# Mile-a-Minute Weed Monitoring Protocol Revised Feb. 2009

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### **Introduction**

Mile-a-minute weed (*Persicaria perfoliata*, formerly *Polygonum perfoliatum*) is a prickly, branching, annual Asian vine that invades a variety of habitats in the northeastern U.S., including forested floodplains, streamside herbaceous wetlands, upland meadows, forest edges, and disturbed sites. A biological control program targeting mile-a-minute weed was initiated by the Forest Service in 1996, with field surveys and laboratory host specificity tests conducted in China and subsequent testing continuing under quarantine conditions in Delaware. A stem-boring weevil, *Rhinoncomimus latipes*, has been determined to be host-specific to mile-a-minute weed and a permit application for field release was approved in July 2004. The following guidelines are intended to help monitor the abundance of both mile-a-minute weed and the weevil, and assess the long-term impact of biological control. Monitoring should ideally be initiated one or more years before biocontrol organisms are released, so that changes can be tracked pre- and post-release.

Mile-a-minute weed (MAM) seeds germinate in early spring, usually in April in the mid-Atlantic region. Vines grow rapidly, climbing over other plants, and attain lengths of 6 meters or more. Flowers are inconspicuous, and iridescent blue berry-like achenes (single-seeded fruits) are produced, usually beginning in mid-summer and continuing until the plants are killed by frost in the fall. Seeds require a cold period before germinating. Many will germinate underneath established patches the following year, while others are spread by birds, mammals, water, and inadvertently by human activities. Seeds can survive for up to 6 years in the seed bank.

Adult *R. latipes* are about 2 mm long, and are black, but may be covered by an orange film derived from plant exudates once they start feeding. Adult weevils eat small holes in young MAM leaves and lay eggs on leaves, stems, and terminals. After hatching, larvae bore into the stem where they complete development, then exit the stem and drop to the soil for pupation. Development from egg to adult takes about 26 days, and they have 3 – 4 overlapping generations during the growing season. Weevils are very small, but can be observed directly in the field, especially at the ends of terminals (Fig. 1). The pale yellow eggs are peanut shaped and covered by thin strips of fecal material (Fig. 2); however they are difficult to spot in the field due to their very small size. Characteristic adult feeding holes ("shot holes" in leaves) are relatively easy to see (Fig. 3). Larval emergence holes at plant nodes (near where ocreae encircle stems or where stems diverge) can sometimes be seen in the field (Fig. 4).



Fig. 1. Adult weevil



Fig. 2. Eggs (with penny)



Fig. 3. Adult feeding damage



Fig. 4. Larval node damage *Photos by Ellen Lake* 

The MAM monitoring protocol outlined here is designed to balance ease of implementation and collection of useful plant and insect data. Plant populations are assessed quantitatively in the spring, when individual small plants can be counted in measured quadrats, along with estimated percent cover of MAM. Counts of overwintered weevils are also done at this time, although numbers are usually low; however, weevil feeding damage is usually obvious if present. Weevil abundance, MAM percent cover, number of MAM seed clusters, and presence of other dominant plants in the community, should then be assessed once in mid-summer (late July or early August), and again in early fall (mid-September).

## **Site Selection and Quadrat Placement**

<u>Materials needed</u>: one 0.5 m by 1 m rectangular quadrat frame, 40 pieces of ½" or ¾" plastic conduit pipe ~1 m long (to mark corners of 10 quadrats), hammer, permanent marker, 50 or 100-m tape measure, GPS unit (if available), camera, work gloves, 10 bamboo poles (~6' long), colorful flagging tape

Select a weevil release/monitoring site that will be protected from other uses that may jeopardize insect establishment and continued monitoring, i.e. where the landowner is willing to make no attempt to control vegetation through mowing, herbicide use, etc., for at least 3 years. The study site should contain an ample population of MAM, but ideally native vegetation should also be present so that control of MAM will result in establishment of a more desirable plant community.

