Purple Loosestrife Project - Rearing and Releasing Natural Enemies to Control Purple Loosestrife
Michigan State University
Michigan State University Extension
Cooperators Handbook Section 2
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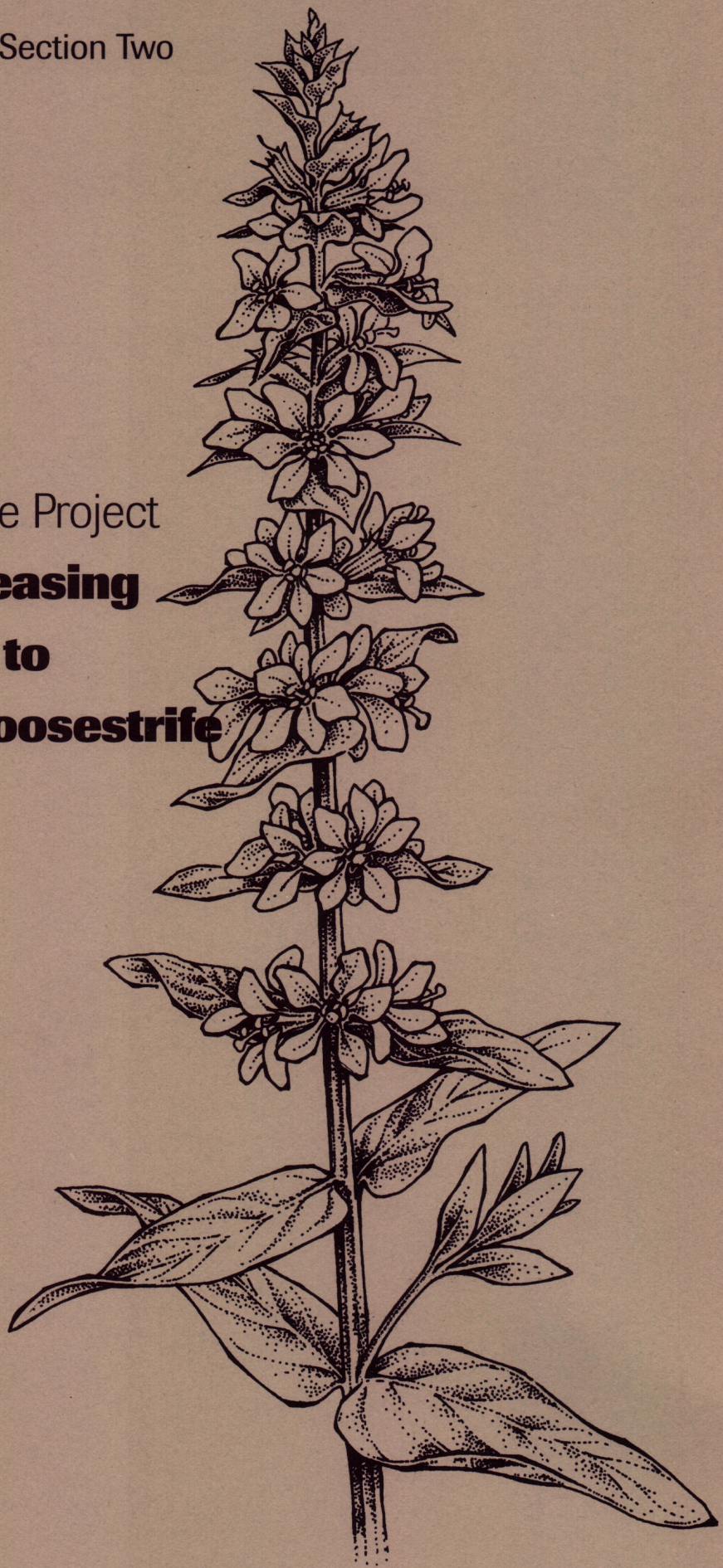
Cooperator's Handbook • Section Two

The Purple Loosestrife Project

Rearing and Releasing Natural Enemies to Control Purple Loosestrife



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Michigan State University
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The Purple Loosestrife Project
Cooperator's Handbook

