Site Evaluation Monitoring Protocol for Common Biocontrol Systems in Montana

OVERVIEW

A critical part of successful weed biological control programs involves evaluating the current state of biocontrol agent populations at release sites. Biocontrol site evaluations are a quick way to inform management decisions and help answer the most common questions – **Are biocontrol agents established at this site? Is the biocontrol agent collectible? Are release practices working?** This protocol has been created to assess current biocontrol populations at historic release sites or sites where biocontrol agent establishment is unknown. Site evaluations are appropriate for the most common established biocontrol systems in Montana – yellow toadflax (*Linaria vulgaris*), Dalmatian toadflax (*Linaria dalmatica*), leafy spurge (*Euphorbia virgata*), and spotted knapweed (*Centaurea stoebe*). Below is the list of biocontrol agent species and associated target weeds that can be evaluated using predetermined thresholds, which is the minimum limit that indicates a biocontrol agent is collectible at a site.

Yellow Toadflax (*Linaria vulgaris*):

Yellow toadflax is an herbaceous perennial that can grow $^1/_3$ to 1 m (1 to 3 ft) tall with numerous stems emerging from a taproot and creeping lateral roots. Leaves are pale green, narrow, hairless, pointed at both ends, and have a large central vein on the underside. Flowers are tightly clustered near the top of the stem and are typically pale yellow with orange throats. The flowers look similar to snapdragons with an obvious upper and lower lip and a spur pointing downward. Each flower produces an oval capsule fruit holding 10–40 viable, flat, disc-shaped seeds. It is usually associated with agriculture fields, rangeland, roadsides, clearings, and clearcuts.

Yellow Toadflax Stem Weevil (*Mecinus janthinus*):

Adult weevils are 2.4–3.4 mm long, bluish-black in color, with elongated bodies and snouts. Adults emerge from stems in early spring and deposit eggs within new growth stems throughout spring. Larvae feed exclusively on tissues inside of the same stem and the damage reduces water/nutrient transport and causes stems to be deformed. New adult weevils overwinter in the same stem until the following spring. When present in high numbers, adult feeding damages growing tips of stems, reducing flowering and seed production. Dalmatian and yellow toadflax weevils look nearly identical. In general, the two *Mecinus* species are differentiated based on the toadflax they are collected from.

Dalmatian Toadflax (*Linaria dalmatica*):
Dalmatian toadflax is a perennial that grows between $^1/_3$ to 2 m (1 to 4 ft) tall. The waxy green leaves are heart shaped and clasp the stem. Flowers are 25 mm (1 in) long (excluding the spur), yellow, often tinged with orange or red, and similar in shape to a snapdragon. Plants flower from midsummer to fall. Seeds are produced in a pod, and a single plant may produce up to 500,000 seeds in a season that may remain viable in the soil for up to 10 years. This plant also reproduces vegetatively by stems that develop from adventitious buds on primary and creeping lateral roots. It is usually associated with sparsely vegetated or disturbed areas such as roadsides, disturbed hillsides, abandoned or unmanaged land, gravel pits, pastures, and rangelands.

Dalmatian Toadflax Stem Weevil (*Mecinus janthiniformis*): Adult weevils are 3–6 mm long, bluish-black in color, with elongated bodies and snouts. Adults feed on toadflax shoots, stems, and leaves, stunting shoots and roots, and reducing flowering and seed production. Adults lay eggs along the stem and larval feeding within the stem damages tissue and reduces water/nutrient transport which may kill some stems. Larval stem mining may also cause upper portions of the stem to become deformed. Adults emerge from stems in late spring through early summer. Dalmatian and yellow toadflax weevils look nearly identical. In general, the two *Mecinus* species are differentiated based on the toadflax they are collected from.

