Garlic Mustard Monitoring Protocol
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Introduction:

Garlic mustard (*Alliaria petiolata*) is a biennial European herb that invades forested communities in North America, especially in the central and eastern part of the US and adjacent Canada. A biological control program targeting garlic mustard was initiated in 1997. Four weevils (*Ceutorhynchus* spp) including two stem-feeders, a seed-feeder, and a root-crown feeder, are under study, and releases of the first insects are anticipated to begin in 2004-05. The following guidelines are intended to help monitor the abundance of both garlic mustard and the biocontrol insects, and assess the long-term impact of biological control. The protocol can also be used to detect change in herbaceous vegetation relative to change in garlic mustard. For maximum information, monitoring should ideally be initiated one or more years before biocontrol organisms are released: the resultant 'pre-release' data will provide a baseline to assess 'post-release' changes. For best results, monitoring should be conducted twice a year; in June to assess garlic mustard density and seed production, and in October to assess rosette abundance and external evidence of insect feeding.

Garlic mustard is an obligate biennial and can only spread by seeds; therefore the goal of biocontrol is population reduction, achieved by reducing total seed production. Garlic mustard seeds germinate in early spring, and form a basal rosette by June. Plants remain as rosettes through the winter, and produce flower stalks the following spring, usually blooming in April - May, depending on the location and temperature regime. Seeds are produced in siliques (linear pods) 4-8 weeks later, usually in June-July. Garlic mustard seeds live $\geq$5 years in the seedbank.

The four weevils are difficult to observe directly. Larvae induce most of the damage, but because they feed inside the plant (in seeds, stems, leaves, and root crowns) they are not usually observed. Adults are small (2mm) and black, and feed on stems and petioles, leaving a 'scraping' mark. In addition, all four weevils produce a characteristic 'window pane' feeding pattern that can be easily observed on the leaves. Under heavy attack by one or more of the weevil species, garlic mustard plants become shorter and less robust, often have tip dieback, and produce fewer flowers and siliques.

Site Selection and Quadrat Setup:

Select a monitoring site that will be protected from other uses that may jeopardize your continued monitoring. It is imperative that the monitoring site be protected from all management that could damage the insects or the garlic mustard plants, in particular burning, herbicide application, and pulling of plants. We do not know how the weevils will respond to fire or flooding, and in the initial establishment phase a fire (which may burn the insects), flooding (which may drown the insects), or removal of garlic mustard plants (with the insect larvae hidden inside) could eradicate small populations. The study site should be sufficiently distant from a trail to limit vandalism.

The study site should contain a well-established garlic mustard population ($\geq$ 0.5 ha). Garlic mustard does not need to form a continuous carpet, but should be present throughout the study area every year, as rosettes and/or adult plants. To determine response of the associated groundlayer vegetation to the anticipated reduction in garlic mustard, it would be beneficial to locate the study site in an area with native vegetation. Avoid establishing plots in a site where garlic mustard has been present for <3 years, as the population should be large enough with a well-established seed bank to maintain a reliable food source for the weevils.

We recommend an open-ended quadrat frame with the fourth side removable. Construct the quadrat frame from a 10' length of 1/2" diameter PVC or CPVC pipe, 4 right-angle elbows of the same diameter, and PVC or CPVC glue. The inside dimensions of the finished frame should measure 1m by 0.5m. 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). Set one piece aside (This will be the fourth side of the frame). Glue the elbows of the other 1m long piece to two 0.5m long pieces to form the open 'u' shaped frame. Using a permanent marker, mark 1 dm intervals on each side to assist with estimating percent cover. In the field,
slide the open-ended U-shaped frame along the ground to avoid disturbing the vegetation. Then, attach the fourth side to the frame.

Materials needed: $0.5m^2$ quadrat frame, permanent marker, GPS unit (if available), 50m tape, conduit and hammer, Form 1, pencils and clipboard, camera.

We recommend a total of 20 permanent $0.5m^2$ (0.5m x 1.0m) quadrats, spaced $\geq$10 meters apart. This allows statistical analysis of the expected decline in garlic mustard, and provides sufficient locations to ensure that garlic mustard is present as adult or seedling in most quadrats each year (in general, once garlic mustard is present, it will continue to be present almost every successive year in that location, although densities may vary significantly).

