3. Place the pots in the pools and fill the pools with water within 2” of the soil line in the pots. There is no need to cover individual plants with netting.
4. Immediately close the cage to keep out predators.
5. Care for your plants as normal, keeping the pools full, and pinching off top buds once the plants are about 12-15” tall to encourage bushiness. Fertilize if necessary to stimulate growth.
6. Add beetles (10x the number of plants) directly to the cage.
7. You may need to move the plants to infested wetlands if the larvae begin to decimate them, or else they may run out of food. If the plants become large, 5-6 feet, with few larvae or adults, indicating that the pots have pupating beetles in the soil, it is also good to move them to the wetlands.
8. When the young beetles begin to emerge (4-8 weeks), damaging the nursery plants and collecting on the top of the cage, collect them by entering the cage and collecting as you would in a wetland by tapping them into beetle traps or using aspirators. Aspirators work well for beetles collecting on the netting.



APPENDIX 6: LATE SUMMER OR FALL ROOT STOCK COLLECTION

It can be a challenge to dig and start your purple loosestrife plants in the north if the spring thaw is too slow or the ground remains snow covered. A late start digging makes it difficult to raise plants that are large enough in time for beetles. Some cooperators choose to give late summer or fall digging a try. There's no guarantee, of course, but we have had some successful projects.

1. Dig your plants during late summer or early fall to give them time to stabilize.
2. Clean off the root crown well and pot in 12" diameter or greater pots as you would for spring plants.
3. Cover the pot with a piece of netting or fabric and hardware cloth to keep out insect predators and rodents.
4. By late fall, cut them back to the root crown and store in the ground or a cool location.
5. In ground options:
    a. Dig holes large enough for each pot and place them in the ground
    b. Pack leaves and soil around the pot
    c. Insulate the pots by covering them thickly with leaves and other natural debris, but do not use straw
6. Cool location option:
    a. An unheated garage or storage building; success may depend on how cold it gets
    b. Dark
    c. Keep moist until dormancy
7. In mid-March, pull the pots from the ground or bring them out to a sunny area and net them; depending on their overwintering success and the weather, you should see shoots within a couple weeks.
8. Initially, instead of pools, it can be helpful to use large, clear storage bins and a few inches of water so that you can bring them in from the cold if there is a severe cold snap.
9. Once spring is clearly here and your plants start to grow, place them in pools and proceed as normal. Adding fish fertilizer has produced excellent results for some who have tried overwintering plants, especially if the plants were smaller to begin with or if you're using plants that have been used for rearing previously.
10. As your plants grow, you may find beetles emerging from the soil around the roots; you want these plants to have a chance to grow. Cage one plant use as a feeding plant for all the volunteer beetles you find. Keep in mind that if there are beetles in these pots, the wetland that your plants came from might be a great place to go back and collect spring beetles for your plants!

APPENDIX 7: WINTER SITE CHECKS

Late summer and early fall are the best time to do site revisits; however, winter can be a good time to monitor purple loosestrife in wetlands, especially if there are large infestations with unmanaged plants

that can be over 6’ tall. Access can improve when the wetlands freeze. The plants turn dark brown and generally stand tall among marsh grasses and cattails which are more likely to fold over.

- If you are monitoring a known site to see how your control efforts are going, use the Site Revisit form found at https://dnr.wi.gov/topic/Invasives/loosestrife.html
- AIS Coordinators: If you are looking for new sites, regardless of the time of year, use an AIS Early Detection form, following normal DNR monitoring protocols
- Random discoveries of new sites should be reported on the AIS Incident form available here
- Data should be entered in SWIMS or sent to the WDNR AIS Coordinator.
- You may want to bring along large garbage bags and clippers to remove stems if you find seed capsules that still hold seed. Double bagging helps prevent stems from poking through.
- Don’t forget to clean your footwear after your visit! Purple loosestrife seeds are tiny and easily hidden in mud.
- Remember, no ice is safe ice. Use best judgement and always put safety first. Reference the WDNR’s Ice Safety webpage for helpful tips: https://dnr.wi.gov/topic/outdoorrecreation/activities/icesafety.html