Leafy Spurge (*Eurphorbia virgata*):
Leafy spurge is an invasive, herbaceous perennial with an extensive creeping root system and deep taproot ranging from 3 to 7 m (9 to 21 ft) in depth. Leafy spurge flowers are minute, bright green to yellow in color, and enclosed by showy green bracts. The stem and leaves of this plant produce a white, sticky sap. Leafy spurge spreads by ejecting seeds and creeping lateral roots. A single plant can produce over 100 seeds, and they may remain viable in the soil for up to 15 years. Leafy spurge belongs to the *Euphorbia* family and may be confused with warty spurge (*E. spathulata*), which is native to Montana.

Leafy Spurge Flea Beetle (*Aphthona* spp.): There are multiple leafy spurge flea beetle species in Montana. The two most common are the brown-legged leafy spurge flea beetle (*A. lacertosa*) and the black dot leafy spurge flea beetle (*A. nigriscutis*). All adult flea beetles are generally 3 mm long, but some species are orangish-brown (*A. nigriscutis*) while others are black with brown legs (*A. lacertosa*). Multiple species may occur at one infestation but they not cross-breed. Adults emerge from the soil and are actively feeding on leafy spurge leaves and flowers

from early to mid-summer. Adult flea beetles lay eggs in the soil and the larvae burrow into roots to feed on root hairs and young roots, which inhibits root function and stunts spurge stem growth. Larval feeding also makes plants more susceptible to soil-based pathogens.

Leafy Spurge Red-headed Borer Beetle (*Oberea erythrocephala*): Adults are slender and 10–12 mm long with long, dark antennae and red heads. Adult bodies are dark with reddish-brown legs. Adults emerge in early to mid-summer and feed on leafy spurge leaves and flowers. Females girdle a leafy spurge stem, chew a hole, and deposit an egg. Hatching larvae mine down the stem until reaching the root crown and nearby lateral roots where they overwinter and pupate in spring. Adults chew through remaining plant tissue and emerge from the soil.

Spotted Knapweed (*Centaurea stoebe*):
Spotted knapweed is an herbaceous, short-lived, perennial that reproduces entirely by seed. Plants can produce up to 25,000 seeds that can remain viable in the soil for up to eight years. Flowers range in color from pink to light-purple and bloom from July to October. The flower head bracts have black tips, resulting in the characteristic "spotted" appearance. Seeds are brown to black in color, smooth, and less than 7 mm ($^1/_4$ in) long. Stems are typically $^1/_3$ to 2 m (1 to 4 ft) tall with lower leaves that are deeply lobed and upper leaves that are more linear. Spotted knapweed prefers moist rangeland habitats, but is common in waste areas, along roadsides, and in pastures.

Spotted Knapweed Root Weevil (*Cyphocleonus achates*): Adult weevils are 13–15 mm long with short, thick snouts and brownish-gray spotting. Adults emerge in late summer through early fall, where they feed on rosettes and lay eggs in notches on the root crown just below the soil surface. Larvae hatch in 10-12 days and mine toward the center of the roots where they overwinter until the following summer. Most damage is done to the plant when multiple larvae occupy a root, which reduces plant biomass and may kill some plants. Larval tunneling in the root also exposes the plant to bacterial and fungal infection that can cause secondary injury.

Spotted Knapweed Flower Weevil (*Larinus* spp.): There are two species of *Larinus* in Montana and they look nearly identical. Both adult knapweed flower weevils are a mottled dark brown color with a large bent snout. Adult *L. minutus* is typically 4–5 mm

long, and *L. obtusus* is typically 5–7 mm long. Overwintering adults emerge throughout summer from dried knapweed seed heads, leaving a visible exit hole in old seed heads. Larval feeding destroys a large portion of developing seeds which reduces the rate of knapweed spread. Adult weevils present in large numbers can cause severe feeding damage to stem and foliage, which can stunt or even kill some plants.