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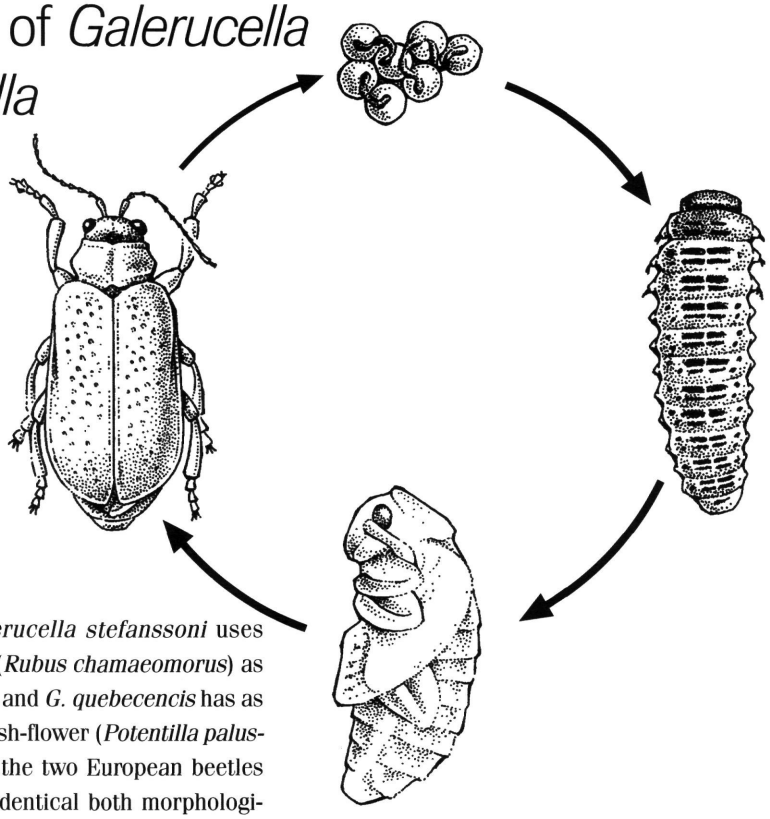

Purple loosestrife (*Lythrum salicaria*) is an exotic wetland perennial first noted in the United States in 1814. Since its arrival, purple loosestrife has established itself over a broad area in the United States and Canada. Due to the fact that it grows in nearly any moist environment and may produce up to 2.5 million seeds per plant, dense stands of purple loosestrife can become dominant in infested wetlands. Purple loosestrife damages wetland ecosystems by displacing native wetland flora and fauna. In an effort to bring this plant's population into a more stable balance, a program of biological control has introduced several natural enemies of purple loosestrife into the United States and Canada. Among these is a root-mining weevil, *Hylobius transversovittatus* which feeds specifically on purple loosestrife, and two leaf-feeding beetles, *Galerucella calmariensis* and *G. pusilla*.

Galerucella spp. are members of the leaf beetle family (*Chrysomelidae*). There are approximately 25,000 known species of leaf beetles worldwide with 1,460 known species of chrysomelids in North America. *Chrysomelid* adults feed on foliage and flowering parts while the larvae eat foliage or mine leaves although, others mine roots and stems. Some leaf beetles are serious agricultural pests.

Galerucella calmariensis and *G. pusilla* are joining three native species of *Galerucella* in North America. *Galerucella nymphaeae* has a broad range of aquatic hosts in the U.S. and Europe including water lily and purple loosestrife. *Galerucella stefanssoni* uses cloudberry (*Rubus chamaeomorus*) as a host plant and *G. quebecencis* has as its host marsh-flower (*Potentilla palustris*). Since the two European beetles are nearly identical both morphologically and in their life cycles they will mostly be treated as a single entity.

Description

Galerucella calmariensis and *G. pusilla* range from 4 to 6 mm in length and are about half as wide as they are long. Coloration is light brown, sometimes with a dark stripe located at the margin of each elytron. It is not possible to differentiate between the two species in the field due to variability in key characteristics. It is sometimes possible to differentiate between the two with some accuracy using spots on the pronotum (one mark for *G. calmariensis* and two tiny dots on *G. pusilla*) although variability is present here as well. Some insects of either species may exhibit no markings and adults emerging from pupation do not harden and develop any markings for up to ten days from emergence.

