

# Bureau of Water Quality Procedural Document

Water Quality Policy Management Team

Wisconsin Department of Natural Resources 101 S. Webster Street, P.O. Box 7921 Madison, WI 53707-7921

# Wisconsin Purple Loosestrife Biocontrol Program Overview and Instructions

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This guide provides step by step instructions and help you decide if a purple loosestrife biocontrol project is right for you and/or your organization.

**Contacts**: There are Aquatic Invasive Species (AIS) specialists who can provide further guidance and assistance.

- 1) WDNR Regional AIS Biologists and County AIS Coordinators: Click <u>here</u> to find the contact information for your County.
- 2) <u>DNRAISinfo@wisconsin.gov</u> a general mailbox. Monitors of this address can direct you.

Any person visiting a site with invasive plants should bring along a brush to clean off shoes, boots, clothing and equipment. Clean water for rinsing is also useful, especially for mud that can hide seeds. It is up to all of us to help slow the spread of invasive species to other sites.

# Acknowledgements

Thank you to the over one thousand volunteers and professional invasive species staff who have contributed countless hours to Purple Loosestrife Biocontrol efforts for over thirty years. In a review of paper files from 1995 to 2010, I found many photos of wetlands, shorelines and roadways where "purple" blanketed the landscape. In my years working with purple loosestrife (2014-2025) and doing site visits across the state, it has been very rare to see sites covered to such an extent. The change is thanks to the diligence and leadership of all of you.

Special thanks to Brock Woods and Richard Henderson who got us all started with tremendous support from others in WDNR, UW Madison Division of Extension, Master Gardeners, the Wisconsin Wetlands Association, GLIFWC and other tribal partners school educators, RC&D's, lake and river organizations, nature conservancies, counties and municipalities, the Wisconsin Waterfowl Hunters Association and other hunting and fishing groups, 4-H and Scouting organizations, and many others. There are several partners who contributed their efforts for over five, ten, and even twenty years. Some are still at it, rearing and releasing our little "Cella" beetles to make sure that purple loosestrife "plays nice" with the other plants and animals in our wet habitats. Even if you've only participated for a year or two, your contribution is greatly appreciated and has made a difference.

Although the information in this guide is research-based, it is also enriched by the hands-on experience of everyone who has participated in the program and shared what they have learned.

Welcome to all who are reading the guide in preparation to start biocontrol. You are joining distinguished ranks! Your help is still needed.

Thank you!

Jeanne Scherer, Statewide Purple Loosestrife Biocontrol Coordinator 2020-2025

#### Introduction

Welcome to the Purple Loosestrife Biocontrol Program, a practice of aquatic plant management (APM) for the wetland invasive plant purple loosestrife (*Lythrum salicaria*), that helps us manage the plant in a wide variety of locations without utilizing chemical herbicides. Biocontrol is the use of one species to manage another and is based on predator-prey relationships. The program relies on two small, leaf-eating beetles, *Galerucella calmariensis* and *Galerucella pusilla*. You may also find references using the species name *Neogalerucella*. People usually refer to them as purple loosestrife beetles or "Cella" beetles.

In Eurasia where the plant and beetles are native, the beetles' entire life cycle depends on purple loosestrife for feeding, egg laying and growing new generations. The beetles' activities stress the



Figure 1 Purple loosestrife beetles feeding on purple loosestrife leaves. Photo: Lawanda Junawirth.

plant, keeping it smaller and producing less seed than it would without the beetles' presence. The plants in Eurasia are part of the native habitat and do not cause problems. However, when the plants arrived in North America, the beetles, weevils and other species that used them for food or shelter did not arrive with them. Purple loosestrife was able to grow to its full potential, as tall as seven feet, drop up to 2.7 million seeds per plant per season, and spread across multiple states and Canada.

Purple loosestrife arrived in North America in the early 1800s and reached Wisconsin sometime after 1900. Seeds were likely carried over accidentally in ballast soil and by settlers, but plants and seed could have also been brought here intentionally. People enjoyed the honey produced by bees that collect purple loosestrife pollen. In the past, it was also available as a garden plant. Without any natural controls

like insects or disease that damage the plants, they gradually began to establish in wetlands and other moist natural areas like shorelines. They also spread to roadsides and other disturbed areas.

Due to its heavy seed production, hardy nature, and lack of predators in North America, purple loosestrife can crowd out native plants, reduce water flow and infiltration in wetlands and streams, and decrease feeding and nesting sites. By the mid-1980s, Wisconsin Department of Natural Resources (WDNR) biologists reported that 3% of Wisconsin wetlands were beyond hope of control with the management methods available at that time. In the mid-1990s, biocontrol gave us a new management option.

Purple loosestrife is listed in <u>Wisconsin's Invasive Species Rule</u>, NR40, as a Restricted Species. It is not illegal to have it on your property, but it cannot be transferred (sold or given away), transported or introduced to other locations without an approved permit.

In place since 1987, <u>Wisconsin's Conservation Chapter 23.235</u> requires the WDNR to conduct control efforts for purple loosestrife and provides the Legislative support to conduct research to find the "most environmentally sound manner" of control. Brock Woods (UW Madison-Division of Extension and WDNR) began and nurtured the Wisconsin Purple Loosestrife Biocontrol Program for more than 25 years, beginning with in-state research conducted with Richard

Henderson (WDNR) once the beetles were approved for importation by the Federal and State government in the mid-1990s.

For decades, biocontrol has been utilized to control pests in agricultural settings as well as in natural settings. Learn about the safeguards and research for biocontrol species in <u>Appendix 7</u>. The first purple loosestrife beetles were released in Wisconsin in June 1994 and are now naturalized across the state, meaning they have become part of our ecosystem. Initially, the

beetles had to be imported to North America, but professional aquatic invasive species (AIS) staff and volunteers have raised and released generations of beetles for more than three decades. Even though beetles are now widespread, there are still areas with large populations of purple loosestrife that we want to control or that could use a "booster shot" of beetles now and then.

Biocontrol is best in areas with dense stands of 50 purple loosestrife plants or more. Even small patches that are less than a quarter acre can be good sites if there are too many plants to dig out and chemical control is not an



Figure 2 Purple loosestrife has 4-sided square stems and lance-shaped pairs of leaves that alternate by 180 degrees up the stem. Photo: Jeanne Scherer.

option. It helps if other patches are nearby for the beetles to travel between for food if needed. The beetles are known to travel up to 5.6 miles according to studies, and some observers report finding them much farther from known release sites. Their ability to fly to multiple patches of purple loosestrife make the beetles especially useful in areas where it's hard for people to get to the plants for digging or treating with chemicals, such as an expansive wetlands or wetlands along lake and river shorelines that are hard to safely reach by foot or boat.

Once enough beetles are introduced to an area, they can sustain themselves for many years, depending on site and weather conditions, and if enough purple loosestrife remains to support them. The beetles are not attracted to native species of loosestrife, except possibly winged loosestrife (*Lythrum alatum*) if it is in the same area as purple loosestrife. During their early purple loosestrife biocontrol studies, Woods and Henderson noted some feeding and egg laying was found on winged loosestrife if it was growing within 50 meters of purple loosestrife where beetles were released. Care should be taken if you know this species is present where you are considering biocontrol, though the beetles prefer purple loosestrife when given the opportunity to choose.

Results can be very noticeable within 2-4 years, depending on site conditions. Biocontrol will not completely eradicate purple loosestrife, but the plants will become smaller and weaker over time. They will flower later in the season and have fewer, shorter flower stalks, reducing the number of seeds they can produce. Extended warm temperatures in fall may give them more time to produce seed, but depending on light conditions and temperatures, the beetles can sometimes raise a second generation in the same year, possibly offsetting this concern.

River shorelines and wetlands that are prone to heavy seasonal flooding can be the most difficult sites to manage and need beetle releases over a longer time than other locations. New plants can die in water that is 12" or deeper, but well-established plants which have strong woody roots can continue to flourish. The beetles tend to overwinter in uplands, so they may not be harmed due to flooding as long as they can reach a feeding site once they emerge in spring. An extreme rain event in early summer that causes flooding may harm beetle larvae which move into the soil at the base of the plants to pupate before emerging as new adult beetles.