SUPPLIES

- Biocontrol Site Evaluation Worksheet
- Canvas sweep net
- Stopwatch
- Pencil
- Clipboard
- GPS/mapping tool for coordinates
- Camera (e.g., phone with camera)
- Clicker counter
- Tray (spotted knapweed only)

SITE EVALUATION PROTOCOL

Site Selection

In general, sites appropriate for evaluation must be accessible during the evaluation season (typically late spring to late summer) and fit into one of the following categories:

A. Sites where biocontrol releases have been made in the past
    a. Agents were released five or more years ago
    b. Agents were released on yellow toadflax, Dalmatian toadflax, leafy spurge, or spotted knapweed
B. Sites where you suspect biocontrol releases have been made in the past but cannot confirm
C. Sites where you are considering utilizing biocontrol and want to evaluate the current state of biocontrol agents, or find out if biocontrol agents have naturally established

Evaluation Timing

To ensure accurate assessment of biocontrol agent populations, evaluations should be conducted during the appropriate season and under appropriate conditions. **Evaluations should only occur when 1) it is not raining, 2) wind speed is no greater than the average for the area, and 3) the previous day didn't receive heavy rain and wasn't unseasonably cold.** The season and conditions vary depending on the biocontrol agent(s) being evaluated. The specific guidelines for each biocontrol agent are as follows:

Biocontrol Agent	Time of Year	Temperature
Yellow toadflax stem weevils (*Mecinus janthinus*)	May	>65°F
Dalmatian toadflax stem weevils (*Mecinus janthiniformis*)	mid-May to mid-June	>65°F
Leafy spurge flea beetles (*Aphthona* spp.)	July	>80°F
Leafy spurge borer beetles (*Oberea erythrocephala*)	mid-June to mid-July	>80°F
Spotted knapweed flower weevil (*Larinus* spp.)	mid- to late-July	>80°F
Spotted knapweed root weevil (*Cyphocleonus achates*)	August	>80°F

Data Collection (Biocontrol Site Evaluation Worksheet)

1. Fill in General Site Information including the site name, date, county, previously monitored (Yes/No), site coordinates, and names of monitors
2. Fill in Site/Monitoring Summary
    a. Time of monitoring
        i. Date and time are meant to capture the monitoring window to ensure the biocontrol agent populations are being assessed at the most optimal time
    b. Target weed being monitored
    c. Biocontrol agents being monitored
        i. Yellow toadflax biocontrol agent
            1. Yellow Toadflax Stem Weevil (*Mecinus janthinus*)
        ii. Dalmatian toadflax biocontrol agent
            1. Dalmatian Toadflax Stem Weevil (*Mecinus janthiniformis*)
        iii. Leafy spurge biocontrol agents
            1. Leafy Spurge Flea Beetles (*Aphthona* spp.)
            2. Leafy Spurge Borer Beetles (*Oberea erythrocephala*)
        iv. Spotted knapweed biocontrol agents
            1. Knapweed Flower Weevil (*Larinus* spp.)
            2. Knapweed Root Weevil (*Cyphocleonus achates*)
    d. Size of weed infestation
        i. Map or estimate size of target weed infestation in acres
    e. Density of target weed infestation
        i. Estimate density by choosing low (1–25% cover), moderate (25–50% cover), or high (50–100%)

ii. Refer to Figures 1A, 1B, and 1C in Appendix for examples

f. Anything to note about site
    i. This is meant to briefly capture site characteristics including but not limited to site description, land usage, other invasive weed species present (in addition to target weed), plant community, notable changes in site characteristics, anything unusual

g. Yesterday's weather conditions
    i. This is meant to capture weather trends and to ensure that the previous day's weather is not impacting site evaluation results (site evaluations conducted when the previous day's weather conditions were unseasonably cold and/or rainy will not be accurate)
        1. High temperature
        2. Low temperature
        3. Precipitation

h. Current weather conditions at the time of evaluation
    i. Temperature
    ii. Wind speed
    iii. Cloud cover
        1. Circle "sunny", "cloudy", "partly cloudy", "rainy", or "other"
            a. If choosing "other", specify conditions