To construct quadrat frame: Using a 10' length of ½" or ¾" diameter PVC or CPVC conduit pipe and 4 right-angle elbows of the same diameter, construct a rectangular quadrat frame with inside dimensions that measure 1 m by 0.5 m. After cutting the pipe to the correct lengths, use PVC or CPVC glue to attach two elbows to each 1-m-long piece, making sure the elbows are perfectly aligned to each other. Glue the elbows to two 0.5-m-long pieces to form the rectangular frame. Using a permanent marker, mark 10-cm intervals on each side to assist in estimating percent cover and seedling numbers.

Quadrat placement: At the monitoring site, establish 10 quadrats, approximately 10 m apart, along a 100-m long transect if the MAM patch is linear, or at random within the mile-a-minute infestation, with quadrat #5 (the release point) located near the center. Make sure you have a good sketch of the location of each quadrat, including any landmarks, to help with relocation of the quadrats.

Locate the quadrats initially during the period of mile-a-minute weed germination and seedling emergence, making sure each quadrat has an ample mile-a-minute weed population. (<u>Note</u>, if other tearthumbs are present you may need to wait until plants have developed characteristic ocreae, encircling stems, before establishing quadrats [Fig. 5]).



Fig. 5. Mile-a-minute weed seedling. Arrow indicates ocrea encircling stem.

Permanently mark the position of each quadrat by placing the rectangular quadrat frame on the ground and hammering a 1-m long piece of conduit pipe into the ground at the inside of each of the four

corners. Write the quadrat number (1 through 10) on <u>each</u> corner pipe with a permanent marker. Place a bamboo pole in one corner pipe and tie brightly colored flagging tape near the top of the pole. Locate permanent photo-points and take photographs of the study site, including one or more quadrats and/or other prominent landscape features that will remain the same each year.

#### Weevil release

<u>Note</u>: Please inform our lab of any new releases as soon as they occur, since we are maintaining a data base of all North American releases. Please send the following information to <a href="mailto:jhough@udel.edu">jhough@udel.edu</a>: date of release, number released, State, County, Location (park, refuge, or municipality); description of exact site; and GPS coordinates if available. Also let us know where you obtained the weevils (e.g. Philip Alampi Laboratory, other rearing, or from an established field site). Please send copies of all monitoring forms to us at the end of the season (note that forms are available in Excel versions to facilitate data entry; e-mail me to request).

Release approximately 500 adult weevils (or more if available) within quadrat #5. Carefully document all releases, including the date, numbers released and exact site of release.

### **Spring mile-a-minute survey (Form 1)**

<u>Materials needed</u>: hand tally, ruler to measure approximate MAM stem lengths, Form 1 (**make copies as needed**), clipboard and pencils, camera, maps, GPS unit (if available), hand tally, work gloves, permanent marker, extra corner pipes, bamboo poles, and flagging tape.

Choose a date in spring (probably between April 15 and May 15) after the main flush of MAM germination is complete, but before vines have become too dense to count. Ideally, MAM stems should be approximately 15 - 30 cm (6 - 12) tall. Measure the height of an average stem (or a range of heights if there is much variation) and note it on the survey form.

<u>VERY IMPORTANT</u>: As you sample each quadrat, renew the flagging tape, replace bamboo poles and corner pipes, and renew permanent marker notation of quadrat numbers as needed. Quadrats are easy to see in the spring, but much harder to locate later in the year as vegetation grows up and around the poles.

Survey each quadrat for the following, recording results on Form 1:

- (1) Number of weevils. Carefully approach each quadrat site (since adult weevils tend to drop from plants when disturbed) and count and record all adult weevils that can be seen on plants within the approximate confines of the quadrat, delineated by the four corner poles. Weevils will generally be found on MAM terminals or foliage, often near characteristic "shot hole" feeding damage. However, numbers may be very low in the spring sample.
- (2) MAM seedlings. Slide the quadrat frame down in place around the four corner pipes, and count the total number of MAM seedlings present in the quadrat, using a tally counter for accuracy. If too many are present to count, you may mark off and count the number in ½ or ¼ of the quadrat and multiply by 2 or 4 to arrive at a reasonable estimate for the entire quadrat.

