

Biocontrol Agent Handling Techniques

For collecting, shipping and releasing in B.C.

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Introduction

There are many invasive plant species occupying lands in British Columbia. Where possible, the province has introduced biological control (or biocontrol) agents for long-term, cost-effective control of individual invasive plant species. A variety of equipment and methods are required to handle biocontrol agents for the intent of managing invasive plant species. The purpose of this document is to provide information that may be useful when collecting, shipping and releasing approved biocontrol agents for general distribution in B.C. Included within are common and specific details for handling biocontrol agents. This document is particularly useful when used in conjunction with the BC FLNRO Biocontrol Agent Lifecycle Schedule located under the Documents and Outreach materials tab of the BC FLNRO Invasive Plant Program web site at <https://www.for.gov.bc.ca/hra/Plants/publications.htm>.

General Collecting Information

Biocontrol Agent Species Suitability

All biocontrol agents.

Collection Containers

The type and size of container depends on how the agent is to be handled, the agent's size, their feeding habits, and the shipping time.

Types of Collection Containers

- Ventilated 1-litre plastic containers (Figure 1)
- Large collection containers (>1 litre)
- Paper bags

Ventilated 1-liter plastic containers:

- Collection container sizes may vary; however, the most common is a 1-liter plastic semi-transparent container that has been fitted with a piece of mesh for ventilation.
- For most biocontrol agents, the maximum number kept in 1-liter collection containers should not exceed 200, however, exceptions do occur. If large quantities are collected, larger containers should also be used. Care must be taken to not overcrowd the biocontrol agents.
- The mesh used needs to be appropriate to the biocontrol agent collected. Some beetles and weevils, in particular *Cyphocleonus achates* and *Larinus* species, are capable chewers, therefore, a light-weight fibreglass mesh is recommended when shipping these types of agents to prevent them from chewing through the mesh and escaping the containers. Moths and flea-beetles can be held in containers with a light weight mesh. The holes of the venting mesh need to be small enough to prevent the agents from escaping.



Figure 1. 1-liter plastic collection container and ventilated lid

Large collection containers (>1 litre):

- Clean, re-cycled 4-liter ice-cream containers (or similar) can also be used. These are usually used to collect large quantities of biocontrol agents.
- The size of the biocontrol agent will determine the number that can be stored in the larger containers. For example, a 4-liter container could hold up to 50 *C. achates* adults. Smaller biocontrol agents such as *Mecinus janthiniformis*, *Rhinusa antirrhini* or *Longitarsus jacobaeae* could be stored in quantities up to a few hundred. Care must be taken to not overcrowd the biocontrol agents.
- Large containers are also used when foliar feeding larvae are collected such as *Tyria jacobaeae*. Foliar feeding larvae consume large amounts of food and can quickly foul containers with their feces and moults.
- It is important to provide the agents with materials (such as firm stems from their host plant – see below in the Add Plant Material to the Collection Containers section) to climb upon to decrease their contact with one another, their feces, and therefore, their stress.
- As with the 1-liter containers, appropriate ventilation is required.

Paper bags:

- Sturdy paper bags can be used to collect biocontrol infested plant material for transfer. Ensure the plant material will fit well into the selected bag with the top securely folded down and/or taped or stapled closed.

Containers made from other materials:

- Light weight cardboard or styrofoam shipping containers, similar to take-out food or ice-cream containers can be used for shipping. These are normally used when shipping a small number of biocontrol agents in a short time span.

Add Plant Material to the Collection Containers

- Clip and fill the collection container with sufficient host plant material for the biocontrol agents to cling to, to feed upon and to prevent them from crawling over each other, fighting and injuring one another.
- The plant material should not encourage oviposition. For example, clip the early floral buds to avert seedhead-feeding biocontrol agents from ovipositing. It is difficult to stop oviposition on leaves when they are required both for oviposition and for food. In this circumstance the egg

laying can be kept to a minimum if the biocontrol agent is collected before the main oviposition activity begins or if a different material is used – see below, this section. It is necessary to store the biocontrol agent in the collection container for as short of time as possible.

- The plant material should not encourage unwanted pest transfers such as generalist feeders, spiders or parasites. For example, seedhead pests can be prevented by removing all the seedheads. Even competing biocontrol agents may be considered as pests if the intention is to redistribute only the single agent. For example, it may be difficult to transfer *Urophora spp.* infested seedheads without some *Metzneria paucipunctella* also present, and vice-versa.
- Plants need to be free of flower heads and seeds to prevent the unintentional spread of seeds.
- Most adult moths do not feed on plant material, but, the vegetation helps create a cooling environment (through transpiration) and provides small amounts of moisture. If the plant material is lush too much moisture can be created and harm the moths. Moths can also be transported with non-plant materials – see below, this section.
- When the duration of shipping times are short, lightly crumpled paper towelling can be used in place of plant material. This method is used only when the biocontrol agent does not feed on plant material (biocontrol moths) or can survive without food, therefore, it is not recommended for larvae. This method could be used for small weevils such as *Rhinusa spp.* when they are collected, shipped, and released within 24 - 36 hours.

Preparing containers with Dalmatian toadflax:

- Dalmatian toadflax plants are prone to heavy transpiration; therefore, it is recommended that the collection containers are prepared slightly differently for shipping.
- Line the bottom of each large container with paper or shop towelling or form a 'vase' inside the 1 – liter containers. Add sufficient plant material to the container or within the 'vase'. Using a vase shape for the paper towelling is limited to adult beetles and weevils. It is not suitable for adult moths because they can become trapped between the towelling and the container wall. It will also interfere with the movement of active feeding larvae.
- Add the pest and debris-free, counted biocontrol agents and cover with a ventilated lid.