Letting a limited amount of purple loosestrife remain at a site can serve benefits. Small areas of plants can be important beetle refuges to maintain the statewide beetle population and provide locations for collecting parent beetles for rearing. Purple loosestrife is also utilized by bees and other insects during its bloom in late July – August, helping struggling pollinator species in areas with few native plants. However, it is important to have a variety of native plants that flower throughout spring, summer and fall. Adding native plants or their seeds can help improve sites where biocontrol is conducted.

Sometimes people are interested in trying biocontrol, but they missed the opportunity for the current year and ask if removing flowers in the meantime will help reduce the plants' impacts. This is a good practice and can be done any time during flowering. Keep in mind that the stalks of flowers bloom from the bottom up, so once the flowers at the top are in full bloom, there can be seed pods drying out and dropping seed at the bottom. Carefully bag all the cut flower stems or full plants that are cut down. The plant material can be burned or the bags labeled, "Invasive plant-WDNR approved for landfilling." Before leaving the site, carefully clean off footwear and tools with a brush and water, so seed is not accidently spread.

If your goal is to completely remove purple loosestrife from a site, consider digging and chemical treatment. For very large, difficult to access sites, biocontrol could be conducted first to reduce the population of plants before using other methods. Combining management techniques is known as Integrated Pest Management (IPM). Contact your <u>WDNR AIS Biologist</u> for details. Click on this link to the <u>Purple Loosestrife IPM Chart</u> which is also available on the program <u>website</u>.

#### CHOOSING SITES FOR PURPLE LOOSESTRIFE BIOCONTROL

Many WDNR staff, partners and volunteers help monitor and report invasive species. Beetle rearing gives people an opportunity to participate in invasive plant management. It's a fun and educational activity, but before deciding to get involved, you need to find purple loosestrife patches in your area that need control.

Your <u>County AIS Coordinator or WDNR AIS Biologist</u> can help you get started. They may have projects you can help with or know others you can join for an existing project. If you are interested in doing a small-scale project that only needs a few beetles or want to do this work for educational purposes, contact your County AIS Coordinator for assistance. The book, <u>See Cella Chow!</u>, is an excellent reference for lesson plans.

#### **SWIMS** and the Lakes and AIS Viewer

These two online sources are primarily used by DNR Staff and AIS Coordinators, although the WDNR's AIS Viewer can be used by anyone without special access.

Purple loosestrife locations have been reported in the WDNR database called the Surface Water Integrated Monitoring System (SWIMS) for decades. Hundreds of SWIMS Purple Loosestrife Biocontrol projects have been set up for data entry and hold valuable reports about purple loosestrife locations and beetle releases. Currently, all reporting is entered into county-based projects. To learn more about how to use SWIMS visit the <a href="WDNR SWIMS webpage">WDNR SWIMS webpage</a> or contact your WDNR AIS Biologist or County AIS Coordinator for assistance.

Some purple loosestrife biocontrol reports in SWIMS go back to the late 1990s. Locations that are reported in SWIMS show up on the WDNR's <u>Lakes and AIS Mapping Tool</u>, also referred to as the Lakes and AIS Viewer. The viewer is a great way to see where purple loosestrife has already been reported and might help you find places to look for beetles, if they are present. If you don't know how to use the viewer, your County AIS Coordinator or WDNR AIS Biologist can help. They can also search the SWIMS database.

# **Finding or Revisiting Sites in Person**

In-person site visits can be done at many times of year, but late July through August, the peak bloom period, is the easiest time to look for purple loosestrife. You can look in the winter, too. See the winter site check tips in Appendix 6.

# Consider these questions:

- 1) How tall are the plants on average?
  - a. You can use your own height to judge or a measuring stick or tape
  - b. Pick a height, such as 4 feet. Are most of them shorter, taller or the same height?
- 2) How large is the patch? Choose an estimate method you're comfortable with.
  - a. Acres, square yards, etc.
  - b. Comparisons to a living room, small yard, or a football field, etc.
- 3) Are there at least 50 plants?
  - a. Are they few enough and easy enough to get to that they could be dug out by a couple people?
  - b. Or so many that digging could take days with a number of people working?
- 4) Are they densely packed or scattered here and there?
  - a. Dense example: plants are growing close together and it can be hard to see individual plants.
  - b. Scattered example: Most of the plants are a few feet apart. Some may be several yards apart.
- 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



Figure 3 Purple loosestrife on the edge of a waterway in La Crosse, WI. Photo: John Sullivan.

Large, dense patches of plants averaging four to five feet tall or greater with abundant flowers (stems loaded with seed capsules if observed after flowering, including in winter) indicate a good location for biocontrol. As long as you can access the edge of the patch, you can release beetles. They'll fly to the rest of the patch on their own. Consider long stretches of purple loosestrife in ditches and any sites an eighth of an acre or larger, especially if it's in an area where water is regularly present to move seed or where people frequently drive or walk through the plants, possibly moving seed on tires or footwear. Sites with large plants will also provide the healthiest rootstock for raising your plants.

# **Sites Exhibiting Good or Limited Existing Control**



Figure 4 Monitoring the status of plants and beetles at a Madison area pond. Photo: Jeanne Scherer.

Does purple loosestrife make up 15-30% of the plants in the area? Are most of the plants under 3-4 feet tall? Healthy plants often have flower spikes that are about 1' to 1.5' long. Are most of

the spikes on plants at the site shorter than this? If there are leaves present, check for many scattered, roundish holes along the veins that indicate beetles feeding. Holes larger than 5mm and oval, especially at leaf edges, indicate a different insect species that is not used for biocontrol. Do you see any purple loosestrife beetles? Keep in mind the time of year you are at the site. By August, the new generation of beetles have usually left to overwinter in the uplands.

If the site only has a few plants, consider removing the plants by digging out or cutting and possible chemical control.

If you are finding a significant number of purple loosestrife beetles or the damage they cause, consider this site for an insectary. Insectaries, locations where purple loosestrife beetles are consistently present each year, are important for collecting parent beetles for rearing projects. The program is actively looking for insectaries around the state and wants to map them. If you have found a potential insectary, let the County AIS Coordinator know the location and if it can be used by others with landowner permission. <u>Appendix 3</u> provides more information about collecting beetles.

#### **Mixed Sites**



Figure 5 A field with a mix of purple loosestrife, cattails, Queen Anne's lace and other plants in a moist field. Photo: Jeanne Scherer.

Some sites may appear to be a mix of short/weak and tall/hearty plants, with or without obvious beetle damage. Sites that have a mix may be locations where beetles are just becoming established, or a population of beetles has been negatively impacted by mowing, chemical control, other predators, severe weather, etc. It may be that the beetles have simply moved to a new location. Here, a "booster shot" of beetles could be helpful. Also consider the size of the site and if there are nearby patches of purple loosestrife. Adding more beetles can also benefit other patches in the area because they will fly between patches as they need to for food.

Once you know that you have a good site to work on, check with your County AIS Coordinator to see if anyone else is working in your area who you can partner with or assist. If your county does not have a coordinator, contact the WDNR AIS Biologist or <a href="mailto:DNRAISinfo@wisconsin.gov">DNRAISinfo@wisconsin.gov</a>.

# **Reporting Sites**

Purple loosestrife sites are updated in the WDNR's SWIMS database based on reports gathered from volunteer reporting and AIS monitoring reports from natural resource professionals. The Purple Loosestrife Biocontrol Program also learns about new sites through people using the Beetle Release form, or the Purple Loosestrife Beetle Presence and Activity form, which is used for visiting sites at times other than beetle releases. Both reporting forms are found on the <a href="Purple Loosestrife Biocontrol Program">Purple Loosestrife Biocontrol Program</a> webpage.

People using either purple loosestrife form will answer questions about plant size and density, if beetles are seen, and if beetle damage is apparent. People who know how to use SWIMS can upload data and photos themselves. Others can provide their forms to their County AIS Coordinator or WDNR AIS Biologist for uploading to the database. Anyone who thinks they have found a new purple loosestrife location can send photos of the plants and location with a clear description of the site, such as GPS points, address or map, to the <a href="Regional WDNR AIS Biologist">Regional WDNR AIS Biologist</a> or <a href="maintenance.invasive.species@wisconsin.gov">invasive.species@wisconsin.gov</a>. If you've also found beetles, send a close-up photo of those, too.