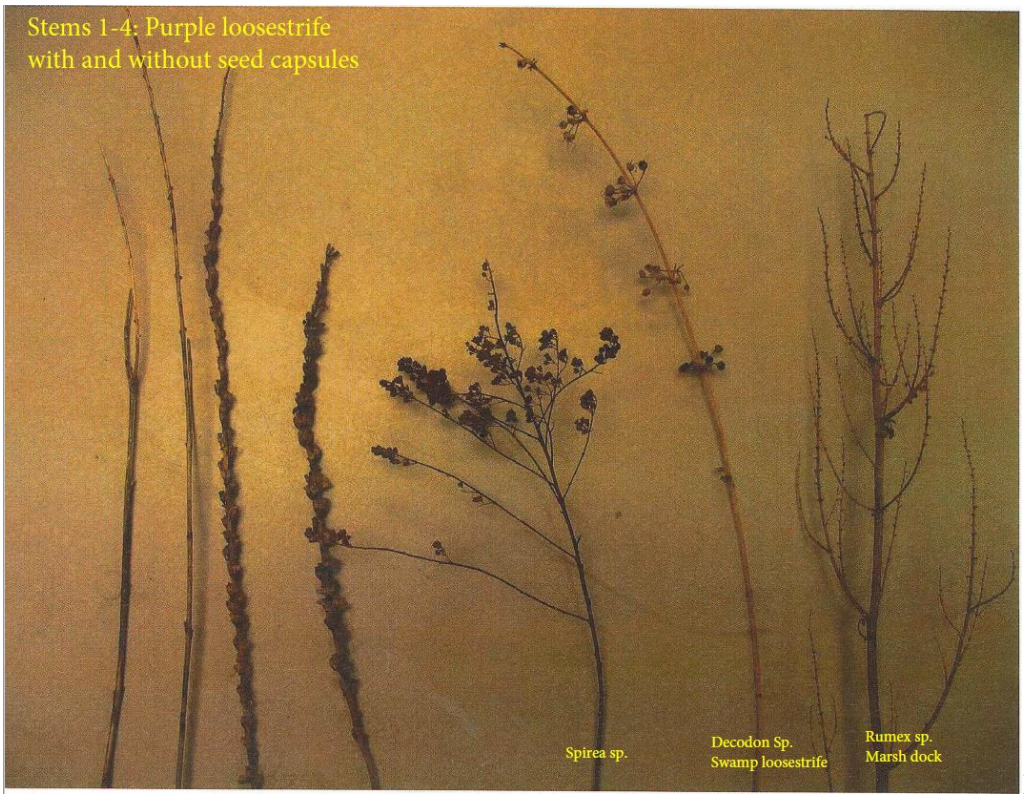
Note how tall the average stems are, an average number of flower stems per plant, an estimate of the number of the plants, and the amount of area covered. Are the stems heavily covered with seed capsules as in the photo below or are they sparsely covered? Are stems only lightly covered or bare?

Brock Woods

Winter site checks, cont.

The plant here is a little about 5’ tall, but notice how few the seed capsules are on the stems compared to the photos above and below. The stems themselves are short. Short flowering stems (6-10”) on candelabra-shaped inflorescences are beetle trademarks.

Often a clump will have stems that are 1, 2 and 3 years old. You are attempting to assess the newest stems. Photo: Paul Skawinski



Brock Woods

Winter site checks, cont.

Chris Hamerla

APPENDIX 8: PURPLE LOOSESTRIFE BIOCONTROL DATA AND THE WDNR SWIMS DATABASE

The WDNR collects water related data, including aquatic invasive species monitoring and water quality data, in the Surface Water Integrated Management System (SWIMS) database. SWIMS includes the beetle release and site revisit data for the Purple Loosestrife Biocontrol Program projects. Data entered into SWIMS can be used by DNR and partners to do program tracking, evaluation and to map the locations of the projects and purple loosestrife sites. On page 5, in the Choosing Sites section, you can see a map from the Lakes and AIS Viewer. Thanks to purple loosestrife monitoring data entered into SWIMS, site locations show up on the map. DNR AIS Coordinators can provide more detailed information for anyone interested in learning more.

When new volunteers send in their applications to participate, a profile is created for them in SWIMS and they are 'associated' to the correct biocontrol project in SWIMS. Prior participants already have profiles in the system. The projects can be for their county, their county's Land and Water Conservation Department or another organization conducting the projects. For example, if they are an individual volunteer in Adams County, they would be associated with the project Purple Loosestrife - Adams County. Being associated with the project lets people enter their beetle release data or site revisit data from the paper forms.

Who should enter the data?

Providing the data collected on the beetle release forms is permit requirement.

Water Action Volunteers, Citizen Lake Monitoring Network volunteers and Clean Boats, Clean Waters volunteers and staff enter their own data in SWIMS multiple times per year. If you are already a SWIMS user, go ahead and enter your data. Data entry is the same process as for the other SWIMS projects. It's a good practice save the original forms or send them to the statewide coordinator as a backup. If you don't see the purple loosestrife biocontrol project in your SWIMS project list, let the statewide coordinator know and it will be added.

If the only project you do that requires SWIMS data is the purple loosestrife biocontrol project, it's likely that you'll only have data to add once or twice a year. Since using SWIMS so seldom means it's easy to forget your password or the data entry steps, you are welcome to send your forms to your local or the statewide program coordinator. If you would like SWIMS training, the statewide coordinator will be happy to help you get training.