General Collection Method Considerations

- Individual biocontrol agent species are handled during different times in their life cycles for different purposes. See the BC FLNRO Biocontrol Agent Lifecycle Schedule located on the Publications and Downloads page of the BC FLNRO Invasive Plant Program web site for these times and purposes.
- Some site characteristics can affect the timing of biocontrol agent life cycles, for example, latitude, elevation, or aspect. Several preliminary site visits may be required to determine when to start collecting. There may be some fluctuation in the start and end collecting dates each year as some agents will adjust their lifecycle according to seasonal climatic conditions.
- Collections are usually done when plants are dry. Accumulated moisture can damage the wings on flies and moths and the wing covers on beetles, weevils and flea beetles if they become stuck to the sides of the container. Pooling water can also increase the risk of drowning.

- If *M. janthiniformis* is collected from wet plants, excess moisture can be removed from the collection container by transferring adults onto paper towelling. By gently lifting and lowering opposite ends of the towelling (rolling the weevils), the moisture is removed and the adults can be placed in a dry container.
- While collecting in the field, keep biocontrol agents cool by placing the containers in a cooler or at the very least, in a well shaded location. In very hot conditions, keep containers out of direct sunlight while collecting by shielding the container with your body as much as possible. During high temperatures, plant respiration and thereafter condensation may also occur and, therefore, should be monitored to prevent biocontrol agent losses. Excess condensation will have the same damaging effects as collecting from wet plants.
- The majority of biocontrol agents are more abundant during the warmest and brightest part of the day. As well, heat trapped under cloud cover, which is often still quite bright, can affect the biocontrol agents the same as direct bright light. An exception to this rule is *M. janthiniformis* adults, which can be found during all types of weather.
- There are some agents that are nocturnal or more abundant during evenings or mornings and their handling should occur during these times. For example, *Sphenoptera jugoslavica* adults are more abundant on warm evenings and *Botanophila seneciella* adults are best found in the early morning when the temperatures are still quite cool.
- In most collection situations the biocontrol agents are usually found on above-ground plant parts and can be found concentrating at certain locations on the plant depending on their individual life cycle position. For example, *L. planus* seed feeding adults can often be found on or near the seedheads when egg laying is occurring. *Aphthona* spp. will also congregate on the upper plant parts during peak mating periods and intermittently move to the soil to deposit their eggs. *T. jacobaeae* larvae concentrate on the upper half of bolting plants whereas the adults tend to seek shelter on lower plant parts including rosettes.
- Gloves should be used when working with plants with spines or thorns, such as gorse, thistles, and teasel.
- Gloves are also recommended when hand picking some agents (larva) such as *T. jacobaeae* or *C. lunula* that are sensitive to, and may be affected by, oils found on human hands.
- Predatory pests such as spiders and ants should be removed as quickly as possible when they become mixed in the field collection container.
- Pests and debris such as plant seeds are often accidentally collected and must be removed before shipping. A second sorting is recommended when excess debris, undesired pests, or multiple biocontrol agents are accidentally collected. Sorting and cleaning can be done in the field when working with biocontrol agents that are less active or prone to escape. Biocontrol agents that are more active, such as weevil adults, can be best sorted in cooler conditions indoors. After field collecting, the filled containers can be placed for a short time in a refrigerator to 'cool' the agents. Ensure the temperature of the refrigerator is not below 4 °C as this will harm or even kill the agents. By removing and handling the containers one at a time, the chilled agents can then be recounted and separated from any contaminants.
- Some biocontrol agents can be collected and released at different stages in their life cycle by using different handling techniques. For example, *M. janthiniformis* can be collected by aspirating or tipping the adult form in the spring and early summer. Larvae and pupae can also be collected by clipping infested stems in early to late fall or early spring. Spring collections must be carried out before the adults have emerged from the stems.

- Some biocontrol agents can be found on the plants with overlapping generations or be present in more than one life cycle stage. For example *Galerucella spp.* can occur as an adult, larva, or egg during late spring or early summer. The different stages can be collected for redistribution, but, each stage needs to be stored separately to prevent the possibility of damaging the other stage during their confinement in collection containers. If one biocontrol agent is collected in more than one life cycle stage, all the stages and the quantities of each stage should be recorded.
- Usually when adults are found at the same time as well developed larvae, the adults should not be collected because they may be 'spent adults' that have already completed their oviposition period, i.e. the females would have dispensed all their eggs.
- Eggs are usually not collected for release because they typically require an egg-to-plant transfer procedure that entails specialized skills and equipment.
- When multiple biocontrol agent species occur on a single site, often they can be collected at the same time. In most cases, individual sorting and counting is done prior to release for biocontrol agents that can easily be distinguished from others. Ensure to record the number of each species separately to prevent inaccurate records and subsequent monitoring results that may prove difficult to interpret.
 - Biocontrol agents that occur on the same plants and at the same time and generally require additional sorting are:
 - *L. planus* and *Rhinocyllus conicus*; and
 - *Brachyterolus pulicarius*, *M. janthinus*, *R. antirrhini*, and *R. neta*
- Some biocontrol agent species are very difficult to distinguish from one another; some require a microscope while others may require DNA testing. Due to the difficulty of separating some species, these are collected and released in mixed populations. Record the population as mixed for future reference. Once the biocontrol agents are released, the individual species will self-disperse and seek their preferred niche habitat.
 - Biocontrol agents that occur on the same plants at the same time and generally do not require additional sorting are:
 - *A. cyparissiae* and *A. nigriscutis*;
 - *Chrysolina hyperici* and *C. quadrigemina*;
 - *L. minutus* and *L. obtusus*;
 - *G. californiensis* and *G. pusilla*; and
 - *U. affinis* and *U. quadrifasciata*.
 - In the adult form, *U. affinis* and *U. quadrifasciata* can easily be identified. Therefore, when collecting *Urophora spp.* in the larvae or pupae stages in seedheads, it is recommended to make a collection of seedheads to be taken back to the office and allow the agents to emerge on their own. This activity will provide an estimate of the proportionate quantity of each species being released. A general guide is to collect about 100 seedheads for this activity.
- Although the best practice is to release the biocontrol agents as soon as possible, it may be necessary at times to store the biocontrol agents before shipping or releasing.
 - Some species of biocontrol agents can be kept for a short time (see below, this section) in a cold environment such as a fridge. To decrease the potential mortality rate,