**Fun Fact:** There is a very similar looking beetle, *Galerucella nymphaeae*, that feeds on waterlily leaves. The easiest way to tell the difference is to notice which plants they are gathering on because dissection is the only way to be certain which species is which. Each of the beetles is very true to their preferred food plants, so it's unlikely you'd find purple loosestrife beetles on waterlilies and vice versa.

# **METHODS OVERVIEW**

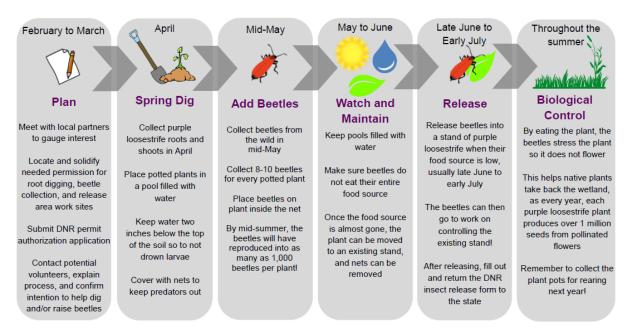


Figure 6 Graphic provided by Golden Sands Resource Conservation & Development Council, Inc., member of the Wisconsin Aquatic Invasive Species Partnership. This timeline may be 2-3 weeks earlier than northern Wisconsin phenology.

Timing can vary depending on location.

- 1) Choose the sites you want to manage and where you will dig rootstock (purple loosestrife roots) the year prior to rearing beetles.
- 2) Dig rootstock as soon as the ground thaws enough to free the roots with a shovel or pitchfork OR dig dormant plants in late fall/early winter and overwinter them. (See <u>Appendix 5</u> for overwintering tips).
- 3) Pot and net your plants as soon as possible plants will need to be at least 18" tall when parent beetles are added.
- 4) Collect parent beetles when they emerge in spring to feed on purple loosestrife usually mid-May to the first week of June depending on region.
- 5) Put 8-10 parent beetles on each plant.
- 6) While beetles are going through feeding, egg laying and larvae are growing into new beetles, keep plants watered and watch them for excessive damage.
- 7) Once new beetles emerge from pupating in the soil or if the plants are significantly damaged by larval feeding, take the plants to release sites.

#### TOOLS NEEDED FOR BEETLE REARING

- Clean up supplies for footwear and tools to be used when leaving dig sites and potting sites to prevent accidentally moving seeds
  - Handheld brushes
  - o Jug(s) of clean water

# Digging

- Waders or rubber boots for wet and mucky sites
- o Shovels and/or pitchforks; pitchforks are excellent for digging intact roots
- o Hand shears, loppers and/or small saws for clipping stems and roots
- o Extra-large contractor bags, tarps, feed bags or large tubs for hauling roots
- Optional: native seed to drop into the holes left from digging

# Potting and growing

- o A mostly sunny area near a water source for refilling pools
- 2-gallon to 5-gallon plastic pots at least 12" across often free from local garden centers or landscape companies; paint buckets with drain holes drilled in the sides also work
- o Wading pools each should hold 4-9 pots without crowding the growing plants
- o Potting soil with a fertilizer about 2 cubic feet per 6 pots
- o Fertilizer if not in the potting soil or to add later if plants are not growing well
- o A sturdy frame: clothesline, fence posts or other support method to hold the tops of the nets up 5-6' above the pots. Examples are shown in Appendix 2.
- Net sleeves (1 net per pot)—unsewn netting is cut to 6-7' lengths and provided by WDNR. <u>Preparing Your Netting Training Video</u>
- O Zip ties, bungee cords, elastic or double-sided Velcro to secure net <u>tightly</u> around the bottom of the pot. Although expensive, some partners have used the same 7/8<sup>th</sup> inch Velcro for multiple years. Duct tape tends to come loose, is difficult to replace when the pots get wet and dirty, and beetles can get stuck on it.
- o Ponytail holders to close the top of net and rope to secure it to supports
- o Fabric glue or needle and thread to repair holes in netting.
- Collecting beetles—see Appendix 3 and the video How to Make Beetle Traps
  - Aspirators and vials used to separate beetles to put on netted plants, not for collecting the beetles
  - o Clean, dry 2-liter bottles
  - Electrical tape
  - Cotton balls
  - o Plastic zippered gallon-sized bags
  - A cooler for collected beetles

#### • Release

- o Garbage or contractor bags for hauling out any flowers and stems you remove
- o Flagging for pots where needed to find them for pick up after releases

 Purple Loosestrife Biocontrol Plant stakes for pots that are left in public areas (see Item Number WY-097 below)

# Purple Loosestrife Biocontrol items you can order from <a href="mailto:DNRAISinfo@wisconsin.gov">DNRAISinfo@wisconsin.gov</a> for free:

Item Number	Item Name	Limits
	Aspirator – includes two vials	1 per group-supplies limited
WY-116		
WY-117	Extra vials: used with aspirators to collect beetles from collection bags or bottle traps to add to each rearing plant	10-15
WY-118	Unsewn netting- pre-cut to 6-7' lengths	30: larger orders are allowed as needed
WY-119	Sewn netting (SUPPLIES LIMITED)	10: Prioritized for those who are not able to find someone to do the sewing
Purple Loosestrife (WI Restricted Species per NR40) Recommend Project approved by the commend Project approved by the of Natural Alterations.  DNHAISINGOR research approved by the of 0.8 band Alterations.  DNHAISINGOR research approved by the of 0.8 200-0081	Purple Loosestrife Biocontrol Plant stakes – approx. 3" x 4" and biodegradable. Placed in the pots with plants left at release sites the public regularly visits, such as parks.	20

# WINTER ACTIVITIES

**Monitoring:** It is possible to do winter monitoring: See the <u>Winter Monitoring Training Video</u> and <u>Appendix 6</u>.

**Volunteer recruitment:** You can start looking for volunteers at any point, but depending on the scope of your project, early is best. Job descriptions for staff and volunteers are available <a href="here">here</a>. Sharing the <a href="Purple Loosestrife Biocontrol website">Purple Loosestrife Biocontrol website</a> and the training videos linked there for specific activities are good recruitment tools.

<u>Netting</u>—check used netting and make repairs or order new, if needed. Small holes can be hand sewn closed. Netting will usually last at least 2-3 years before wind and sun have damaged them beyond repair.

- Preparing Your Netting Training Video
- Written sewing directions: New netting is cut to 6-7' lengths. 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 or second straight seam next to the first. It's OK if the ends aren't completely even. The netting is cut off of large rolls and most pieces won't be perfectly straight on the ends. <u>DO NOT sew the top or bottom of the nets closed.</u>

O Stapling and hot gluing do not make a sufficient seal to keep beetles in and predators out. If you do not sew or have someone who sews in your group, consider asking for volunteers from local 4-H clubs, 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.

<u>Pots</u>—check with local garden centers, landscapers, local botanical gardens and County Parks Departments at any time of year to see if they have used two- to five-gallon pots available. Five-gallon are preferred, but smaller pots may be easier for some people to manage. The pots must have drainage holes.

<u>Pools</u>—Pools can become brittle and cracked over time, so check them to see if new pools are needed. Individuals and groups buy their own pools. The water in the pools should be kept 2" below the top of the soil in the pots to prevent drowning pupating larvae when they move into the soil. Once you have your pools, <u>drill a few large holes in the sides about 5" up from the bottom</u>. The holes should be at least 1" in diameter to reduce clogging. The holes will let the pools drain to a safe level once beetle larvae go into the soil in your pots.

<u>Frames</u>—Plan 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 <u>Appendix 2</u>.

Mass rearing cages—A few groups have mass rearing cages. The cages are 12'x12'x6' heavy duty frames covered by a resin-coated tent. See Appendix 4 for more information. If you have a mass rearing cage, check both the zipper, netting and framing every year for needed repairs. Use dish soap, WD40 or another option on the zipper to keep it moving freely. Pieces of canvas, weatherproof tape applied to both sides of holes, or heavy window screen material are all good options for hole repairs. Window screening is easy to hand sew onto the tents. If there is damage that is too extensive for repairs, contact your WDNR AIS Biologist to learn if a replacement is available. The mass rearing cages have become extremely expensive, approximately \$1350 for a tent and framing in 2023. The program may not be able to replace tents or parts in the future. If you have a cage and are done using it, contact your Biologist and AIS Coordinator to arrange returning it or transferring it to another group.