- (3) Percent cover of MAM. Standing over the frame, look straight down and estimate how much of the quadrat is covered by MAM foliage and vines.
- (4) Note presence or absence of characteristic weevil feeding damage ("shot holes"), and if present rate damage as low (holes in a few scattered leaves); medium (holes in many leaves); or high (extensive damage on most leaves).

#### Summer/Fall Assessment of Weevil Abundance, MAM % Cover, and MAM Seed Clusters

<u>Materials needed</u>: Form 2 (**make copies as needed**), clipboard and pencils, GPS unit (if available), hand tally, permanent marker, work gloves; extra corner pipes, bamboo poles, and flagging tape; detailed sketch map of quadrat locations

Once in summer (mid-July to early August), and once in early fall before frost (approx. mid-September), return to each quadrat site and survey for the following within each quadrat, recording the results on Form 2. As you visit each quadrat, replace corner pipes, re-number the pipes, and replace or add more flagging, as needed.

- (1) Number of weevils. Carefully approach each quadrat and first count and record all adult weevils that can be seen within the approximate confines of the quadrat.
- (2) Percent cover of MAM. Slide the quadrat frame down in place around the four corner pipes. Standing over the frame, look straight down and estimate how much of the quadrat is covered by green (not senescent) MAM foliage and vines.
- (3) Note presence or absence of characteristic weevil feeding damage ("shot holes"), and if present rate damage as low (holes in a few scattered leaves); medium (holes in many leaves); or high (extensive damage on most leaves).
- (4) Seed clusters. Count the number of mature (containing at least one blue seed) and immature (all green seeds, at least some near full-size) MAM seed clusters within the confines of the quadrat.
- (5) Other vegetation. Note what other plants are dominant in each quadrat, and at the site overall.

<u>Note</u>: if you cannot find a particular quadrat, simply mark "not found" on the form, and continue to sample as many quadrats as can be found. Most likely the quadrat will reappear the following spring, when it should be marked with additional flagging tape on surrounding vegetation and with careful notation on the quadrat sketch map as to surrounding landmarks.

If a quadrat has no MAM present, mark it as 0% cover, note the dominant plants that <u>are</u> present, and move to the next. However, be sure to return the following spring, since MAM seedlings may well be present again from the seed bank.

# Form 1: Spring mile-a-minute weed survey

(To be conducted after flush of MAM germination but before vines become too dense, when stems are  $\sim 15 - 30$  cm (6 – 12"); approx. May 15 in DE)

Approx. length of MAM stems:  Person(s) conducting sample:	Site:	<u> </u>	
Person(s) conducting sample:	Approx. length of MAM stems:		
Contract Con	Person(s) conducting sample:		

Quad #	Number of weevils (count first)	Number of MAM stems originating in quadrat	% MAM cover in quadrat	Weevil feeding damage (none, low, med., or high)*
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

<sup>\*</sup>low = holes in a few scattered leaves; medium = holes in many leaves; high = extensive damage on most leaves.

**COMMENTS:** 

Form 2: Summer/Fall Weevil and Plant Monitoring (To be conducted once in mid-July to early August and once in mid-September)								
	-			1	,			
conducting s	sample:							
Number of % MAM weevils cover in	cover in	Weevil feeding damage	Number of seed clusters		Other dominant vegetation			
first)	quadrat	med., or	Mature** Immature**					
		,						
e seeds have at	least one blue	seed; immatur	re have all gree	en seeds, at leas				
	Number of weevils (count first)	Number of weevils (count first)  noles in a few scattered leave e seeds have at least one blue	Number of weevils cover in quadrat (none, low, med., or high)*  moles in a few scattered leaves; medium = holes e seeds have at least one blue seed; immature occording to conducting sample:  Weevil feeding damage (none, low, med., or high)*	Number of weevils (count first)  Number of high)*  Number of weevils (amage (none, low, med., or high)*  Number of weevils (amage) (none, low, med., or high)*  Number of weevils (amage (none, low, med., or high)*  Number of Mature**	nducted once in mid-July to early August and once in mid-September of conducting sample:    Number of			