- gradually cool down and warm up the biocontrol agents when transferring them to and from storage and field temperatures.
- During the stored period, biocontrol agents may be kept in smaller quantities within their holding containers and then just before shipping larger numbers of the agents may be included inside containers.
 - A few extra biocontrol agents are usually added at the time the collection is made to compensate for any that may escape or perish during the handling process.
 - Although the biocontrol agents will be significantly slower in the cooler temperature, fresh food should be supplied regularly to sustain the agents.
 - The collection containers may also need regular cleaning or the agents may need to be transferred to clean containers if kept for any length of time.
 - ***B. seneciella*** adults can be kept up to one week in a cool environment.
 - ***Mogulones crucifer*** adults will store in their containers inside a refrigerator for up to one week.
 - ***M. janthinus*** or ***U. cardui*** larvae and pupae collected in the fall can be stored over the winter in a cold environment such as in an unheated building like a shed or in the fridge; however, care must be taken to prevent condensation and the possibility of mould developing during storage. Moisture (typically in the form of snow melt or rain) may be required the following spring for the biocontrol agent to emerge – see below.
 - Adult moths must not be kept in collection containers or cool storage for any significant length of time.
 - Large larvae, such as ***T. jacobaeae*** and ***C. lunula*** also should not be kept for prolonged periods of time because daily cleaning and supplying new food is required and excessive handling causes stress to the larvae.

Specific Collection Methods

Aspirating

Portable hand-held vacuum

Biocontrol Agent Species Suitability

Beetles

Brachypterolus pulicarius – adults

Galerucella calmariensis – adults

Galerucella pusilla – adults

Larinus minutus – adults

Larinus obtusus – adults

Larinus planus – adults

Longitarsus quadriguttatus – adults

Mecinus janthinus – adults

Mogulones crucifer – adults

Rhinusa antirrhini – adults

Rhinusa conicus – adults

Rhinusa neta – adults

Rhinocyllus conicus - adults

Moths

Agapeta zoegana – adults

Aplocera plagiata - larvae

Required equipment

- Portable hand-held vacuum and apparatus (Figures 2 and 3).
 - The size of the tubing used in the apparatus may need to be adjusted to accommodate agents of various sizes.
- Prepared 1-liter plastic collection containers containing plant material or other materials as described above in the General Collection Information section with tight fitting ventilated lids.
- Ensure the mesh used with the aspirator apparatus tubing has very small openings to prevent the biocontrol agents from becoming pulled into the motor.



Figure 2. Portable vacuum apparatus: vacuum, tubing attachment, collection lid and collection container



Figure 3. Assembled apparatus

Procedure

- This method is only used for agents that are small enough to fit through the aspirator tubes.
- Aspirating allows for relatively clean, somewhat pest-free collections. The biocontrol agents can be counted as they are aspirated, later in the field, or later indoors.
- With a portable hand-held vacuum and prepared collection containers, carefully move through the invasive plant patch, moving slowly to avoid bumping the plants and disturbing the biocontrol agents. The slightest movement of plants and shadows may cause them to habitually drop to the ground, feign death, leap away, or take flight.
- Directly ‘vacuum’ (aspirate) the biocontrol agents off plants.
- Dalmatian toadflax plants tend to accumulate water in their leaf axils. The water can often be unintentionally aspirated at the same time as the weevils. Placing paper towelling into the bottom of the collection container will absorb the moisture.
- It appears that two to three weeks after the *L. minutus* and *L. obtusus* population has increased, the weevils begin to tenaciously cling to the flowers and are difficult to collect with sweep-nets. This may coincide with ovipositing. Once this occurs, it is more efficient and less harmful to the insects and the host plants, to aspirate or handpick them from the plants although aspirating may also become difficult.
- When a portable hand-held aspirator is used for collection, excess condensation can be removed by engaging the motor full power for a short time which causes the air to swirl within the container. This cannot be done with moths because the increased air movement damages their wings.

- The suction strength required varies depending on the biocontrol agent:
 - Moths are always aspirated head first into the collection containers to not damage their wings. Adult moths can be removed from the plant with relatively light suction. It is best to engage and release the aspirator in short bursts and use the latter part of the suction power to remove the moth from the plant to prevent damaging their wings. When collecting **A. zoegana**, excessive suction power can cause them to lose their wing scales which can be seen as yellow dust that accumulates on the container sides. Keep knapweed plants inside the container to help reduce the air swirling within the container and provide the moths a surface to which they can cling.
 - Adult flies are delicate and also require light suction to prevent damaging their wings and their soft bodies.
 - Adult weevils and beetles may require more suction, depending on their clinging habits and where they are located on the plants. When aspirating out of flowers or crevices, more suction is often required.
- When collecting moths (for example, **A. zoegana**), an accurate count must be maintained during collection because it is difficult to re-count them later and should not be done because extra handling increases the risk of losing and damaging them.
- Use a leather glove on one hand to carefully bend or move thistle plants about to allow access into hard to reach locations and collect **L. planus** or **R. conicus**.
- This aspirating method can be used to collect small populations of **M. crucifer** as this agent is relatively difficult to collect in this manner. To collect, crouch or kneel on the ground and aspirate adults directly off the plants. Aspirating the soil around the plant base and in soil crevices will yield some adults. See the Large Scale Aspirating and Biocontrol Agent Self-Sorting section below for an alternative collection method for **M. crucifer**.
- **G. californiensis** and **G. pusilla** adults, eggs and larvae stages can slightly overlap. It is not recommended to aspirate the eggs or larvae.
- Powerful aspirators are required to aspirate **R. antirrhini** and **R. neta** from flowers as the adults cling tightly inside.
- When the appropriate numbers have been counted into the collection container, exchange the aspirating lid with a ventilated shipping lid.

