

Purple Loosestrife Monitoring Protocol

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Introduction:

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

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

Site Selection and Quadrat Setup:

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

We recommend using a two-piece quadrat frame composed of two open-ended “U” halves that snap together to form a square. Construct the quadrat frame from two 10' lengths of 3/4" diameter PVC or CPVC pipe, 4 right-angle elbows and 2 connectors of the same diameter, and PVC or CPVC glue. The inside dimensions of the finished frame should measure 1.0m by 1.0m. After cutting the conduit to the correct lengths, glue two elbows to each 1m long piece (make sure the elbows are perfectly aligned to each other). Then, glue each elbow to a 0.5m long piece to form two open ‘U’ shaped half-frames. Glue the connectors to the short sides of one of these half-frames. Using a permanent marker, mark 1 dm intervals on each side to assist with estimating percent cover. The frame can be filled with foam insulation to create a floating quadrat for use in flooded sites. In the field, slide one of the half-frames into position, and then attach the other to it.

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

A minimum of 5 1.0m² permanent quadrats should be established at each site, and **10** if possible (more are even better). This allows statistical analysis of the expected decline in purple loosestrife density and performance, the abundance of biocontrol insects, and the change in associated vegetation. Experience shows that control agents reduce the number of purple loosestrife stems and the number of plants per area, to as little as 5% of the original abundance. Quadrats smaller than 1 square meter in size have a high probability of losing all purple loosestrife plants, which in turn would require a change in sample unit size. To avoid this, the recommended quadrat size is 1 square meter. Quadrats should be placed at random **into the purple loosestrife infestation. ALL quadrats must contain purple loosestrife; if necessary, shift the location of the quadrat so that purple loosestrife covers at least 30% of the quadrat.** Various methods are available to randomize the quadrat placement. The easiest is a transect running through the vegetation with quadrats placed at predetermined intervals (e.g. every 5, 10, or 20 meters). This method works well in most sites and facilitates relocation of the permanent quadrats. **Start at least 5m from an edge** (road, stream, upland etc.) Record the position and numbers of the quadrats on the vegetation map on Form 1. Use GPS coordinates for easy relocation in dense vegetation.

To establish the permanent quadrats, first locate the position of each quadrat, then place the 1m² frame into the vegetation, carefully inserting the arms of the first U-shaped frame through the vegetation and as close to the ground as possible. Then, attach the second half of the frame. Avoid trampling vegetation in and near the quadrat. At each of the four corners drive a 1-2m (5-8") long plastic or galvanized steel pipe into the ground (galvanized metal electric conduit or PVC pipe are inexpensive and readily available at hardware stores). This will allow exact placement of the quadrat in future years. Write the quadrat number on each conduit with a permanent marker or other means. Allow the pipe to stick up high enough to facilitate relocation, and low enough to minimize vandalism. Quadrats can also be marked with fence poles, and flagging tape can be attached to help relocation. Be aware that too obvious markings can attract vandalism - poles are used for target practice by hunters (personal experience). We have had good success using GPS data to relocate our permanent quadrats even in dense and tall vegetation.

Data Collection

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

Instructions for Form 1: Site location, background information

Site Location:

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

Contact Person and Legal Landowner:

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

Site Characteristics:

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

Road Map:

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

Site and Vegetation Map:

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

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

Insect Release History:

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

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

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

The first counts should be made in late spring/early summer, 2-3 weeks after *Galerucella* adults appear after overwintering at your site (the average height of purple loosestrife shoots should be at least 20-30 cm). This will vary depending on latitude and local annual weather, **and therefore no specific dates are provided**. This first site visit is intended to estimate the abundance of biocontrol insects (adults of *Galerucella* and *Nanophyes* are easily spotted feeding on shoot tips, eggs and larvae of *Galerucella* can be counted). *Hylobius* adults are largely night active but you will encounter them on overcast days, early in the morning or late in the evening (no need to adjust site visits to their activity periods but record time of observation on data sheets). Begin with quadrat 1 and fill out Form 2, then move to the next quadrat. Use new data sheets each year. Monitoring is easier with two people, one to make the observations and the other to record data.