3. Count biocontrol agents and record data in table(s) on datasheet

a. Conduct three-minute timed counts to evaluate either **yellow toadflax stem weevils** (*Mecinus janthinus*) or **Dalmatian toadflax stem weevils** (*Mecinus janthiniformis*)
    i. Set a timer for three minutes and visually count without collecting the number of stem weevils seen on plants
        1. Continuously move through the infestation while counting during the three-minute sample
        2. Insects may be tucked into crevices, such as where the leaf meets the stem
    ii. Record the total number of stem weevils counted during the three-minute sample on one table under Count Site #1
        1. Note the Biocontrol Species on the table
    iii. Move to an adjacent area of infestation that has not yet been sampled
        1. This ensures that biocontrol agents are not counted more than once
    iv. Repeat steps until you have completed six samples and filled one table

b. Conduct sweep net samples simultaneously for **leafy spurge biocontrol agents** (i.e., flea beetles (*Aphthona* spp.), borer beetles (*Oberea erythrocephala*)) or for **spotted knapweed biocontrol agents** (flower weevils (*Larinus* spp.), root weevils (*Cyphocleonus achates*))
    i. Sweep a canvas net across target weed plants in the infestation
        1. A sweep is defined as one, 180 arc in one direction in front of your body
        2. One sample consists of 10 sweeps

3. Aim the sweep net so that the opening hits the middle and the top of the target weed
4. Continuously move through the infestation after each sweep to ensure that biocontrol agents haven't been knocked off the plant from previous sweeps
5. Use enough force to knock insects off the plant and into the canvas net but take care to not break plants (flowerheads and debris will likely come off the plant and that is normal)
6. After completing 10 sweeps, hold the canvas sweep net closed to keep biocontrol agents from escaping

ii. Count the number of biocontrol agents in the canvas net

Timed Samples (species)	Sweep Net Samples (species)
Yellow toadflax stem weevil	Leafy spurge flea beetle
Dalmatian toadflax stem weevil	Leafy spurge borer beetle
	Spotted knapweed flower weevil
	Spotted knapweed root weevil

1. If monitoring leafy spurge biocontrol agents:
   a. Open the sweep net and immediately count the number of leafy spurge borer beetles (*Oberea erythrocephala*) individually as they fly out of the net
   b. Count the remaining flea beetles (*Aphthona* spp.) by counting individually or estimating as the flea beetles climb up the sides of the sweep net
      i. Refer to Figures 2 in the Appendix for example
2. If monitoring spotted knapweed biocontrol agents:
   a. Open the sweep net and dump into a tray
   b. Sift through the debris and immediately count flower weevils (*Larinus* spp.) individually before they fly away
   c. Sift through debris and count the remaining root weevils (*Cyphocleonus achates*), they do not fly away

iii. Record the total number of each biocontrol agent on separate tables under Count Site #1
   1. Note the Biocontrol Species on each table

iv. Move to an adjacent area of infestation that has not yet been sampled to ensure that biocontrol agents are not counted more than once

v. Repeat steps until you have completed six samples and filled one table

4. After six samples are collected, an average is calculated

10 sweeps repeated 6 times (for AP, OBER, CYAC, LA) OR a 3-minute timed count repeated 6 times (for MEJA)	
Biocontrol Species: **Flea Beetles (AP)**	
Count site	Insect count
1	**270**
2	**58**
3	**145**
4	**115**
5	**60**
6	**220**
Average:	**144.6**

10 sweeps repeated 6 times (for AP, OBER, CYAC, LA) OR a 3-minute timed count repeated 6 times (for MEJA)	
Biocontrol Species: **Borer Beetles (OBER)**	
Count site	Insect count
1	**2**
2	**5**
3	**9**
4	**1**
5	**1**
6	**2**
Average:	**3.3**

5. Compare the calculated average to the appropriate biocontrol agent collection thresholds on the back of the worksheet

Biocontrol Agent Site Saturation Densities/Collection Thresholds:

a) Aphthona spp. sweeps (10 sweeps x 6 times): average 100 beetles/10 sweeps
b) Oberea sweeps (10 sweeps x 6 times): average 5 beetles/10 sweeps
c) Cyphocleonus sweeps (10 sweeps x 6): average 4 weevils/10 sweeps
d) Larinus spp. sweeps (10 sweeps x 6): average 20 weevils/10 sweeps
e) Mecinus spp. using timed counts (3-minute x 6): average 20 beetles in 3 minutes

APPENDIX

Infestation Density Example Images

Target weed infestation densities are estimated based on the overall average infestation density. Infestations may be comprised of low, moderate, and high-density areas.