Large scale aspirating and biocontrol agent self-sorting

Biocontrol Agent Species Suitability

Mogulones crucifer – adults

Longitarsus spp. – adults

Required Equipment

Modified gas leaf blower and apparatus

- Modified gas leaf blower and apparatus or electric shop vacuum and portable generator (Figure 4)
- Temporary collection container for field use (tubs or bags)
- Separator box

- Prepared collection containers containing plant material or other materials as described above in the General Collection Information section with tight fitting ventilated lids



Figure 4. Modified gas power leaf blower (AAFC)

Procedure

- The procedure includes three steps: field site vacuuming, self-sorting, and final collection. Note: This procedure was designed for *M. crucifer* collections and has not been used on other biocontrol agents to date. However, it is believed this method will also work for mass collection of other agents such as those instinctively drawn to light such as *Longitarsus spp.*

Field site vacuuming:

- Manoeuvre the suction nozzle over the areas the target biocontrol agent generally occupies. Use gentle movements to avoid damaging the plants.
 - For *M. crucifer* this includes over the upper and lower sides of the leaves, between leaves and surrounding any buds, into leaf axils, around the root crown and outward from the root crown over the soil to the drip line of the leaves.
 - For *Longitarsus spp.* vacuum rosette crowns, basal leaves and the soil area surrounding the plant leaves.
- Continue vacuuming until the catch container is sufficiently full. As the catch container becomes full, transfer its contents into a temporary collection container. When collecting agents in damp weather conditions, frequent collection content inspections should be done to ensure moisture does not accumulate in the containers and subsequently cause agent losses due to drowning. If moisture does accumulate, clean, sort, or sift the collection contents more frequently.

Dark and light self-sorting and final collection:

- The first model for mass collecting and sorting was developed in 2004 by Ministry of Forests and Range and is documented in the Operational Field Guide to the propagation and establishment of the biocontrol agent *Moqulones cruciger* (root-feeding weevil), and is available on the BCFLNRO Invasive Plant Program website. (*Moqulones cruciger* was formerly named *Moqulones cruciger*.)
- Since then, other sorting methods and equipment have been tested. The separator box system is now commonly used and is also the most simple and least expensive to prepare:

○ Separator box method:

A cardboard box with a capacity of 2.0 cu. feet (0.5 cu. m) is appropriate for both *M. crucifer* and *Longitarsus spp.* Cut a hole into one side and use tape or glue to attach the open end of a large, clear plastic bag over the opening. Clip off the bottom of the bag and add host plant material with a small amount of damp paper towelling to the bases to keep the plants from drying out. Close the bottom of the bag with a twist tie. Open the cardboard box and add up to 2 inches (5.0 cm) of vacuumed field material. Wait 24 hours before revisiting to allow the biocontrol agents to separate themselves from the field material and find the clean host plant material in the plastic bag. Remove the twist-tie and shake the contents into a large empty container. It may be necessary to cool the agents briefly in a fridge if they are moving quickly. At this time, the biocontrol agents can be counted and aspirated with portable hand-held vacuums or handpicked directly into prepared collection containers. Rapid collection is required because the *M. crucifer* weevils will become active again once they stop feigning death and *Longitarsus spp.* flea beetles tend to leap away quickly. Prolonged sorting periods may increase *M. crucifer* mortalities. Replenish the plant material and damp paper towel and wait for more agents to accumulate.

- The number of sorting boxes required will depend on the quantity of field collected material. Storing the agents and debris for too long can increase mortalities so it is recommended to prepare enough sorting boxes in advance.
- This collection method may not be suitable for biocontrol agents with soft bodies or scaled wings that are prone to damage.

Handpicking

Biocontrol Agent Species Suitability

Beetles

Cyphocleonus achates – adults

Larinus minutus – adults

Larinus obtusus – adults

Galerucella californiensis – adults

Galerucella pusilla – adults

Rhinocyllus conicus – adults

Moths

Aplocera plagiata - larvae

Tyria jacobaeae - larvae

Required Equipment

- Prepared collection containers containing plant material or other materials as described above in the General Collection Information section with tight fitting ventilated lids

Procedure

- Carefully move through the invasive plant patch, moving slowly to avoid disturbing the plants and the biocontrol agents.

- The handpicking method is combined with the tipping method during dry weather or when biocontrol agent populations are plentiful.
- Collection containers may be prepared slightly different for the handpicking collection method. Prepare collection containers with a small amount of plant material for the biocontrol agents to cling to and to prevent them from crawling over each other, fighting and injuring one another. Start with some plant material in the bottom making sure it does not reach the top, i.e. acting as escape ladders. As more agents are caught, add plant material and be ready to use the lid to prevent escapes.
- For most applications, hand picking is best done without gloves. However, the collector may determine their best practices based on the biocontrol agents' requirements or the collectors preferences. Keep in mind that some biocontrol agents are sensitive to the oily contaminants found on human hands.
- The biocontrol agents can be carefully teased off the plants, tipped over and dropped into the collection container, or by cupping your hand below the adult and allowing it to drop into your hand.
- If the contents of the collection container do not contain pests, seeds or debris it will not be necessary to recount and clean later.
- Some unique removal techniques may be needed to tease biocontrol agents off plants.
- **C. achates** adults can cling tenaciously and may require gentle coaxing to avoid damaging them.
- **L. minutus** and **L. obtusus** begin to tenaciously cling to the flowers and are difficult to collect with sweep-nets two to three weeks after the weevil population has increased. This may coincide with ovipositing. Once this occurs, it is more efficient and less harmful to the insects and the host plants, to handpick them from the plants.