1) Before collecting data, please record in spaces provided: site name, date (year, month, day), and the names of the observers (last name, first name), as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. This information needs to be collected at each visit. It will allow for later corrections of observed insect abundances due to prevailing weather patterns. *Hylobius* adults, for example, are more likely to be observed in the morning or on overcast or rainy days. The opposite is true for the two *Galerucella* species and *Nanophyes marmoratus*.

2) The first task is to assess insect abundance using counts. The most useful approach to standardize among observers is using counts in fixed time intervals. Use a 1 minute total search time for each insect species released and for each life stage that can be observed. For example, at a site where only *Galerucella* (one or both species) was released, 3 minutes total will be spent searching for eggs, larvae, and adults (one minute for eggs, one for larvae, and one for adults). At a site where only *Hylobius* was released, 1 minute total search time will be spent looking for adults. Where *Galerucella* and *Hylobius* have both been released, 3 minutes will be spent searching for *Galerucella* and 1 minute for *Hylobius*, etc. It is recommended to do the field work with 2 people observing the sample quadrat from different sides. Total search time then has to be divided by the number of observers (i.e.; 1 minute total for all observers to search for a life stage of *Galerucella*). Do not attempt to count *Galerucella*, *Hylobius*, and *Nanophyes* at the same time, this will get too confusing; rather, look through the quadrat several times. First look for adults, which will most likely be spotted sitting on shoot tips; look for *Galerucella* eggs and larvae last. Eggs and larvae of the leaf-feeders can be found on any plant part (including the base of the stem and under leaves).

3) Now, carefully approach the quadrat and watch for adults of all three species when you slide the quadrat frame into position. *Hylobius* and *Nanophyes* adults often drop from the vegetation once you touch stems (or even as you approach the quadrat). The two *Galerucella* species can **NOT** be separated in the field, therefore, they are counted together. It is impossible to observe eggs or larvae of root feeders or of flower feeders, but it is possible to count eggs and larvae of *Galerucella*. The very first instars of the *Galerucella* species feed internally in shoot tips. You can note their presence but should not spend search time opening each tip (larvae are very small!). This may interfere with their development.

4) Next, count or estimate the number of each insect species present. As long as you are able to count the exact number of adults, eggs, or larvae please provide that number. If the allowed search time does not enable you to count all present individuals, use estimates in Chart A. For example if you were able to count 35 insects in the entire quadrat, enter 35. If you were unable to count all insects in one minute, but you had already counted 35 insects in ¼ of the quadrat, then estimate the total number of insects in the entire quadrat (35 * 4 ~ 140) and enter the Roman Numeral IV (between 100 and 500 insects present: Chart A) under *Galerucella* abundance. During the first years, you will be lucky to find more than just a few adults, if any, so be patient. Over time (months and years), insects will travel quite large distances (several miles). If you monitor field sites within the travel distance of these insects, you

need to spend time searching for insects, **even if they have not been released at your site**. We now frequently encounter insects far beyond their initial release sites.

5) Next, scan the purple loosestrife for any damage to the leaves or shoots. After insect release, look especially for the ‘shotgun’ feeding pattern of the *Galerucella* beetles. Estimate the percent leaf area of purple loosestrife removed by insect feeding integrated over the entire quadrat, using Chart B. Initially, this will be very low or non-existent. Estimating the amount of leaf area removed by insect feeding will initially be difficult because you need to scan through the vegetation, and leaves and plants will show different amounts of feeding damage, but you will get better over time. Experienced observers should introduce new personnel to the methods and to their assessments to increase the accuracy of reported results. We expect to observe large differences over time, especially following high abundance of *Galerucella* larvae and adults.