DIGGING, POTTING AND CARE OF YOUR PLANTS BEFORE ADDING BEETLES Video Training: <u>Digging</u>, <u>Potting and Care of Your Plants</u>

**Digging plants-Spring vs late Fall?** Generally, new purple loosestrife roots for potting are dug in late winter or very early spring, as soon as a pitchfork or shovel can cut into the soil. Changes in weather patterns have led many people to dig their plants in late fall and overwinter them, especially in the northern half of the state. By digging in fall once plants have died back or in early spring, you reduce the risk of damaging new shoots. It's also less likely that beetle

predators will be present. The goal is to have plants that are 18" or taller when adding the parent beetles in spring. See <u>Appendix 5</u> for overwintering plants.

A Message for Group Leaders: As one long-time coordinator says, "As Aquatic Invasive Species 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 know that you might have to reschedule if the purple loosestrife is ready to dig sooner than you expected, which can impact other plans. Keep volunteers in the loop by giving them a list of possible work dates and do regular check-ins. This timing challenge may come up when it is time to collect beetles to add to the plants as well. Give your volunteers as much lead time as possible for making changes to plans.

# The Dig

- 1) Have your planting supplies/frames ready in the location where you'll do potting and growing.
- 2) Gather the tools, boots or waders and head out to your digging site. Take large garbage bags or tubs to hold your roots. Contractor bags and livestock feed bags also work well.
- 3) Have brushes and clean water to clean off your tools and footwear when you leave the dig site. See #10 below.
- 4) Plan to dig 12-15 plants for every 10 you intend to pot. Small plants can be combined. Large roots can be split with a shovel or handsaw. Extra plants can be raised for feeding the larvae/beetles if they need more food before you can get them to your beetle release site.
- 5) The best plants for digging will have tall, strong-looking stems still standing after last year and have a minimum of **6-8 stems** covered in dry seed capsules. Break off the stems, leaving about 8-10" of the stems to act as a handle for moving the rootstock.
- 6) Pitchforks are preferable over shovels where the ground is wet and loose because they will do the least damage to roots. With the pitchfork, loosen the soil around and under the plant. Then you should be able to rock the pitchfork under the plant to pop it out.
- 7) Shovels will work best 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 you plan to use, you can use the shovel or handsaw to split the plant in two or more. Often, a large clump is made up of two or more plants and can be pulled apart.
- 8) Pull the stems and roots of any other plants off of 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 all the soil from the roots, especially if your plants are from a wetland. Wetland soils are rich with nutrients. However, removing most of the soil will reduce the odds of purple loosestrife seeds or the seeds of other invasive plants, such as wild parsnip, ending up in your pots and growing. Leave all the removed material at the dig site.
- 9) If you have native wetland plant seeds, drop some into the digging holes. The seeds can be uncovered or covered with a very light layer of soil. Wetland seeds need light to grow.

10) Clean up: To the best of your ability, make sure not to spread soil with seeds or pieces of roots that could sprout between the dig site and where you will raise the plants. Contain the plants in bags, tarps or tubs; thoroughly brush and use plain water to rinse footwear and gear before leaving the site. If available, handheld steamers can help kill seeds and other potentially invasive organisms. Refer to the WDNR disinfection website for further information. Later, any plants that are not needed can be burned or bagged and labeled 'Invasive plant-WDNR approved for landfilling' and put in the trash if allowed in your municipality.

# **Potting**

- 1) In a kiddie pool or other large tub, spray wash the top of the root ball with a garden hose to remove any eggs of insect predators; bag the waste for the landfill labeling the bag "Invasive Species-WDNR Approved for Landfilling."
- 2) Mix potting soil with water so it is thoroughly wet but still easy to move with a shovel or trowel.
- 3) Add some soil to the bottom of the pot and place your roots on top, trimming the roots as necessary to 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. If the roots look very healthy, it may be OK to have fewer.
- 5) Loosely pack the soil into any air pockets, water and keep adding soil until the roots are well-covered without air spaces. Do not pack the soil too hard. The larvae will need to dig into the soil surface later.
- 6) If the soil does not have fertilizer included, sprinkle slow-release fertilizer (amount as shown for pot size) onto the soil surface and mix in about 1" deep.
- 7) Cover the plants with netting.
  - a. Secure the bottom of a net sleeve around the pot with elastic, zip ties, double-sided Velcro or bungee cords pulled very tightly around the pot and the net; pots often have a lip around the top that will help keep the net from slipping off. Rope is not recommended because it can become loose and stop holding the netting in place. It is difficult to untie and replace ropes once they are wet. Duct tape can also come loose or slip off. The pot and bottom of the nets will become very wet and dirty, making duct tape almost impossible to replace.
  - b. Close the top of each net with a ponytail holder 5-6" from the top; it needs to be tight enough to keep predators like spiders out and the beetles in. You will be opening the top of the nets to drop in your parent beetles later.
- 8) Place your pots in the pools near a watering source in a sunny location. Try to avoid areas that get strong winds. One pot (two if the pool is large) can go in the middle. Circle the rest around it. Don't crowd the pots. The plants need good airflow and sunlight.
- 9) You can loop twine or rope through the ponytail holder to then tie the top of each net to your support structure, whether it's a clothesline or rope strung between fence pots or another structure; the goal is to have the net tall enough (5-6') to give your plants maximum growing room and the beetles easy access around the plants.



Figure 7 Nets closed with ponytail holder. Photo: Jeanne Scherer.

10) Add water to the pools so that it comes up to the drilled holes; <u>remember the water level</u> should stay 2" below the top of the soil in the pots.

# **Plant Care**

The rate of plant growth will vary depending on weather and location.

- 1) Plant crowns can take at least a week or two to sprout and then they usually grow quickly.
- 2) Regularly check your pools to make sure there is always water. If it is very hot or there is a drought, check plants at least twice per week.
- 3) To avoid mosquito larvae living in the pools, occasionally fill the pools to just above the drain holes to flush out any larvae. You can also add a small amount of a BTI mosquito larvae killing product, such as Mosquito Dunks, to the water without risk to your beetles or their larvae.
- 4) When stems are 12-15" tall, spread the small leaves at the tops 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. Cutting back the tops will give you bushy plants with lots of foliage for the beetles and keep the plants from getting too tall for their nets.
- 5) If plants are not growing well after two weeks, add some fertilizer.



Figure 8 Snipping off the tips of each stem will help the plants grow bushier, providing more food for the beetles. Photo: Chris Hamerla.

#### COLLECTING AND REARING BEETLES

In southern Wisconsin, beetles may show up on purple loosestrife in wetlands, ditches and on shorelines by mid-May or slightly later in May. By Memorial Day, they are usually out in force. In the far north, depending on the weather, they may not start showing up until the first week of June. Check early and often until you find what you need.

- 1) Plants are ready for beetles when they reach at least 18" tall and start to become bushy.
- 2) There are three options for getting beetles.
  - a. Most people collect their own beetles once they find places to collect them.
     Previous release sites are often excellent. There are detailed directions in
     Appendix 3. Also see the videos Making a Beetle Trap and Collecting Beetles.
  - b. You can also work with a local coordinator to find beetles. Many County AIS Coordinators can assist with beetle collections as needed.
- 3) Once you have your beetles, you must get them into the nets as soon as possible. Until they go into the nets, keep them cool and out of direct sunlight. A cooler is helpful for this. If they are in a zippered bag and have eaten the leaves provided, you can carefully open a corner and slip in a few more purple loosestrife stems with leaves for them to eat until they are put on the plants. If the bag is deflated, open a corner and blow in some air like you're blowing up a balloon, but be careful. They are escape artists! An aspirator comes in handy to catch them.
- 4) If someone else is providing your beetles and you receive them in small vials or bags with about 10 beetles per vial or bag, open the top of each net and shake the beetles onto the plants. Close the net.
- 5) If you have large bags of beetles, you can use the aspirator to 'suck' beetles out of the zippered bag either directly into the net, or to be sure they don't escape, into an attached vial. Aspirator instructions are in Appendix 3 or see the video Collecting Beetles.
- 6) The advantage of having an aspirator is that you can often catch the escapees, since they tend to collect on netting because their favorite food is inside. If you happen to have them escape a bag indoors or vehicle, they usually head toward a bright window. Local coordinators are provided aspirators to help those in their areas, and one can be sent directly to groups. Those

who decide to stop participating should return aspirators to their County AIS Coordinator or WDNR AIS Biologist.