Infested Plant Material

Biocontrol Agent Species Suitability

Flies

Botanophila seneciella – larvae/pupae
Terellia ruficauda – larvae/pupae
Urophora affinis – larvae/pupae
Urophora cardui – larvae/pupae
Urophora quadrifasciata – larvae/pupae
Urophora stylata – larvae/pupae

Moths

Cochylis atricapitana – larvae

Beetles

Mecinus janthinus
Rhinocyllus conicus – larvae/pupae

Rusts

Puccinia spp. – all stages

Required Equipment

- Sharp pruning clippers
- Twine
- Collection containers, bags, or boxes

- Portable hand-held vacuum and apparatus (if rearing from larvae/pupae to adults from infested material)
- Host plant material
- Rearing cages

Procedure

- Collect infested plant material at times recommended for specific biocontrol agent presence; see the BC FLNRO Biocontrol Agent Lifecycle Schedule located on the Publications and Downloads page of the BC FLNRO Invasive Plant Program web site.
- Sometimes biocontrol agent presence can be observed on the plants. For example:
 - *R. conicus* egg clusters can be seen on seedheads,
 - *M. janthiniformis* oviposition marks can be seen on stems, and
 - *U. stylata*, *L. planus* and *B. seneciella* oviposition points or presence can be seen on seedheads or flowerheads.
- If the infested plant material does not have visually obvious biocontrol agent presence, the plants should be sampled to determine establishment and to provide an average biocontrol agent count that can be used to estimate their population. For example, Dalmatian toadflax plants sampled yielded an average of five *M. janthiniformis* larvae or pupae/stem.
- Infested plant collections are done with dormant or actively growing plants. Some infested material will be seedheads, stems, leaves, or whole plants. For example, *Puccinia* infests all plant parts, therefore, clipping stems with buds and leaves intact is necessary.
- Infested plant collections are usually used for mass collecting and releasing and the amount of infested plant material required will depend on the biocontrol agent, for example:
 - New colonies of *B. seneciella* can be established with 1000 to 1500 seedheads.
 - 25 – 50 plants from a well-established site and transplanted into a new site is required for the best chances of success for *C. atricapitana* establishment.
 - More than one *U. affinis* and/or *U. quadrifasciata* larva or pupa can inhabit each suitable seedhead, therefore, new sites can be established with 500 to 1000 seedheads.
 - Potentially only one to three *M. paucipunctella* larvae/pupae will occupy a seedhead, therefore, a large quantity of infested seedheads are required to ensure good establishment.
 - *T. ruficauda* will usually have only one or two larvae present per seedhead, therefore, a large quantity of infested seedheads are required to ensure good establishment.
 - Observe the stems and choose those with *M. janthiniformis* oviposition marks that indicate biocontrol agent presence.
 - The strain *P. jaceae var diffusa* will not develop on spotted knapweed, therefore, collections should be made from the same species it is intended to be released upon.
 - Collect all plant parts that are infested with the *Puccinia spp.* biocontrol agent. The rust can be easily seen on leaves, stems and buds.
 - In crowded conditions, *R. conicus* will develop in the upper most part of the stem, just below the seedhead. This can be observed as abnormal swelling. When this occurs, clip the stem a short distance below the swollen point to capture the maximum number of potential weevils.

- It is highly recommended to collect an agent from the same host plant it is intended to be released onto. For example, *R. conicus* collected off Canada thistle should be released onto Canada thistle.
- Multiple *U. stylata* larvae/pupae are usually present in each seedhead and new releases can be made with 20 to 50 seedheads.
- When collecting bundled plant material, the bundles are tied into manageable ‘bouquets’. The bundle quantity can vary because some plants are large and can become hard to manage if too many stems are tied together. Clipped material must be done in dry conditions to prevent moulding.
- Individual infested seedheads can be counted directly into collection containers.
- It is best to release the biocontrol agent infested plant material as soon as possible as pests can occur in bulk storage. *M. paucipunctella* infested knapweed is susceptible to the predatory mite, *Pynotes* spp., which is capable of spreading rapidly through collected plant material.
- If clipped plant material is stored overwinter, it must be stored in a cold, dry location to prevent moulding and further biocontrol agent development prior to releasing them. Storing the infested material in warm conditions may encourage adult emergences which will not be synchronized with their host plant.
- Store and ship the clipped plant material in collection containers, bags or boxes.
- Handling the infested plant material for natural adult emergence in a controlled environment:
 - The preferred method is to store the infested seedheads in bulk quantities in a dry location.
 - It is common to use rearing cages and allow the adults to freely emerge on their own.
 - As they emerge, the adults are aspirated with portable hand-held vacuums into prepared containers.
 - This method allows for clean collections and for the new adults to naturally complete their development in the plant.
 - This method can be used when multiple biocontrol agents are present in the host plant and a pure population of one agent species is desired for a release as it allows for separating the species upon emergence.
- Handling the infested plant material by extracting adult biocontrol agents from seedheads:
 - A second method, though less preferred, is to break open the seedheads and extract the new adults. This is often done to prevent seed transport to new locations.
 - This method yields fewer adults because there may be numerous biocontrol agents present in different stages of development. Substantial losses will occur because the immature larvae and pupae cycles will be interrupted and the biocontrol agents will die.
 - Fully developed *R. conicus* normally remain inside seedheads until their bodies harden; pre-mature extraction interferes with this process and may increase adult mortalities.
 - This method allows for clean counted collections.
- Infested seedhead/gall collections for transfer into the field:
 - Seedheads/galls collected in the fall or winter can be released immediately or stored until early spring. Seedheads can be stored over winter in a cool environment (such as a shed) and kept for spring release, but, it may be difficult to keep them from excessive