APPENDIX 9: PURPLE LOOSESTRIFE BIOCONTROL SAFETY AND REFERENCES

Prior to *Galerucella* spp. beetles being allowed into Wisconsin in the mid-1990s for biocontrol, a strict process took place and the safety was assessed at multiple levels. Any potential biocontrol species must be reviewed by the USDA Technical Advisory Group for Biological Control Agents of Weeds (TAG). Information about the TAG process can be found here. The TAG provides guidance to those conducting the research and must sign off on the safety of the species' release. The Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP) must also approve of the use of the species prior to release and permits specifically for research are required to bring a biocontrol agent into Wisconsin.

Although purple loosestrife biocontrol has been thoroughly researched, sometimes people are still concerned about introducing one species not traditionally found in Wisconsin to control another non-native species. Brock Woods, researcher and program coordinator from 1997-2019, addresses the concern based on his research and that of others specifically for purple loosestrife biocontrol:

> *Classic biocontrol depends on close coevolution between an exotic pest and its control organisms so that the control reproduces exclusively on the pest. This is clearly the case for our two species of imported Galerucella beetles. Groups of our plants closely related to purple loosestrife, other wetland plants and economically important plants--including wild rice--were all tested in Europe and in the US to be sure of our beetles' behaviors. Research suggested only Lythrum alatum (winged loosestrife) and Decodon verticillatus (swamp loosestrife) might be utilized--our closest related plants to purple loosestrife. The one and only plant here that I have seen the beetles utilize is Lythrum alatum, a native found mostly in Southern Wisconsin wet prairies and the closest relative to purple loosestrife. Even then, this species was only attacked when it grew adjacent to purple loosestrife. Plants as close as 20-30 meters were left alone. By the way, we also have native Galerucellas that do feed on other plants. From time to time we get a report of cross-over feeding on native plants, but follow-up has always shown native Galerucellas (present in essentially all extant wetlands) as the plant predators involved and they're actually feeding on the native plants they have evolved with as expected.* (email to Jeanne Scherer and Dara Fillmore 11/8/2019)

APPENDIX 10: CONDUCTING BIOCONTROL IN ROAD RIGHT OF WAYS (ROWS)

Input provided by Christa Shaefer, WI Dept. of Transportation: Christa.Schaefer@dot.wi.gov

We regularly find purple loosestrife along roadways. There are several special considerations for roadside work, including checking with your County Highway Department to determine who has jurisdiction or maintains the roadsides you are interested in for biocontrol.

Keep in mind that there could be active but not obvious roadwork plans for a site. Some may even be for managing invasive species. Because County aquatic invasive species or Invasive Species Management Area staff might have an approved plan in place for the county with their highway department and other local road managers, it is important to check in with them. They might even have project that you could join as a partner. If your county does not have a coordinator, check with the Regional DNR AIS Coordinator for advice, and you should also contact your highway department or municipalities to learn about the site and what activities are allowed. The bottom line is that we do not wish to get in the way of a road project, put ourselves into an unsafe or illegal situation, or work hard on a biocontrol project only to learn later that someone already has the site covered or an upcoming road project will tear out the site.

A free permit is required from WisDOT to work on state managed roadsides. State managed roads have a number for their name. The permit, DT1812 Work on the Right of Way Permit, needs to be approved by WisDOT and a copy should be with you when accessing the roadside. Visit https://wisconsindot.gov/Pages/doing-bus/real-estate/permits/work-on-hwy.aspx for more information and to apply. You may contact Christa Schaefer if you have issues getting the permit but understand that it may be because the regional staff have determined it is not a safe location or there is another restricted access issue.

As long as you stay in the ROW, you do not need to contact landowners for permission.

How far a ROW extends from the center line of a road depends on road width: and can vary greatly. Look at the County GIS site to see the full extent of the right of way width. There are usually markers, and when there is a fence, they are most often (though not always) installed 3 feet inside the WisDOT right of way. If you are not able to check the GIS site, check with the local road department for guidance.

Safety ALWAYS COMES FIRST for any purple loosestrife related work:

1) Check out the Adopt a Highway Safety video on their safety page.
2) Know and follow the required safety protocol of any organization you are working with as staff or a volunteer; this appendix document does not override any County or other local laws and rules.
3) Never work in medians. If you are working in conjunction with DOT or the County highway department, they may allow this if you are working directly with them and following their protocols.