Figure 9 On a shoreline with a wetland and consistent water levels. Photo: Cathy Higley (Vilas County).

#### 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, depending on how much damage there is to the plants. It is not uncommon to make a plant check and discover that one or more plants look dead or nearly dead overnight because larvae or the new beetles are feeding so heavily. Or you may find the top of your beetle nets 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 <u>Appendix 1</u>. The following directions are for traditionally reared beetles in individual nets, not those in mass rearing cages.

- 1) Transport your pots with nets on. If carrying in an open pickup bed or trailered boat, lay them down to avoid wind injury or toppling.
- 2) Sometimes people cut the plants off at the base and just take out the netted tops and active beetles. That may work, but it is very likely that pupating larvae are still in the soil, so you may be leaving many beetles behind using this method. It is not recommended.
- 3) Release where access and footing are safe. You want to place the pots with beetles next to healthy purple loosestrife, but it is OK if you can only reach the plants at the edge of the site since the beetles will be able to fly to plants you can't reach.
- 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 want to find multiple locations to cluster 2-3 pots. The adults will fly to new plants as needed.
- 5) If you are releasing along shorelines, do your best to keep the pot out of water to avoid drowning pupae still in the soil.

- 6) For each pot, uncover your plant next to a purple loosestrife plant and as much as possible, entwine the branches of the two plants. This is vital. If there are still larvae on leaves or in the soil, they can crawl over to fresh plants to eat.
- 7) If possible, you should leave your pots and mark where you leave them to be retrieved two to three weeks later. This is important because it's likely there are still larvae or pupae in the soil. Sometimes the pot will fall off the root ball when you place it. In that case, you can slip the pot back on. If that is not possible, after shaking and tapping the pot well to get any larvae along the sides out, you can take these away at the time of release.
- 8) Complete the beetle release form found on the <u>Purple Loosestrife Biocontrol and You</u> webpage for each site. Enter the data into SWIMS or return the form to your County AIS Coordinator or WDNR AIS Biologist for data entry. One to two pictures of the plants and your site would be appreciated. If your site hasn't been previously entered into SWIMS by WDNR, the photos will be used to complete that step.

#### BEETLE PRESENCE AND ACTIVITIES CHECKS

Checking beetle activity can be done any time you want to see if your project is working, or when looking at new sites or looking for insectaries. The Beetle Presence and Activity form for reporting is found on the <u>Purple Loosestrife Biocontrol and You</u> webpage. Year-to-year photos of sites are very helpful for doing comparisons. If you take site photos, find a landmark to have in each photo to use as a reference point and keep the distance between the photographer and the site as similar as possible for good comparisons.

Checking your sites after releases each year will help you determine if biocontrol is making the desired impact. It can take two or more years to see a significant difference in sites that started with dense populations of purple loosestrife and depend on how many beetles are released each year. These visits can indicate if you will need to do more releases, possibly bigger in the future, or if the site has reached a state of balance where the beetles are keeping the plants at a level where they are "playing nice with others." Even balanced sites may need an infusion of beetles in the future, so continued monitoring is valuable.

- 1) During visits to retrieve pots or later in the summer/early fall in years when you have done a beetle release, note the plant conditions you find. Is there significant damage to the plants from the beetles? Are they discolored? By mid-September, the plants will be drying out and dying back for winter. Do they seem to be dying back earlier?
- 2) During a winter or early spring visit, check the remains of the flower spikes. Healthy plants will have spikes heavily covered with dried seed capsules. Impacted plants will have sparse to no seed capsules. Stems that are light in color instead of dark are probably older stems, not those from the previous summer.
- 3) The best time to determine if the beetles you find on plants are beetles that have overwintered in the area is when they emerge in spring. Late season beetles may have only been there from a release or may have flown in from other patches. It's good to consider spring monitoring if you are hoping a site will be a good insectary.

Like the beetle release data, the Beetle Presence and Activity data should be entered into the SWIMS database. SWIMS users can enter the data themselves. Others can provide their form to their County AIS Coordinator or WDNR AIS Biologist.

#### A NOTE ON SITE GOALS AND NATIVE PLANTS

Consider the overall goals for managing your biocontrol location, not just the purple loosestrife. If restoration is the goal, planting native plants is important. A different goal might be limited to reducing purple loosestrife even where the location is unlikely to be returned to a natural state. For example, purple loosestrife along roadsides and in medians have a high risk of spread on mowing equipment or water flowing through them. It may not be possible or practical to add native plants, but biocontrol can help weaken the plants, reduce the seeds they can produce and reduce the spread.

Research has shown that it can take as long as ten years for native plants to return to a purple loosestrife management site on their own, even where biocontrol has been very successful. If an area originally had many native plants, there may still be a good native seed bank in the soil. If a faster return to native plants is the goal, seeding or planting will be needed. Other invasive species on site may also need to be managed in the meantime.

Remember that eradication of purple loosestrife is extremely rare using biocontrol and site goals may need to include loosestrife living alongside the native species present at the site. Reducing density or loosestrife height may be good goals depending on the site's specific needs.

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# APPENDIX 1: GALERUCELLA SP. BEETLES LIFE CYCLE

1) Each summer's new beetles overwinter in upper soil layers of the wetland or surrounding uplands and emerge to feed and mate in late spring. Spring's parent beetles tend to be a darker brown than young beetles seen the previous summer. They are 4-6 mm long and half as wide. *G. calmariensis* has a wide dark stripe on each wing. Bugwoodcloud.org has an excellent ID guide with photos for both beetle species and the two weevil species that were approved for purple loosestrife biocontrol. These are *Galerucella calmariensis*, *G. pusilla*, the small *Nanophyes marmoratus* (see <u>Appendix 11</u> to learn about the success of these tiny, flower eating weevils.), and the larger *Hylobius transversovittatus*, which spends its time mining the roots of the loosestrife. The beetles proved easiest to rear.

The beetles 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 nets, check the netting 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. Escapees will often stay on the outside of the nets, wanting to get back to the plants. You can use cup or an aspirator to catch them safely. If a plant appears to



Figure 10 Black-margined purple loosestrife beetle. Photo: Paul Skawinski.

have no beetles left, you could collect a few from your other plants or you can save the plant to use as food for beetles eating their plants too quickly.

2) The beetles will live about 40 days after emerging to feed and mate. Females can lay up to 400 eggs each which can be found on the plants leaves. The eggs are tiny, less than 1 mm



Figure 11 Purple loosestrife beetle eggs. The black lines are beetle excrement, not part of the eggs. Photo: Paul Skawinski.

and cream colored with an uneven black line of frass (insect excrement) deposited on them. They are usually laid in bunches of 2-10 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 approximately 2 weeks after laying.

3) The larvae are very small and hard to see at first. Larvae damage in the shoot tips, called "tip-feeding," is usually obvious when the new leaves are gently separated, especially due to

visible frass. The larvae are yellow with a dark head capsule. They grow and then molt, each



Figure 12 Purple loosestrife beetle larva climbina up a stem. Photo: Paul Skawinski.

time increasing in size. Each of these growth stages are called an instar. Their feeding damage is described as window-paning because the leaf tissue is left brown, thin and translucent. If your plant appears to be dying due to extreme window-paning, get it out to a release site as soon as possible. At the release site, take off the net but leave the plant in the pot to protect larvae that have already moved into the soil to pupate. Check the net for larvae. Make sure to entwine the branches of the potted plant with one on site so the larvae can crawl to new leaves.