- moulding and potential predation such as from mice. Spring collections, however, must be released right away.
- This method allows for adults to emerge over a longer span of time, giving the population some overlap of new and older adults.
 - This method also allows the agents to become synchronized with the local plant cycle when released early in the spring.
 - Early release of infested galls is required, particularly for species such as *U. cardui*. Moisture from melting snow and rain in the early spring soaks into the galls. The woody tissue remains hard but the callus tissue formed by the larvae tunnelling into the plant softens, degenerates and separates from the rigid gall. Once air reaches the mature larvae, pupation begins (Lalonde and Shorthouse 1982).
 - Handling the infested plant material for live whole plant transfers:
 - When using the method of transplanting live infested plants directly into a new release site, it is important that the plants remain in good health to allow the biocontrol agent sufficient time to complete development. If possible, when live plants are transplanted, limit any unnecessary aerial growth by clipping. Do not clip plants when the biocontrol agent may be located in the aerial growth such as some generations of *C. atricapitana*. It is best to transplant live plants as close as possible to the time when the target biocontrol agent is ready to emerge. Maintaining the plants' health is critical for successful transfers. This method can be used for *B. seneciella*, but care must be taken to avoid collecting plants that the fly larvae have already vacated.
 - Sometimes infested plants are excavated and biocontrol agents are partially reared in captivity. This method is also used for *B. seneciella*. The plants are excavated at the collection site and transplanted horizontally into moist sand in a shallow tray (with drainage). Securing the plants with bent wires may be necessary to keep the roots in contact with the sand. Damp paper towelling can be placed loosely over any part of the exposed roots to prevent them from drying. By keeping the sand moist and not allowing the roots and, therefore, the rest of the plant, to become dried, the larvae can exit the seedheads, move into the sand to pupate, and later emerge as adults. Once the larvae have moved to the sand, remove the plant. The following spring, the tray of sand containing the pupating biocontrol agent can be moved to the release site to allow the adults to freely emerge on their own, or the tray can be kept in a rearing cage where the adults will be collected and moved to the new site.
 - Currently there are no biocontrol agents for general distribution that are handled with the method that utilizes water-absorbing floral foam or sponges. It is important to not use this method for longer term storage of agents where oviposition may take place on the plant parts.
 - Water-absorbing floral foam can be used for biocontrol agents that require the host plant to maintain vigor during the shipping process. This method is recommended in particular for sap sucking agents. Some plants wilt quickly after they are clipped and stop producing the nutrients necessary for the biocontrol agent's survival during transport. An adequately wet floral foam piece is wrapped in plastic wrap. The plastic wrap is stretched around the foam carefully ensuring no parts of the floral foam are left uncovered. The plant clippings should be clipped on an angle to allow the stem to pierce through the

plastic wrap and into the foam. If the stem is too fragile to pierce the plastic wrap a small hole can be poked through the plastic to allow the stem to be inserted without damaging the stems. The floral foam along with the collected stems is then put into a collection container or bag that is an appropriate size to prevent the foam from sliding or shifting during transport.

- An adequately moist sponge can be added to biocontrol agent collections that require the leaves they occupy from desiccating during transport. Ensure the sponge is not too moist or moulding may occur. Alternatively, a dry sponge may be added to a leaf collection for any plants that are prone to heavy transpiration when clipped. Ensure the sponge does not absorb excessive moisture that may cause premature leaf desiccation.
- The simplest infested plant handling technique is done when the plant is no longer actively growing and whole plants or plant parts can be clipped. However, senescent plant collections may not coincide with the biocontrol agents handling requirements.

Sweep-netting

Biocontrol Agent Species Suitability

Beetles

Aphthona cyparissiae – adults
Aphthona nigriscutis – adults
Chrysolina hyperici – adults
Chrysolina quadrigemina – adults
Larinus minutus – adults
Larinus obtusus – adults
Sphenoptera jugoslavica - adults

Flies

Botanophila seneciella – adults
Urophora affinis – adults
Urophora quadrifasciata– adults

Moth

Aplocera plagiata - larvae

Required Equipment

- Light to medium weight cotton canvas sweep-net (Figure 5).
- Portable hand-held vacuum and apparatus
- Prepared collection containers containing plant material or other materials as described above in the General Collection Information section with tight fitting ventilated lids



Figure 5. Lightweight canvas sweep-net

Procedure

- Using a sweep-net allows for rapid collection over a large area of biocontrol agents that are less prone to physical damage caused by the procedure.
- Plants that damage easily (Dalmatian toadflax, purple loosestrife,) or accumulate too much litter (thistles, hound's-tongue) are not suitable for sweeping methods.
- Use a sweep-net with appropriately weighted fabric for the biocontrol agent and the plant. A light weight cotton canvas material has proven suitable for most collections.
- Sweep during dry periods to prevent the bag from becoming water-logged. Excess water can drown biocontrol agents and can damage them as they and debris become stuck to the sweep-net bag.
- The sweep-net can be hard to manage in heavy wind gusts or during steady windy periods. In addition, the movement of the plants in the wind discourage the adults from rising from the soil or lower plant parts.
- Collect biocontrol agents by sweeping host plants, stopping to check the sweep-net bag frequently for target biocontrol agents. By performing a consistent number of sweeps and obtaining subsequent counts of agents in the net, an average population quantity can be derived which is useful for estimating and comparing populations at collection sites.
- Sweeping for flies, for example *Urophora spp.* or *B. seneciella*, is less preferred than other methods because they can easily become damaged from the sweeping action or the debris in the net.
- *A. plagiata* is a one of the few species that can be collected in the larva stage using the sweeping method. The larva is quite tolerant of the sweeping motion, however, it is necessary to sort and clean the sweep-net more frequently than when sweeping for other agents such as flea beetles.
- Sweeping is followed by aspirating. Use the hand-held portable vacuum and, with quick action, aspirate the adults from the net.
- Most biocontrol agents will move towards light and climb upward to the sweep-net bag opening. On extremely bright days, the sweep-net bag can be held almost completely closed with only a small opening to provide light. *L. minutus* and *L. obtusus* have been observed to quickly move towards the light and through a narrow opening where they can be rapidly collected. *Aphthona spp.* adults are quick to leap away and out of reach towards bright light so rapid, steady suction may be necessary.
- Count the biocontrol agents as you aspirate them into recommended numbers in the prepared containers.
- When appropriate numbers are aspirated into the container, replace the aspirator lid with a ventilated shipping lid.
- A second sorting is recommended to remove any pests, excess debris and to separate biocontrol agent species that may have been unintentionally aspirated.
- Clean, counted field collections are required for *Aphthona spp.* because flea beetles are difficult to sort later and, therefore, a second sort is not recommended.
- Clean, counted *U. affinis* and *U. quadrifasciata* collections should be attempted in the field because the flies are very delicate and a second sorting can increase the risk of damage. Sweep-netting is less preferred because of the increased damage to the biocontrol agents,

however, it is preferred over other methods that may increase the spread of seeds to sites where the host plant from which the biocontrol agents were collected differs from the one the agent is going to be released upon.