Quadrats can be located in several ways: along two parallel transects, in 4 rows of 5 quadrats, or completely randomly. Relocating the quadrats is easier using parallel transects, and this method will be outlined here. Randomly establish two parallel transects, at least 100m long and $\geq$10 meters apart. Locate quadrats at fixed intervals $\geq$10 meters apart along each transect. **ALL quadrats must contain garlic mustard**; if necessary, shift the location of the quadrat so that garlic mustard covers at least 25% of the quadrat. In sites where both age classes (adults and rosettes) are present, makes sure that these age classes are represented in the 20 quadrats. Record the position and numbers of quadrats on the vegetation map on Form 1. Use GPS coordinates for easy relocation in dense vegetation. Locate permanent photo-points and take photographs of study site, including one or more quadrats.

To establish the permanent quadrats, first locate the position of each quadrat, then place the quadrat frame on the ground, and mark the four corners by driving a 30-50cm long and 1/2" diameter plastic or aluminum conduit into the ground. 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. In areas with high public use and potential vandalism, conduits should be short and difficult to see. Obvious markings can attract vandalism and 'helpful protectors' who remove the conduits. Avoid trampling vegetation in and near the quadrat.

Data Collection

Assessment of the plants and insects will occur twice each growing season. Four data forms are provided and described in detail on the following pages: Site location (Form 1); Summer monitoring (Forms 2a and 2b), and Fall monitoring (Form 3). In addition, 'Quick Reference' sheets are provided to use in the field. To assess the growth and abundance of garlic mustard, and growth of other groundlayer 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.

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 garlic mustard infestation outlined, other vegetation types, trails, creek etc. An arrow should indicate North on the map. Paste map into space provided. Once insects are available for release, indicate with Arabic numerals (corresponding to numbers under Insect Release) points of single or multiple control agent releases

Photographs of changes in vegetation over time are a powerful tool for presentations or to re-enforce quantitative data. One or several permanent photo-points should be marked in the monitoring area using flagging tape or stakes driven into the ground. The position of these photo-points should be indicated on the vegetation map, and 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 garlic mustard 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, how individuals were released, time of day and weather conditions. Code each release with an Arabic numeral and insert number at the release point on the vegetation map (see above).

Instructions for Form 2a: Garlic Mustard Biocontrol Monitoring (Summer)

Materials needed: 1 meter stick; $0.5m^2$ quadrat frame; data sheets (Form 2a and several copies of Form 2b), pencils and a clipboard, camera, permanent marker to refresh quadrat numbers.

Summer data should be recorded when garlic mustard has completed flowering and has fully formed green siliques, but before the siliques turn brown and start to disperse seed. In northern locales this is usually in mid to late June, while in southern locales this may be as early as mid May. Begin with quadrat 1 and fill out both Form 2a, and then Form 2b (if adult garlic mustard are present), then move to the next quadrat. Use new data sheets each year. Summer 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. Take photographs at permanent photo points.

2) First, slide the frame into position. Standing over the frame, and looking straight down, estimate how much of the quadrat is covered by garlic mustard and, independently, how much is covered by all other vegetation (Use cover estimates in Chart A, 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 garlic mustard and other vegetation are abundant, these estimates may total >100%, due to layering. Next, focus only on garlic mustard. If adult garlic mustard plants are uncommon or small, or if only seedlings are present, you may need to carefully move vegetation to determine how much garlic mustard is actually present in each age class. Estimate the actual percent cover (using the cover classes in Chart A) of all garlic mustard; of only adult garlic mustard; and of only seedling garlic mustard. Often, adult garlic mustard will overtop seedling garlic mustard, and their combined cover will therefore exceed the 'all garlic mustard' cover. That is okey, as we are interested in monitoring how much of each size class is present.

3) Next, scan the garlic mustard for any damage to the leaves, shoots, or siliques. After insect release, look especially for the 'window pane' feeding pattern of the biocontrol weevils. Some windowpane feeding is already present but in ---- This may originate from native species or accidental introductions. Estimate the percent leaf area of garlic mustard removed by insect feeding integrated over the entire quadrat, using Chart A. Initially, this will be very low or non-existent. After weevil populations build up you may find as much as 50% of the leaves are damaged. Next, indicate what type of damage is visible, such as leaf miners, deer browse, disease, etc., using a 'check' or '+' in the appropriate box. This may be omitted if feeding damage is very low (<1%) and not clearly discernible. Make a note if some other type of damage is present, and include a sketch or photograph of the damage.

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 *Ceutorhynchus* larvae and adults.

4) Count the number of seedlings. If seedling density is very high, count the number of seedlings in a section of the quadrat, and then use this density to estimate the total number of seedlings in the quadrat. If time does not allow counting individuals or a subset of the population, use Chart B to estimate seedling density. Estimations are never as accurate or powerful as actual counts, so count actual seedling density whenever possible.

5) Looking below all vegetation, estimate the cover of soil, wood, leaves and rock using Chart A or actual percent cover: This should total 100%. Often, sites with abundant garlic mustard have little leaf litter.