- 4) Larvae become pupae after 2-3 weeks when they move down to the soil after intensive feeding. It will seem as if most of your larvae have disappeared, but they are 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 become saturated. If the leaves of your plant have been severely damaged, not leaving enough food for young adults, take the plants out for release. Take off the net but leave the plant in the pot to protect the pupae in the soil. If your plant appears completely dead at this point, put it out anyway. We have reported cases of plants looking dead for as long as two weeks and then new beetles appearing on the dead plant material anyway. The larvae are very likely to still be in the soil regardless of the plant's appearance.
- 5) For every ten parent beetles placed in the netted plants a few weeks earlier, as many as 800-1000 new adults can emerge. They will be light tan with no dark lines on the wing covers but will darken in a few days. They often collect at the top of the net, ready to disperse and eat. Soon, there will be hundreds emerging. If you can't get out to the release site immediately, adding fresh stems from other loosestrife plants can help them if rearing plants are dying rapidly; cut tall stalks of loosestrife and put them in buckets with water to help them last longer. If you find hundreds of beetles at the top of the net, it is ideal to get them to a release site as soon as possible.



Figure 13 Left to right: Newly emerged beetles feeding. Photo Credit: Amanda Smith.

Newly emerged beetles filling at net. Photo Credit: Mike Alaimo.

# **APPENDIX 2: FRAMING FOR NETTING**

Nets need to be held tall to give the plants plenty of light and room to grow. The plants need 5-6' of available height. Putting them under a home clothesline often works. One group put their pools into an unused corn crib and tied the tops to pipes above them, using the open wire of the crib to support the pipes. Others have run a rope between two trees with the pots set in kiddie pools below the rope or between trees on the edge of a wetland where the bottoms of the pots can sink into saturated soil so that watering is not needed.



Figure 14 Clockwise from left: A wood structure designed for the project. Photo: Wendling; An old wooden swing set repurposed. Photo: Jeanne Scherer; Pole run between the sides of a dog run. Photo: Jeanne Scherer; Conduit structure with guide wire. Photo: WDNR; Tall metal fence pots and rope. Photo: Amanda Smith.



Figure 15 Pools and nets set up without a support structure. Photo: Derek Thorn.

Placing a post or bamboo pole directly into the pot and tying at the top has worked for some people where other methods aren't practical. Windy areas need to be avoided because this method can make the pot unstable and likely to blow over. Tying the plants together with twine or plastic tape as shown helped overcome the risk of tipping over. If you try this option, try looping the twine or tape around the top of each plant and then to each other for even more stability.

# **Video Training:**

Making a Beetle Trap Collecting Beetles

# **Beetle Traps**

Beetle traps made from 2-liter bottles are very efficient for collecting your spring parent beetles. Some people will collect beetles directly into zippered plastic bags. Try both to find your preference. When using a bottle trap, you'll dump your collection into a 1-gallon zippered plastic bag whenever the bottle appears too full to safely hold all the beetles. Beetles in the large bags are then transferred to vials or small plastic bags using an aspirator and then placed with each plant.



Figure 16 Bottle trap made with a soda bottle. Photo: Brock Woods.

# **Bottle Trap Directions**

- 20 oz and/or 2-liter bottles (preferred)
- Scissors or X-Acto knife
- Electrical tape
- Cotton balls
- Gallon-sized zippered plastic bags for field use
  - 1. Discard the cap. Wash and dry the bottle thoroughly.

- 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, set it in place and it falls into the bottle, you've cut too high and will need to start with a new bottle.
- 3. Once the top fits snugly, use electrical tape to secure it to the bottle. Electrical tape works best because it is easy to remove when you're ready to dump the beetles into a 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 not actively catching beetles.

# When and Where to Look for Beetles

In southern and central Wisconsin, start checking known purple loosestrife sites for beetles in mid-May, once purple loosestrife shoots are appearing and easily found. When there is an exceptionally warm spring, start looking a week or two earlier. Farther north, the best time for collection tends to be the first week of June, but checking sites earlier is best. Large numbers of beetles may collect on stems even when the plants are only a foot tall.

- 1. Keep in mind that the plants being reared should be at least 18" tall before adding beetles.
- 2. Check as often as you reasonably can. To maximize egg production, your goal is to find early beetles that are just starting to mate but haven't laid eggs yet.
- 3. Sunny, warm days with a very light breeze are best. Collect after dew has dried. Usually the optimal time is between 9:30 a.m. and 2:00 p.m. Earlier, dew can end up in the trap causing the beetles to stick to the inside.
- 4. Start with sites where beetles have been released before or where past surveys indicated beetle damage. You may need to make repeat visits or expand the search to new areas depending on how many beetles are needed. Since they often overwinter in uplands, some beetles may have moved to nearby sites instead of where you expect to find them. Heavy winds can also relocate them.

#### What to Look for

- 1. Look for feeding damage indicated by 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 beetles on the plants, there are likely to be even more hidden at the stem tips and under the leaves, generally in the top third of the plant. You can test for more beetles by tapping a stem over your open bottle to see if some fall out of hiding.
- 3. If you see damage, but few or no beetles, check back after a few days to see if more have emerged. If you still can't find many beetles, the site may still be developing a sufficient population. Widen your search.

# **Trapping your Beetles**

1. Purple loosestrife beetles escape predation by simply dropping off the leaves 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, hold the open bottle or zippered plastic bag below the stalk or stem. Then gently tap the stem or blow on the leaves to send the beetles falling into it. Even if you don't see beetles, it's good to tap the stems because they are often hiding. 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 often crawl up or fly to the top of the bottle. The inverted top slows them down, so you can usually get them to drop to the bottom again with a gentle tap or two. TO MOVE A STUBBORN BEETLE, BLOW ON IT OR USE AN ASPIRATOR, DON'T PUSH WITH YOUR FINGER. The beetles are very fragile.
- 5. Once you have around 100 beetles in the bottle or they seem too crowded, it's time to put them into a zippered plastic bag. Remove the electric tape and set to side. Quickly insert the bottle top in the bag and dump the beetles in, tapping the bottle to get remaining beetles out. Leaving a small corner of the bag open, gently blow into the bag to fill it with air like a balloon before closing it.
- 6. Remove/squish any possible predators like spiders that have gotten into the bag.
- 7. Keep the filled bags in a cooler and out of the sun. Blow in more air as needed.
- 8. If your beetles need to be stored for travel or shipping to others, bring some stems of purple loosestrife back to your sorting site for them. Put the stems in a bag with a little water for travel, and if you need to hold them for a few more hours, they will do well in a vase of water.
- 9. When collecting beetles at a good insectary site, leave at least a third to half of the beetles you find there. If too many beetles are collected, the site may not have enough to maintain the population for biocontrol and to continue as an insectary. If you are concerned about taking too many, you can also restock 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, plan to put 10 beetles per vial/bag, but 8-10 is fine. If you have some smaller looking plants, the vials of 8 beetles can be used on them. The aspirators used by the program are similar to the image of one 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, take a quick breath in and the beetle will be drawn into the vial. A fine screen protects you from drawing the beetle into your mouth, but you may start to taste the frass and want to blow the tube out every few minutes. This technique is demonstrated in the Making a Beetle Trap video. Remember to take breaks so you don't get light-headed using the aspirator.

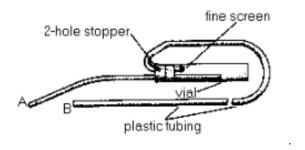


Figure 17 Aspirator. Photo: University of Kentucky.

- 2. You can also use an aspirator to collect on the walls of mass rearing cages. It's great for collecting escapees indoors or in vehicles. They are not efficient for catching large numbers of beetles at a time though.
- 3. Counting a large bag of moving beetles is tricky, but you can get an estimate if you need to, such as if you are collecting beetle for others who will be raising the plants. In a cool place (coolness reduces movement), let some of the air out of the bag. Use a pencil or some other lightweight straight edge to hold over, not on, the bag to visually mark off areas. Count each area as you move the straight edge around over the bag. OR you could draw a grid of 2" x 2" or 3" by 3" squares on the bag before using it.
- 4. Beetles kept in large bags need to be placed on plants again within 2-3 days, but sooner is much better because they can die. Until the release, you will need to add purple loosestrife leaves to the bag(s) and blow air into them as needed, which can be every few hours 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). Since stems may have eggs on them, add them to your potted plants with the beetles.
- 5. Dump your beetles from the small bags or vials into the net on the plants ASAP.

# **APPENDIX 4: MASS REARING CAGES**

There are a limited number of mass rearing 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 wetland site over in-ground purple loosestrife plants or on a flat, dryland site using up to nine kiddie pools (depends on diameter) or lined with a waterproof tarp with edges slightly raised on the sides to create a shallow pool where you set the potted plants without kiddie pools.