6) After you have completed the insect counts, stand near the frame, and looking straight down, estimate how much of the quadrat is covered by purple loosestrife and, independently, how much is covered by cattail (Use cover estimates in Chart B, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc). If both loosestrife and cattail are abundant, these estimates may total >100%, due to layering. That is okay, as we are interested in monitoring how much of each is present. We use cattail (*Typha* sp.) as the most common associate in wetlands across North America. If you do not have cattail, leave this category blank or substitute with the most common species at your site.

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

8) Count the number of cattail stems following the same procedure.

9) Record information about other insects using purple loosestrife, if any. With the increase in control agent abundance we might see the number of other herbivores increase, and potentially, the number of predators using an abundant food source. We would like to evaluate some of these potential changes. If you frequently observe species, take pictures and collect samples for identification. Freeze insects or store individuals in alcohol. Record whether the particular species is present, abundant, or very abundant. Outbreaks of other species on other wetland plants can be recorded on either the spring or fall sampling form.

10) Other Observations: Record any general observations or useful information; disturbances, flooding, fire, bird nests etc., for the sample quadrat or the site in general. Most of this information will be difficult to evaluate, therefore do not spend too much time on this.

11) If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat using Form 4. Use cover estimates included on the form, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc).

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

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

The second site visit should be from late August to early October to measure performance of purple loosestrife (height and reproductive effort). We do not record insect presence during this visit. Monitoring is easier with two people; one to make the observations and the other to record data.

1) Before collecting data, please record in spaces provided: site name, date (year, month, day), and the names of the observers (last name, first name), as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. This information needs to be collected at each visit. Locate permanent photo points and take photographs of the study site.

2) Slide the frame into position, as close to the ground as possible; move stems in or out of the frame so that all stems originating in the quadrat are included. Standing near the frame, estimate how much of the quadrat is covered by purple loosestrife and, independently, how much is covered by cattail (Use cover estimates in Chart B, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc). If both species are abundant, these estimates may total >100%, due to layering. That is okey, as we are interested in monitoring how much of each is present.

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

4) Count the number of cattail stems following the same procedure.

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

6) Count the number of cattail inflorescences. The number of fertile cattail stems often increases as purple loosestrife declines.

7) Select the 5 tallest purple loosestrife stems in each quadrat (if there are fewer than 5 stems/quadrat, measure all that are present); four measures will be made on each stem.

- a. Measure the stem height (to the closest cm).
- b. Count the number of inflorescences on that stem (including all side branches).
- c. Measure the length (to the closest cm) of the longest inflorescence on this stem (this will generally be the terminal inflorescence).
- d. Remove the central 5cm portion of this inflorescence. Count the number of flower buds in this 5cm length of inflorescence. If the plant did not produce any inflorescences or if they are shorter than 5cm please record this in the appropriate form. Repeat this process for the remaining 4 loosestrife stems.

Note: The attack of all biocontrol insects, but especially the flower-feeder, changes the number of flower buds producing seeds. This measurement allows us to assess the impact of these insects.

- 8) Select the five tallest cattail stems in each quadrat. Measure height of each stem (to the nearest cm) and indicate if sterile or fertile.
- 9) Record information about other insects using purple loosestrife, if any. With the increase in control agent abundance we might see the number of other herbivores increase, and potentially, the number of predators using an abundant food source. We would like to evaluate some of these potential changes. If you frequently observe species, take pictures and collect samples for identification. Freeze insects or store individuals in alcohol. Record whether the particular species is present, abundant, or very abundant. Outbreaks of other species on other wetland plants can be recorded on either the spring or fall sampling form.
- 10) Other Observations: Record any general observations or useful information; disturbances, flooding, fire, bird nests etc., for the sample quadrat or the site in general. Most of this information will be difficult to evaluate, therefore do not spend too much time on this.

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

If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat on Form 4.

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

PURPLE LOOSESTRIFE
Spring Monitoring Quick Reference (Form 2)

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

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

PURPLE LOOSESTRIFE
Fall Monitoring Quick Reference (Form 3)

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

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