6) Measure litter depth to the closest cm in the center of each half-quadrat.

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

8) Other Observations: Record any general observations or useful information about the site; windfall, flooding, deer herbivory, insects etc. Most of this information will be difficult to evaluate, so do not spend too much time on this.

Instructions for Form 2b: Garlic Mustard Biocontrol Monitoring (Adult height and number siliques)

Use this form when adult garlic mustard are present in the quadrat. Write the quadrat number in the appropriate box at the top of the sheet. Then, beginning at one corner of the quadrat and working systematically across the quadrat, measure the height in cm, and count the number of siliques, of each garlic mustard stem. Record this information in the appropriate boxes below the quadrat number. Record each stem that originates from the ground as a separate stem, even if you suspect that some stems may originate from a single root. When a stem branches >2cm above the ground, then the branch is counted as part of the single stem. Also, look carefully for short, frequently sterile stems. These small plants are usually overlooked, but it is important to record their presence. Record every stem, using several columns if necessary, and writing the quadrat number above each column. To be counted, a stem must originate within the quadrat; if it originates under the frame, then it is not recorded.

If you see overt damage or anything unusual on a stem, you can record this in the same box, by using an asterix, or a letter, or other symbol, and defining it in the box labeled "notes". For example, if you see leaf mining on a stem 30cm tall with 7 siliques, you could record this by writing "30-7 *" on the data sheet and writing in the notes box " * = leaf mining".

It is important to measure every stem in the quadrat, even if some quadrats have numerous plants. We anticipate that under heavy insect attack garlic mustard plants will decrease in density, height, and silique production, and will also change in plant architecture and produce more small side branches. Therefore it is very critical to have accurate baseline data to compare to 'post-release' data, and accurately assess the impact of the weevils on garlic mustard.

Instructions for Form 3: Garlic Mustard Biocontrol Monitoring (Fall)

Materials needed: 1 meter stick; 0.5m^2 quadrat frame; data sheet (Form 3), pencils, clipboard.

These are similar measures to those collected in summer, except that flower stem density and height are not measured. Because only one size class (rosette) is present, the autumn monitoring takes less time than the spring monitoring, and can be conducted by one individual. Monitoring should occur about the time deciduous trees lose their leaves. Indicate in the 'notes' box whether trees have lost some, all, or none of their leaves (this helps with interpretation of leaf litter depth, and of garlic mustard percent cover, as small rosettes are often covered by new leaves and will be missed in sampling).

1) First, if insects have been released, approach the quadrat slowly and observe for weevils. Typically, only the rosette-feeder *C. scrobicollis* will be active at this time. You may see these small (2 mm) black insects near the center of a rosette.

2) Next, slide the frame into position. If insects have been released, count number of weevils observed in one minute. As long as you are able to count the exact number of weevils, please provide that number. If the allowed search time does not enable you to count all present individuals, use estimates in Chart B. Standing over the frame, and looking straight down, estimate how much of the quadrat is covered by garlic mustard and, independently, how much is covered by all other vegetation (Use cover estimates in Chart A, 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 rosettes are uncommon or small, or tall vegetation is present, you may need to carefully move vegetation to determine how much garlic mustard is actually present. If both garlic mustard and other vegetation 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) Next, scan the garlic mustard for any damage to the leaves, shoots, or siliques. After insect release, look especially for the 'window pane' feeding pattern of the biocontrol weevils. Some window pane feeding is already present but in low abundance. Autumn is when this feeding pattern is most distinct if the rootcrown feeder *C. scrobicollis* is present. Estimate the percent leaf area of garlic mustard removed by insect feeding integrated over the entire quadrat, using Chart A. Initially, this will be very low or non-existent. After weevil populations build up you may find as much as 50% of the leaves are damaged. Next, indicate what type of damage is visible, such as slugs (round holes >1 cm diameter), deer browse, disease, leaf miners, etc. using a 'check' or '+' in the appropriate box. This may be omitted if feeding damage is very low (<1%) and not clearly discernible. Make a note if some other type of damage is present, and include a sketch or photograph of the damage.

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 *Ceutorhynchus* larvae and adults

4) Count the number of rosettes. If rosette density is very high, count the number of rosettes in a section of the quadrat, and then use this density to estimate the total number of rosettes in the quadrat. If time does not allow counting individuals or a subset of the population, use Chart B to estimate rosette density. Estimations are never as accurate or powerful as actual counts, so count actual rosette density whenever possible.

5) Looking below all vegetation, estimate the cover of soil, wood, leaves and rock using Chart A. This should total 100%. Often, sites with abundant garlic mustard have little leaf litter.