When not in use, a large storage tub for the tent is recommended. Rodents will eat their way through a folded tent stored in the open.



Figure 18 Mass rearing cage. Photo: Stephanie Boismenue.



Figure 19 Roughly 75plants can be grown using nine 4-ft. diameter pools. Photo: Dara Fillmore.

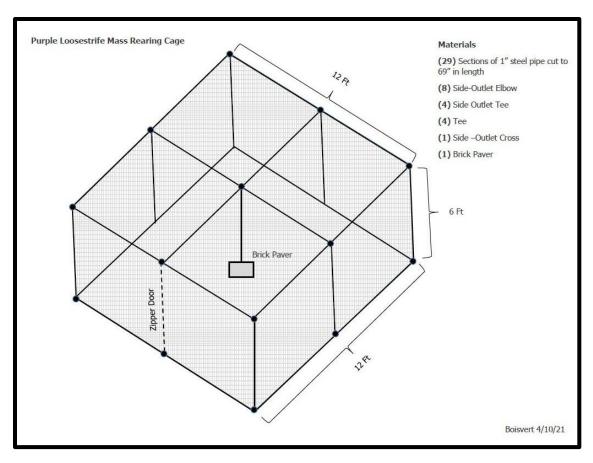


Figure 20 Mass rearing cage diagram. Created by: Thomas Boisvert.

#### Wetland sites

Start with a level site in full sun 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.

# 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. You can't count on beetles overwintering at the base of the plants and will need to add them.
- 2. Remove all above ground vegetation including the purple loosestrife in winter or very 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 at least 18" tall. Example: 80 plants will need 800 beetles.

6. When the young beetles begin to emerge (3-4 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. Outside of the cage, it is recommended to have a full kiddie pool of plants, netted as usual, to have on hand if beetles in the cage feed too quickly on rearing plants and need more food.

- 1. Lining the bottom of the cage will prevent grass and weeds from filling in spaces between the pools and help keep out predators. Set up the cage with kiddie pools. Many of the older cage fittings are secured to the pipes by Allen screws, so you may need an Allen wrench and a product like WD40 to loosen and tighten the fittings. Zippers can also be loosened with WD40 or a dish soap.
- 2. Dig and pot purple loosestrife as you would 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 inside the mass rearing cage.
- 4. Immediately close the cage to keep out predators and help keep the growing plants warm. The cage acts as a greenhouse.
- 5. Care for your plants as normal, keeping the pools full and snipping off top buds once the plants are about 12-15" tall to encourage bushiness. Fertilize if necessary to stimulate growth.
- 6. When the plants are at least 18" tall, add beetles (10x the number of plants) directly to the cage.
- 7. Watch for heavy feeding by the larvae once they hatch. If the plants are turning brown and have little to no green left, you either need to bring in the spare plants or get the pots out to release sites. In either case, entwine the branches of the dying plants with healthy purple loosestrife so that the larvae can get to their food. You may also need to move the plants to release sites or bring in more healthy plants, if the rearing plants appear to be dying and are not putting out more green stems and you stop seeing larvae, indicating that they have moved down to the soil to pupate.
- 8. When the young beetles begin to emerge (3-4 weeks) and have eaten enough that they begin to leave the nursery plants, they often begin collecting in the upper corners of the cage. When the day is still cool, the beetles are likely to be on the plants, making it easier to collect them by entering the cage and collecting as you would in a wetland by tapping them into beetle traps or using aspirators. Later, when the day warms and the sun is out, they tend to collect on the tent walls facing the brightest sun.
- 9. Some people use a narrow sock of netting tucked into a shop vac hose to vacuum them up when they congregate on the cage walls and ceiling. They can be collected in multiple sleeves or dumped into large, zippered plastic bags to take to release sites. Some people have great success with this method. Others worry about harming the beetles or have problems getting the knack of it.

If using a shop vac, you will inevitably crush some beetles. It is important to only collect 50-100 at a time and then get them into bags so they are not being sucked into the net sock for more than a couple minutes. When the shop vac is shut off, they will immediately begin to crawl out, so the zippered bags need to be ready to go.

10. You can collect off the walls with an aspirator, but this is probably the most time consuming and difficult method for large numbers of beetles.

Although we have yet to confirm it with research, we seem to be having increased matching issues with plant and beetle phenology across the state: beetles emerging before we can grow big enough plants to support them in our rearing projects. Often, it has been particularly challenging in the north if the spring thaw is too slow or the ground remains snow covered, but then beetles emerge as soon as they can get out and about on a warm day. More and more people are choosing to dig their plants once they go dormant for the year and overwinter them to get a head start the following spring. There's no guarantee, of course, but we have had some very successful rootstock overwintering projects with 80-90% of the rootstock surviving winter and flourishing the following season.

You can dig plants in late summer to late fall. Ideally, you should wait until flowering is complete and the plants die back, otherwise, you may need to keep cutting off flowers and disposing of them. Often, digging in the last week of October or the first week of November works well in the northern part of the state. Waiting until November is better in the south. Cut the stems down to 6-8 inches to give yourself a "handle" for hauling. If you dig later in the fall, do so before regular hard freezes start so the plants can stabilize in whichever storage situation you choose.

# **Option 1:** Potted plants

- Potted plants that have already been used once generally, it's best to use newly dug plants, but some people successfully get a second year out of their plants if they haven't been too destroyed by a heavy beetle crop. Provide water until the plants have died back for winter.
- One group successfully keeps their potted plants outdoors in front of a light-colored wall that faces the winter sun. The sun and reflected heat usually keep the plants sufficiently warm. Snow will provide insulation, also. It helps to put a tarp under the pots to reduce rodents trying to get in during the cold. You can add some insulation around the pots, such as leaves or soil, but do not use straw as this seems to attract rodents.
- Others put the potted plants in an unheated building for the winter. People who have access to a greenhouse can move their plants into it in later winter, but doing so earlier might interfere with the plants' needed dormancy period. However, some people have had good success by keeping the plants in an unheated greenhouse all winter.
- Other people will use a "digging in" method. Dig a hole to set the pots into and cover them with some netting or screen to help keep rodents out.
- Regardless of how they spend the winter, you can bring the pots out in March to start growing in the south, and often by mid-April in the north. Put the nets on the pots right away. They will help warm the soil and roots besides keeping predators out. If a late season polar vortex comes through your area, you could tarp the plants or move them into a shed, barn or garage until it passes. You do not want to lose sprouts early in the spring because this will set the plant growth back by a couple weeks.

- You'll need to start watering as winter comes to an end. You may want to put the plants
  in the kiddie pools or in tubs that are easier to slide out of the cold, if necessary. Having
  them on a cart you can move can help. Make sure you have added the side drain holes if
  using kiddie pools.
- All plants can benefit from a fertilizer, such as a fish emulsion or Osmocote, but plants that were used for beetle rearing the previous year really need this treatment.
- If you happen to find beetles that spent the winter with the rootstock are now feeding on the sprouts in your nets, you may want to designate one or two plants as nurseries and move them to it, but it will depend on how fast the plants are growing and the number of beetles feeding. Overwintering beetles is a hot topic and as of this guide's revision in early 2025, tried-and-true methods have not been established.

Option 2: Digging rootstock and mounding them on tarps or in kiddie pools



Figure 21 Some overwintering methods have included covering shorter piles with a second pool and adding a weight, as well as a tarp placed on the ground and mounded with 50-80 rootstock and covered with the rest of the tarp. Photo: Dara Fillmore.

Dara Fillmore, WDNR, began rearing beetles and purple loosestrife in 2019 in Douglas County and the following advice is based on what she has learned through some long and very cold winters. She and her partners have used multiple mass rearing cages as well as traditional rearing with individually netted plants. As a result, she has been gathering hundreds of plants and overwintering them each year. Keeping plants nestled in piles has worked better for her than having them in pots overwinter.

Once plants are dug, they are mounded on either a tarp or in a kiddie pool. If using new pools, make sure there are drainage holes in the pool sides, just as for summer and that you keep them clear. You can mound 20-30 plants on the tarp or in the pool. Keep the stem tops uncovered and facing in or up, so when weather warms enough for the plants to start sprouting they get light.