Figure 1A

Low-density Leafy Spurge (0-25% cover)

Figure 1B

Moderate-density Leafy Spurge (25-50% cover)

Figure 1C

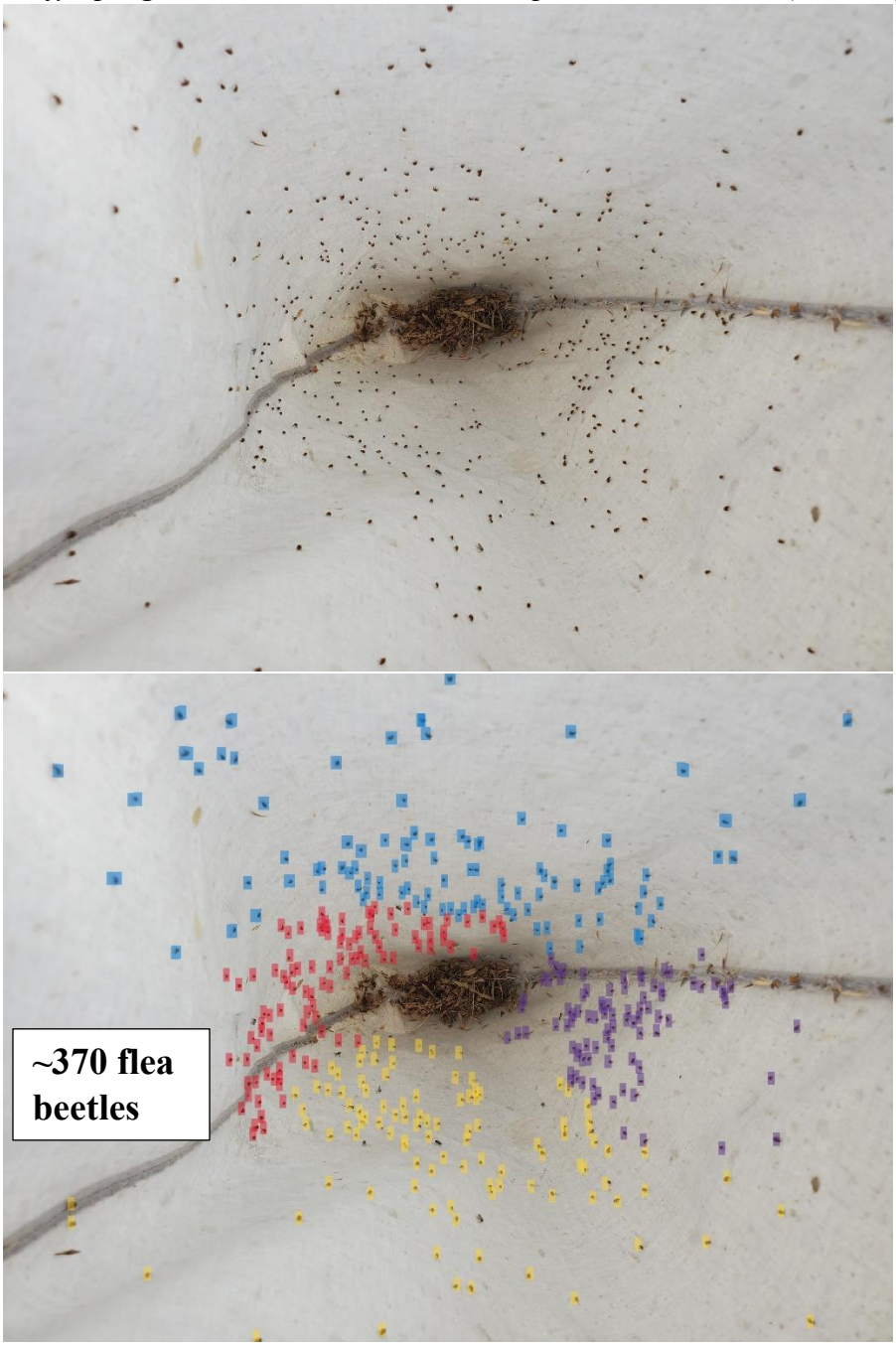
High-density Leafy Spurge (50-100% cover)