4) Have a reflective, florescent orange or yellow vest to wear when out of the vehicle. Sometimes the DOT can give volunteers safety vests that are also used by Adopt A Highway volunteers.
5) Follow all state and local laws that may restrict parking along a road; Stopping along some roads, even local roads, may be prohibited.
6) DO NOT stop on interstate highways.
7) Pull off the side of the road as far as safely possible and do not block a lane.
8) Use your hazard lights when stopping; if staying to do work, such as beetle collection, have safety cones to put out.
9) Use your hazard lights when driving slowly to look for a site where you will work.
10) It is safer to work with at least one partner, so that one can always keep their eyes on the road.
11) Wild parsnip is present along many roads and can cause rashes, blisters and skin discoloration. Learn to identify it to avoid it.

Digging plants and collecting beetles:

1) As noted above, check with road managers first and be sure the work can be done safely.
2) ROWs are mowed at least once a year, so be sure it is a site worth the visit. If you just see a short, single plant here and there along a stretch of road, they may not be worth the risk of a stop for removing plants or looking for beetles.
3) If the plants are sparse, consider checking with the County or local highway department to see if its ok to stop and remove them or cut off the flowers to reduce seed production. You may be able to dab cut stumps with herbicide. Only licensed and certified applicators (in the appropriate certification category for the site) may apply herbicides on WisDOT state highways.
4) If there are easily and safely accessible dense patches, talk to the highway manager and let them know you are willing to get a permit if required to work on them.
5) If there are large plants in the adjacent property, it is possible you will find beetles to collect from smaller plants within the ROW, even if they are regularly mowed. If you want to dig or collect from the adjacent area, landowner permission is needed.

Releases:

Many highway departments and their contractors do their mowing around the same time that we are releasing beetles, so check in with them before you consider working on a ROW site. If they do say no, consider other sites.

1) Check with your highway department to learn who mows and coordinate your timing.
2) Make sure your pots are outside of any obvious mowing path; in this case you may need landowner permission if they are beyond the ROW
3) Do not leave the pot: If you think there could still be many larvae on your plants or in the soil, make very sure your plant is right up against and entwined with a purple loosestrife on site

Reporting new sites: If you feel you have found a patch of unreported purple loosestrife, contact your DNR AIS Coordinator.

REFERENCES:

Biocontrol Populations Flourish While Purple Loosestrife Populations Diminish Several Years Later
Landis, D. A., Sebolt, D. C., Haas, M. J., & Klepinger, M. 2003. Establishment and impact of Galerucella calmariensis L. (Coleoptera: Chrysomelidae) on Lythrum salicaria L. and associated plant communities in Michigan. *Biological Control*, 28(1), 78–91.

Biological Control Keeps Non-Target Species Safe
Blosser, B., Casagrande, R., Tewksbury, L., Landis, D. A., Wiedenmann, R. N., Ellis, D. R. 2001. Nontarget Feeding of Leaf-Beetles Introduced to Control Purple Loosestrife (*Lythrum salicaria L.)*. *Natural Areas Journal,* 21(4), 368-377.

Lindgren, C. J. 2000. Performance of a Biological Control Agent, Galerucella calmariensis L. (Coleoptera: Chrysomelidae) on Purple Loosestrife Lythrum salicaria L. in Southern Manitoba (1993-1998). 16.

Biological Control of Purple Loosestrife: Identifying, Removing, and Rearing
Loos, A., Ragsdale, D. 2001. Biological Control of Purple Loosestrife: A guide to Rearing *Gallerucella* spp. 1-9.

Biological Control Overwinters and Oviposit only on Purple Loosestrife
Malecki, R. A., Blossey, B., Hight, S. D., Schroeder, D., Kok, L. T., Coulson, J. R. 2006. Biological Control of Purple Loosestrife. *BioScience* 43(10), 680-686.

Blossey, B., Schroeder, D., Hight, S. D., Malecki, R. A. 1994. Host Specificity and Environmental Impact of Two Leaf Beetles (*Galerucella calmariensis* and *G. pusilla*) for Biological Control of Purple Loosestrife (*Lythrum salicaria*). *Weed Science* 42(1), 134-140.

Other Region's Guides for Choosing and Applying Specific Controls of Purple Loosestrife
Minnesota Sea Grant. 2017. Aquatic Invasive Species: Purple Loosestrife: What you should know, what you can do. *University of Minnesota.*

Warne, Amanda. 2016. Purple Loosestrife (*Lythrum salicaria)* Best Management Practices in Ontario. Ontario Invasive Species Plant Council, Peterborough, ON.

Long Term Impacts of Using Biological Control of Purple Loosestrife
Blossey, Bernd. 1992. Impact of *Galerucella pusilla* and *G. calmariensis* (Coleopetra: Chrysomelidae) on Field Populations of Purple Loosestrife (*Lythrum salicaria*). *Lincoln University*, 27-31.