You can give the mound a light covering soil or old beetle netting. Once hard freezes start, it will work best to cover with a tarp which you will remove as soon as practical once severe cold weather has passed. Pot the plants once the soil is sufficiently thawed and proceed as usual.



Figure 22 Purple loosestrife rootstock (roughly 30 pots-worth) mounded into a 4ft pool, with stalks mostly facing in and up, is covered with soil for some winter insulation before being covered with plastic or a tarp and tightly secured around the base of the pool with twine. Photo: Dara Fillmore.

# **APPENDIX 6: WINTER SITE CHECKS**

Video Training: Winter Monitoring

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 tall (5-7') populations of loosestrife. The plants' stems turn dark brown and remain standing among marsh grasses and cattails which are more likely to fold over. Frozen wetlands can be easier to access.

- If you are monitoring a known site to see how your control efforts are going, use the Beetle Presence and Activity form found at the <a href="WDNR Purple Loosestrife Biocontrol webpage">WDNR Purple Loosestrife Biocontrol webpage</a>. You may still be able to see evidence of damage on the dried leaves.
- AIS Coordinators: If you are looking for new sites, regardless of the time of year, use an AIS Early Detection form, following normal WDNR monitoring protocols.
- Random discoveries of new sites where you don't do a beetle presence assessment should be reported on the AIS Incident form available <a href="here">here</a>. If you are unable to access the form, you can email photos and the specific location (address, GPS point, road crossing, etc.) to the WDNR AIS Biologist for your county.
- Data should be entered in SWIMS or sent to a WDNR AIS Biologist.
- Don't forget to clean your footwear after your visit! Purple loosestrife seeds are tiny and likely to be picked up on your boots, possibly along with the seeds of other invasive species.
- Remember, no ice is safe ice. Use best judgement and always put safety first. Reference the WDNR's Ice Safety webpage for helpful tips.

Take note of how tall the average stems are, the average number of flower stalks per plant, an estimate of the number of the plants, and the amount of area covered. Are the stalks heavily covered with seed capsules as in the photo below, or are they sparsely covered? Old flower stems that have become lighter in color and are bare or mostly bare are probably two or more years old. The darkest brown plants are usually from the current year, and if they are generally shorter than the old plants around them, this MAY be a positive sign of beetle activity.



Figure 23 Flower stalks on an older plant that flowered weakly. Photo: Paul Skawinski.

The plant here is not quite 5' tall but notice how few seed capsules are on the stems compared to the photos below. The flower themselves are short. Short flowering stems (6-10") on candelabra-shaped inflorescences are beetle impact trademarks. The lack of seed pods and the light stem color can indicate these stems may be two or more years old.

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



Figure 24 Clockwise starting on the left: A first-year plant in winter covered with seed pods. Photo credit: Jennifer Froehly, USFWS. Dark stems of purple loosestrife standing out in the snow along the Mississippi River. Photo: Jennifer Froehly, USFWS. Dark stems of purple loosestrife standing in a wetland in very early spring. Photo: Chris Hamerla.

# APPENDIX 7: HOW BIOCONTROL SPECIES ARE CHOSEN SAFELY

Prior to *Galerucella* spp. beetles being allowed into Wisconsin in the mid-1990s for biocontrol, the safety was assessed at multiple levels and a strict approval process took place. 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 <a href="here">here</a>. 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 (DATCP) must also approve of the use of the species prior to release and <a href="permitts specifically for research">permitts specifically for research</a> are required to bring a biocontrol agent into Wisconsin.

Although purple loosestrife biocontrol has been thoroughly researched, sometimes people are still concerned about introducing a species not traditionally found in Wisconsin to control another non-native species. Brock Woods, researcher and program coordinator from 1994-2019, 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 8: CONDUCTING BIOCONTROL IN ROAD RIGHTS OF WAYS (ROWS)

Input provided by Christa Shaefer, WI Dept. of Transportation (1/8/2025): 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 road work 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 projects that you could join as a partner. If your county does not have a coordinator, check with the WDNR AIS Biologist for advice, and 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, e.g. Highway 8, Highway 53. 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 this page 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 within the right of way (ROW), you do not need to contact adjacent landowners for permission. How far a ROW extends from the centerline 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 the WisDOT 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 <u>directly</u> with them and following their protocols.
- 4) Wear a reflective, fluorescent orange or yellow vest 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 on 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, put out safety cones behind your vehicle.
- 9) Use your hazard lights when driving slowly to look for a site where you will work. A slow moving vehicle sign for the back of your vehicle can also help.
- 10) It is safer to work with at least one partner, so that one person 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 <u>identify</u> it to avoid it. Wear protective clothing.

# 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 moved 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 stopping to remove plants or to look for beetles.
- 3) If the plants are sparse, consider checking with the County or local highway department to see if it's 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) As a reference, check out the Roadside Mowing App from DOT
- 2) Check with your highway department to learn who mows and coordinate your timing.
- 3) 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.
- 4) 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's rootstock is right up against and entwined with a purple loosestrife on site so you don't need to return for the pots.

# Integrated Pest Management Options for Purple Loosestrife, including Biocontrol

Options to use alone or in combination: Chemical Cutting Digging Beetles	Scattered Individual Plants	Small Populations Less than one acre	Medium Populations 1-10 acres	Large Populations More than 10 acres
<b>Low Density</b> 0-33% Coverage	les.			lex.
Medium Density 34-66% Coverage				**
High Density 67-100% Coverage If the area is very small and there are no nearby patches for beetles to relocate to if food runs out, use an option other than beetles.		lex.	**	**

# **Other Considerations**

<u>Consider Access</u>: If you are unable to safely access a site (extreme muck, water levels, etc.), beetles are probably your best option.

<u>Survey time</u>: If possible, base your ratings during peak bloom time. This may be July-August in southern Wisconsin or August-September in far northern Wisconsin.

<u>Chemicals</u>: If you are in an area with wet ground, contact your WDNR Regional Lakes Biologist to determine if a permit is needed.

Images: sprayer by Unknown Author is licensed under <u>CC BY-ND</u>. Shovel by Unknown Author is licensed under <u>CC BY-SA-NC</u>, Lopper by Unknown Author is licensed under <u>CC BY-SA</u>, beetle by Emily Heald

Author: Jeanne Scherer, UW Madison Division of Extension: Natural Resources Institute-AIS Program (2022)

#### APPENDIX 11: NANOPHYES MARMORATUS-PURPLE LOOSESTRIFE FLOWER EATING WEEVIL

# Provided by Dara Fillmore

Although this small biocontrol weevil was released into Wisconsin wetlands at the same time as Cella beetles, they did not seem to be as easily reared as the beetles, and some early research suggested that the weevils were not able to flourish in stands already containing beetles. However, in 2022, Dara Fillmore found hundreds of them on the outside of her rearing cage one day, trying to get in, so she looked them up. She had only seen a handful of the weevils prior to this on various loosestrife plants. Since 2022, the weevils have been seen at nearly every site she worked on around northern Wisconsin, and they seem to overwinter with her beetles and do well on the sites that they are most common. The weevils have also been reported by USFWS staff along the Mississippi River.

The loosestrife weevil is less than half the size of the Cella beetle. Most of its life cycle is focused within the flower bud of purple loosestrife. Eggs are laid inside the flower bud and larvae eat the buds from the inside, causing the flower to die or sometimes making it unable to produce seed.



Figure 25 Left: Nanophyes marmoratus weevil. Right: Weevils breeding in mid-July on loosestrife. Photos: Dara Fillmore.

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Figure 26 Top: A healthy flower stalk with spent flowers. Bottom: A flower stalk interrupted by the feeding of Nanophyes marmoratus weevils with very few flowers making it to bloom. Photo: Dara Fillmore



Figure 26 Weevils on purple loosestrife buds. Photo: Dara Fillmore.

The lifecycle of the weevil is a bit later than the beetles; the weevils often do not show up in large numbers until later in the summer (July in northern WI) and are breeding into late August and early September among the buds of the loosestrife flowers.

The practice of cutting flower stalks before they bloom may result in lower quantities of weevils finding stands of loosestrife to live in, so if you begin seeing many weevils in your bags of removed loosestrife flower stalks, you may want to release them into areas where it is harder to get to loosestrife or larger wetlands where flower removal is not feasible.