Leafy Spurge Flea Beetle Estimation Example

Flea beetles are typically estimated when numbers exceed 100 in one single sample (10 sweeps). It is best to estimate flea beetles after they have all started climbing up the net and then counting by 5s or 10s, and/or taking a picture.

Figure 2

Leafy Spurge Flea Beetle Evaluation Sample – Count Site #1 (10 sweeps)



Note. The figure shows the same sample of leafy spurge flea beetles. Each color signifies approximately 100 flea beetles.

Biocontrol Site Evaluation Worksheet

REQUIRED Check off:

- ☐ Pictures
  - ☐ site overview
  - ☐ representative weed infestation density
  - ☐ anything of interest
- ☐ Coordinates
- ☐ Weed infestation polygon/acreage estimate
- ☐ Sweep net or timed counts

GENERAL SITE INFORMATION

Site Name: _____

Date Monitored: _____ **County:** _____

Previously Monitored: YES or NO

Site Location (Coordinates): _____

Monitor(s): _____

SITE/MONITORING SUMMARY

Time of Monitoring: _____ **Target Weed Being Monitored:** _____

Biocontrol Agent(s) Being Monitored: _____

Size in Acres of Weed Infestation: _____ **Density of Weed Infestation:** _____

Anything to Note About the Site: _____

_____

Yesterday's Weather Conditions (high/low temp, rain): _____

Current Temperature: _____ **Current Wind Speed:** _____

Please Circle One:

Sunny	Cloudy	Partly Cloudy	Rainy	Other_____

10 sweeps repeated 6 times (for AP, OBER, CYAC, LA) OR a 3-minute timed count repeated 6 times (for MEJA)	
Biocontrol Species:	
Count site	Insect count
1	
2	
3	
4	
5	
6	
Average:	

10 sweeps repeated 6 times (for AP, OBER, CYAC, LA) OR a 3-minute timed count repeated 6 times (for MEJA)	
Biocontrol Species:	
Count site	Insect count
1	
2	
3	
4	
5	
6	
Average:	

Field Notes & Map (if needed):

Overview Picture Taken? YES or NO

Direction of Overview Photo: _____

Description of Other Photos Included (anything of interest):

Biocontrol Agent Codes:

AP: Aphthona spp. (Leafy Spurge Flea Beetle)
OBER: Oberea erythrocephala (Leafy Spurge Red-headed Borer Beetle)
CYAC: Cyphocleonus achates (Knapweed Root Weevil)
LA: Larinus spp. (Knapweed Flower Weevil)
MEJA: Mecinus janthinus (Yellow Toadflax Weevil) OR *Mecinus janthiniformis* (Dalmatian Toadflax Weevil)

Biocontrol Agent Site Saturation Densities/Collection Thresholds:

a) Aphthona spp. sweeps (10 sweeps x 6 times): average 100 beetles/10 sweeps
b) Oberea sweeps (10 sweeps x 6 times): average 5 beetles/10 sweeps
c) Cyphocleonus sweeps (10 sweeps x 6): average 4 weevils/10 sweeps
d) Larinus spp. sweeps (10 sweeps x 6): average 20 weevils/10 sweeps
e) Mecinus spp. using timed counts (3-minute x 6): average 20 beetles in 3 minutes