- Clean sweep-net bags thoroughly between sites to avoid transferring invasive plant seeds.
- Sweep-nets can be ordered and purchased online from companies selling biological sampling products.
- During optimal collection periods the plants can be repeatedly swept because the fallen adults are quick to rise on upper plant parts.
- It appears that two to three weeks after the *L. minutus* and *L. obtusus* population has increased, the weevils begin to tenaciously cling to the flowers and are difficult to collect with sweep-nets. This may coincide with ovipositing. Once this occurs, it is more efficient and less harmful to the insects and the host plants to handpick them from the plants. Aspirating the weevils directly off the plants is not as effective when they are really clinging.

Tap and tray

Biocontrol Agent Species Suitability

Currently there are no biocontrol agents for general distribution that are handled with this method.

Required Equipment

- Drop sheet or tray
- Tapping or beating stick, size appropriate to plant
- Portable vacuum aspirator and apparatus
- Prepared collection containers containing plant material or other materials as described above in the General Collection Information section with tight fitting ventilated lids

Procedure

- This method is normally used for sturdy host plants such as trees or shrubs.
- Tap or beat the host plant, allowing the dislodged biocontrol agents to drop to either a tray held below the beating point or to a large drop cloth or sheet laid on the ground.
- Examine the collection tray or sheet for target biocontrol agents.
- Once a suitable amount of biocontrol agents have dropped, individually handpick them or use the portable vacuum aspirator to collect them from the tray/sheet and store the agents in prepared collection containers.
- This method is more useful for spring and early summer collections because more pests (aphids in particular), are more abundant later in the season. Sorting to clean the collection can be difficult and time consuming when large quantities of pests or debris are present.

Tipping

Biocontrol Agent Species Suitability

Beetles

Larinus planus – adults

Mecinus janthinus – adults

Rhinocyllus conicus – adults

Required Equipment

- Prepared collection containers containing plant material or other materials as described above in the General Collection Information section with tight fitting ventilated lids

Procedure

- Carefully step through the invasive plant patch, moving slowly to avoid nudging the plants and disturbing the biocontrol agents.
- Tipping can be done with gloves for protection and is recommended when working with plants with thorns or spines.
- The collection container should be prepared with enough host plant food to cover its base. As more biocontrol agents are added, more plant material is also added. Be careful to not add too much plant material as it could cause harm to the biocontrol agent or create escape routes.
- The collection container usually needs to be covered between each tipping to avoid losing biocontrol agents that are prone to flight or crawling out during prolonged collection periods. However, the tipping method is usually used in situations where the target biocontrol agent populations are plentiful and any losses due to escapes usually are outweighed by the volume of biocontrol agents obtained.
- Plants can be tipped over a cupped palm to catch the biocontrol agents which are then transferred to the containers.
- Tipping can be difficult to use if two hands are needed to handle the plant while also manoeuvring the collection container.
- Plants with a thick growth habit or leafy stalks can prevent the biocontrol agents from dropping directly into the container. For example, the leafy growth habit of Canada thistle can interfere with *L. planus* or *R. conicus* collections and cause the weevils to drop onto lower or less accessible leaves, into leaf axils or bounce subsequently to the ground.
- As a slight variation to the tipping method, the plant can be shaken into a container to dislodge the biocontrol agents.
- Accurate biocontrol agent counts usually cannot be obtained in the field and will require a second sort and recount for accuracy.
- Pests are often collected with the target biocontrol agent and predatory pests may need to be removed while still in the field to prevent biocontrol agent losses.

Packing, Shipping and Transporting

Procedure

- Include details of the collection:
 - Use a felt tipped marker, omni-chrome pencil, or printed label to mark each collection container with:
 - Biological control agent name or Invasive Alien Plant Program application (IAPP) 7-letter agent code;

- Number of adults in the container;
- Collection date;
- Collection location and IAPP Site ID number; and,
- Host plant species name or Invasive Alien Plant Program application (IAPP) 7-letter plant code.
- Tape the edge of the ventilated lids closed in two or more places, this prevents dislodging during shipping. Do not tape over the venting mesh.
- Secure the opening of paper bags by rolling them tightly, taping or stapling them closed to prevent the material from becoming loose in the shipping box.
- Transporting and Shipping:
 - Coolers:

Often electric coolers do not have a thermostat; therefore, the temperature may need to be monitored if the agents are transported for any significant length of time. Protect the collection containers in the cooler from getting too cold or developing condensation by placing an item between them and the cooling vent to avoid cold air blowing directly onto them. In standard coolers the freezer packs need to be put inside a closed freezer bag and then wrapped with paper towel to absorb the subsequent condensation formed as they defrost. Distribute the freezer packs around the containers to provide a cool environment throughout the cooler. Keep the containers from direct contact with the freezer packs. Monitor the containers for any condensation during transport. If condensation occurs, rotate the containers/freezer packs or temporarily unplug the cooler.
 - Shipping boxes:

Use the same procedure as outlined above for sturdy cardboard shipping boxes or Styrofoam containers and incorporate the following process. Extra packing material, shredded paper, crumpled newspaper, or Styrofoam pieces should be packed inside the box to protect the containers from damage if the integrity of the box is compromised. A small amount of packaging between the containers is recommended and may prevent the containers from squeezing together which can cause the lids to dislodge. Once the shipping carton is packed, thorough taping of all seams and flaps is necessary. This will increase the shipping container's overall strength and prevent the biocontrol agent from escaping should they get out of their collection containers within. If the box is to be shipped, label the outside with appropriate labels as required or recommended by the courier.

General Releasing Methods

Procedure

- Before releasing, determine the status of any other agents dispersed or released at the proposed release site. This is usually done prior to arriving on the site with the new biocontrol agents but a quick observation prior opening the containers is worthwhile.
- Before releasing, investigate the biocontrol agent and its preferences to ensure that the best possible point of release is selected. For example, do not release into areas with saturated soil if the agent will be unable to relocate itself to more desirable locations.
- Keep the biocontrol agents cool and shaded until they are released.