Life Cycle in the Midwest

In spring (late April or early May) overwintering adults emerge from soil and litter below or nearby old loosestrife plants to feed for several days on new foliage and then begin to reproduce. Females lay eggs in masses averaging 5 to 7 eggs per grouping, which may be placed low on the stem, at leaf axils, or on leaves. The egg-laying period occurs from mid-May to mid-July with the peak in May and June. An individual female may lay up to 500 eggs during this period. Larvae emerge from eggs in 7–10 days and feed in and on shoot tips. When larvae commence feeding, adults disperse, migrating to other nearby plants, possibly to reduce competition for food. Competition is also reduced by differential feeding

(wherein younger larvae feed only on shoot tips and flower buds while later instars are less descriminant). Defoliation of plants by larvae can be complete resulting in their migration to nearby plants. Larvae feed and molt for about three weeks before moving down into the soil or litter to pupate. Where water levels are higher, larvae may pupate inside the stem by burrowing into aerenchyma tissue (plant tissue containing cells composed primarily of air giving aquatic plants buoyancy, for example). Upon emergence the new adults harden in about seven to ten days, then feed until moving into the litter or soil below to overwinter. This period of emergence occurs typically from July to September. When warm periods are extended there may be a short period of some mating and oviposition prior to entering into hibernation. The total maturation time from egg to adult is approximately 30–40 days.

Ecology

The two *Galerucella* species are found inhabiting loosestrife throughout the plant's natural range in Europe and Asia. In nature, both species are host-specific in regards to oviposition (i.e., will lay eggs only on purple loosestrife) and are primarily specific feeders as well. In research trials where purple loosestrife has been completely defoliated, newly emerging adults have been noted feeding to some extent on winged loosestrife (*Lythrum alatum*) and swamp loosestrife (*Decodon verticillatus*). This type of feeding is reported to be rare under field conditions.

Both *Galerucella* species occupy the same level in the food chain but a number of specializations allow them to avoid competition. These include species-specific attractants (pheromones) used for aggregation and mating, male courtship behavior, adult dispersal, and differential feeding of larvae.

Impact on Host

The *Galerucella* beetles can cause significant damage to purple loosestrife, being capable of complete defoliation and photosynthetic suppression of the plant as well as rendering it incapable of flowering. Adults inflict a shothole feeding pattern eating small (1–2 mm) holes through foliage. Larvae produce a skeletonized feeding pattern on the leaf, eating the softer tissues and leaving the more lignified (tougher, less digestible) veins. Significant impact can also be seen where loosestrife infests permanently flooded sites. Larval damage to flower and shoot buds reduces plant growth and inhibits flowering. Adult and larval leaf damage greatly reduces the photosynthetic capability of purple loosestrife. This may lead to reduced starch stores in the roots which can result in overwintering mortality in the plant. Photosynthetic inhibition results in reduced stem height and root length, both essential to overall plant vigor. The resultant weakening and/or death of the loosestrife plants provides an opportunity for native plant species such as the cattails (*Typha latifolia and T. angustifolia*), grasses, and sedges to return.

The beetles have the ability to fly between plants or plant clusters and larvae, pupae, and adults can float, allowing current or wind to move them to nearby plants. Impact on flooded sites is not as likely using another approved insect, the root-mining weevil *Hylobius transversovittatus. Hylobius* larvae are unable to survive in the roots under permanently flooded conditions. Unfortunately, long-term standing water may be detrimental to *Galerucella's* development as well since pupation is difficult under these conditions.

It is expected that the establishment of permanent stable populations of *Galerucella* on purple loosestrife will reduce this plant to levels that may be tolerated by North American wetland ecosystems.