6) Measure litter depth to the closest cm in the center of each half-quadrat.

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

8) Other Observations: Record any general observations or useful information about the site; windfall, flooding, deer herbivory, insects etc. Most of this information will be difficult to evaluate, so do not spend too much time on this.

Summer Monitoring Quick Reference (Forms 2A and 2B)

Materials: 1 meter stick; $0.5m^2$ quadrat frame; data sheets (Form 2A and several copies of Form 2B); pencils and clipboard, camera

1. Take photos at permanent photo points.
2. Walk to quadrat 1. Slide quadrat frame into location. Fill out Form 2A first, then Form 2B.

Form 2A:

3. Write Site name, date, and names of investigators, state, and GPS coordinates if known.
4. Estimate Vegetation Cover: Use Chart A.
    a. Estimate total vegetation cover (maximum 100%). Write "0" if no vegetation present.
    b. Estimate total garlic mustard cover. Write "0" if no garlic mustard present.
    c. Estimate cover of adult garlic mustard. Write "0" if no adult garlic mustard present.
    d. Estimate cover of seedling garlic mustard. Write "0" if no seedling garlic mustard present.
5. Look for evidence of leaf attack.
    a. Estimate percent of garlic mustard leaf area removed by insect feeding, estimated over the entire quadrat (use Chart A).
    b. Indicate type of damage visible and/or insects present in quadrat: check or write "+" for each type present.
6. Count the number of garlic mustard seedlings present in the quadrat. If too many to count, estimate density using Chart B.
7. Measure litter depth to the nearest 0.5 cm in the center of each half-quadrat.
8. Looking below all vegetation, estimate percent cover of bare soil, leaf litter, down wood, and rock. Use Chart A or visually estimate so all 4 categories add up to 100%.
9. Optional: Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Chart A or other scale.
10. If adult garlic mustard are present in the quadrat, fill out Form 2B

Form 2B:

11. Write Site name, date, and names of investigators, state, and GPS coordinates if known.
12. Write quadrat number at top of the column. Start at one end of the quadrat and for each adult garlic mustard in the quadrat, record the:
    a. Height (in cm) of stem, measured to the top of the growing point.
    b. Number of siliques (seedpods). Count only siliques that have at least one seed; do not count very small or empty siliques.
13. After completing Forms 2A and 2B for quadrat 1, proceed to quadrat 2, and repeat the process (steps 3-11, above). Continue until all quadrats have been located and recorded.

Autumn Monitoring Quick Reference (Form 3)

Materials: 1 meter stick; $0.5m^2$ quadrat frame; data sheet (Form 3); pencils and clipboard; stop watch (after insect release)

1. Write Site name, date, and names of investigators, state, and GPS coordinates if known, at the top of Form 3.
2. Walk to quadrat 1. If insects have been released:
    a. Approach the quadrat slowly and observe for weevils. Slide quadrat frame into location.
    b. Count number of weevils seen in the quadrat in one minute (use stopwatch). Record actual number of weevils seen, or use Chart B to estimate density.
3. Slide quadrat frame into location.
4. Estimate Vegetation Cover: Use Chart A.
    a. Estimate total vegetation cover (maximum 100%). Write "0" if no vegetation present.
    b. Estimate total cover of rosette garlic mustard. Write "0" if no garlic mustard present.
5. Look for evidence of leaf attack.
    a. Estimate percent of garlic mustard leaf area removed by insect feeding, estimated over the entire quadrat (use Chart A).
    b. Indicate type of damage visible and/or insects present in quadrat: check or write "+" for each type of damage or insect seen.
6. Count the number of garlic mustard rosettes present in the quadrat. If too many to count, estimate density using Chart B.
7. Measure litter depth to the nearest 0.5 cm in the center of each half-quadrat.
8. Looking below all vegetation, estimate percent cover of bare soil, leaf litter, down wood, and rock. Use Chart A or visually estimate so all 4 categories add up to 100%.
9. Optional: Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Chart A or other scale.
10. After completing Form 3 for quadrat 1, proceed to quadrat 2, and repeat the process (steps 2-9). Continue until all quadrats have been located and recorded.

FORM 1: GARLIC MUSTARD biocontrol monitoring (site location)

Site Name:_____ State:_____ GPS: N _____º_____'

Town: _____ County: _____ W _____º_____'

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

CONTACT PERSON:

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

LEGAL LANDOWNER:

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

SITE CHARACTERISTICS:

Habitat type: ___Upland forest ___Floodplain forest ___Field ___Roadside ___ Other _____

Road Map to Site

N

Site and Vegetation Map

N

INSECT RELEASE HISTORY:

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