- When making a biocontrol release the shipped plant material (food) is typically left at the new site if:
 - No seeds are included (with the exception of some agents, for e.g. some knapweed seedfeeders);
 - The new host plant species is the same as what the agent was collected from so its potential reproduction on the site is of lower concern;
 - The plant part does not contain adventitious buds that may generate new plants; and,
 - The plant parts being left do not encourage the agent to use it for ovipositioning.
- Depending on the biocontrol agent's lifecycle, the release techniques differ, for example, actively feeding larvae are released differently than dormant larvae, pupae, or mature adults.
- Releasing adults:
 - Liberate the biocontrol agents from collection containers by tipping them at 45 degree inverted angles. Gently tap the container's sides to encourage adults to fall or fly out. Adults are usually deposited at a single plant so they will be in close contact with one another if the release occurs during their breeding cycle. If they are not actively breeding they have the ability to self disperse if they desire.
 - Adult moths often require additional encouragement and must be handled gently to prevent damaging them. The container can be placed upright to allow the moths to fly out. If some still remain inside the container after a few minutes they can be encouraged to leave by gently tapping the side of the container or 'chasing' them with your finger or a piece of the host plant stem upward and outward.
 - Occasionally during shipping eggs may be laid on leaves and stems and those plant pieces containing the eggs can be lodged or laid into plants at the new site, preferably touching the same part on the plant growing at the site, i.e. place leaves on leaves, stems on stems and seedheads next to seedheads to allow hatching larvae to transfer to the new plant. Newly hatched larvae are incapable of travelling significant distances.
 - If eggs are visible in large quantities, an egg-to-plant transfer may be required.
 - After completing a release, check the equipment and yourself for any biocontrol agents. Adult flea beetles and moths are notorious for attaching themselves to clothing and hitch-hike off a site.
- Releasing actively feeding larvae:
 - Foliar feeding larvae that are actively feeding at the time they are released need to be scattered on healthy, green plants over a large area where they can come in contact with enough food to allow them to complete their development. Most larvae prefer dense stands of plants where they can transfer easily from plant to plant. Larvae are incapable of moving great distances for food.
 - From the time the larvae are collected to the time they are released, several may have prepared for pupation. When this occurs, place those that are pupating in what may be their preferred pupation environment. For example, if they normally pupate on the plants, lodge the plant part with the puparium attached into the plants at the new site. If they pupate under sticks or debris, move them to locations that will offer that preference.
- Releasing infested plant material:
 - Transferring infested plants or parts of infested plants usually requires unique release techniques depending on the biocontrol agent.

- When large quantities are released, it is recommended to divide the material into more than one (or numerous) parts. This may offer some protection from predation, moulding or pests.
- Predation can be a problem when transferring larvae infested material:
 - Rodents are attracted to the larvae and will readily feed on them, therefore, the infested plant material should not be released in large concentrated quantities where snow or debris will cover them and create a 'cache-like' situation.
 - Birds consume thistle seeds; therefore, some of the infested thistle bundles may become consumed in the spring when other food sources are low. Rodents are also known to collect thistle seed for food and bedding and in so doing may also consume larvae or pupae.
- If a biocontrol agent requires snow cover for protection from winter elements, the collection must be placed where it will receive adequate insulation.
- If seedheads are released at a new release site they can be broadcast spread (scattered). This is commonly used for *U. stylata* and *R. conicus* but may also be used with other biocontrol agents that have been collected in seedheads such as knapweed seedfeeding agents like *Urophora spp.* and/or *M. paucipunctella*.
- Bundles of infested stems with or without seedheads intact can be tied upright to stiff, mature plants, stakes, posts or trees to prevent them from blowing away or dropping to the ground and potentially moulding. In the autumn, tie the bundles to existing plants. If the plants typically fall over from snow press then the bundles will also be on the ground. Insulation from cold winds and fluctuating winter temperatures will be provided to the biocontrol agents under the snow. When bundles are tied to trees it is recommended to return later and remove the securing twine to prevent girdling the tree. When possible, choose to tie the bundles to a tree because livestock, cattle in particular, may be attracted to the bundles when they are tied to a stake.
 - This method is most often used with knapweed seedfeeding agents such as *Urophora spp.* and *M. paucipunctella*.
 - When tying upright bundled plants containing *B. seneciella* to a stake or a tree, secure the plants so the base of the stems touch the ground. This will allow the larvae to exit the seedheads and pupate in the soil.
- Infested plant bundles can also be scattered onto the ground or propped into the same species plant over the release area. This method is often used when *M. janthiniformis* is collected as larvae or pupae in plant stems. Occasionally bundles of knapweed stems with intact seedheads may also be collected with agents such as *Urophora spp.* and *M. paucipunctella* and scattered at the site.
- When *Puccinia* infested plants are transferred, the stems should be lodged into the new plants. The direct contact with new host plants allows the spores to easily transfer. *Puccinia* releases are best done shortly before light rain or before dew accumulations because the added moisture helps to transfer spores and multiply the biocontrol agent populations.
- Sometimes infested plants parts are released by securing the clipped infested plant pieces within protective cages to prevent predation but allow for the effect of the local elements. This method is often used when releasing galls that contain *U. cardui* larvae or pupae.

- When biocontrol agents have been allowed to move into sand trays to pupate, the sand tray can be placed at the release site to allow the new adults to emerge into their new environment. It is recommended to later return to the site and retrieve the tray. This method is often used for soil pupating agents such as *B. seneciella*.
- When transferring live transplants of larvae-infested roots or stems with biocontrol agents such as *C. atricapitana*, dig a sufficiently large hole to accommodate the transplant root ball and backfill the excavated area with enough soil to keep the plant crown positioned naturally upright with the root crown at ground level and no roots exposed above the soil line. Also ensure the transplant is not planted too deep. Place new transplants near established target plants to allow the larvae to exit the transplanted host plants if they become dry or perish. If possible, provide water to the infested plant at the time of transplanting. Note: not all larvae are capable of transferring themselves through the soil to new plants.

Useful links and information

Operational field guides, manuals, handbooks, info pages and newsletters

<https://www.for.gov.bc.ca/hra/Plants/publications.htm>

Invasive plants with biocontrol agents in B.C.

https://www.for.gov.bc.ca/hra/Plants/Agent-Plant_Matrix.htm

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