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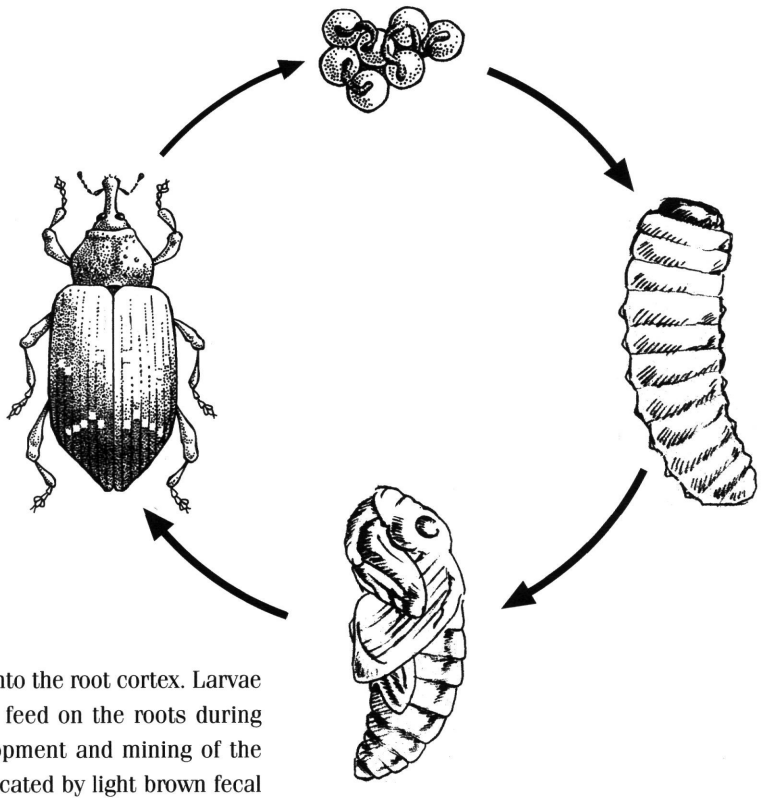
Life History and Ecology of *Hylobius transversovittatus*

H. transversovittatus is a member of the weevil family of beetles (*Curculionidae*). This family includes approximately 40,000 known species of weevils worldwide with 2,500 known species in North America. Weevil adults feed on foliage and flowering parts while the larvae mine roots and stems. Some weevils are serious agricultural pests. The weevils of the genus *Hylobius* are primarily found on conifers in Europe and North America where some are considered significant forest pests.



Description

H. transversovittatus adults average 10 mm in length, but can range from 5 to 15 mm in length. Coloration is dark brown with white tufts of hair arranged in two rows running laterally across the elytra (hard outer wings). The thorax and head taper to a downward-curved snout.

Life Cycle

The nocturnal adults emerge from overwintering in mid-April or May and feed on purple loosestrife foliage for about three weeks before females can lay eggs. Females place 1–3 eggs/night preferably in soil close to roots and in purple loosestrife stems close to the base. When stems harden eggs are laid exclusively in the soil. Up to 200 eggs may be produced by a mature female during the summer season with the peak period being June and July. Upon hatching from eggs larvae laid in stems feed in the pith and burrow downward to the roots while larvae hatching in soil feed on the tender rootlets, then burrowing into the root cortex. Larvae continue to feed on the roots during their development and mining of the roots is indicated by light brown fecal material left in the wake of their feeding. Complete maturation takes 1–2 years at which time larvae pupate at the top of the root, then emerge as adults and feed on leaf edges. Larvae in all stages (instars) are capable of overwintering in the root tissue.

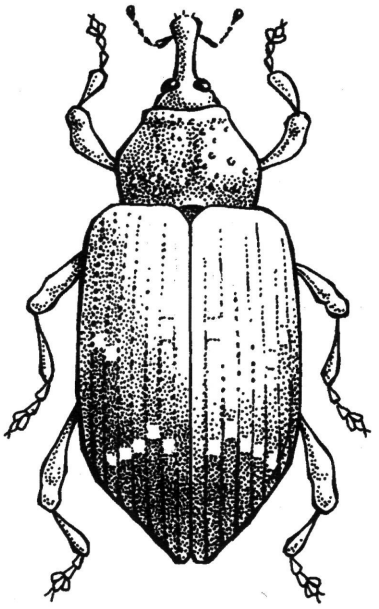
Ecology

H. transversovittatus is found occurring throughout purple loosestrife's native range in Europe and Asia, inhabiting the plant everywhere it is found except in sites which are shaded or permanently flooded. Adults and larvae can survive submergence for a few days, however, permanent flooding results in mortality. Adults disperse twice in a season; after emergence from overwintering in the spring and at the end of the summer, when offspring emerge from pupation. The primary means of dispersal is by walking which makes for slow spread of the insect.

Impact on Plant

Adults feed on leaves of the plant, feeding at the edges and working inward towards the mid-vein. Key impact on the plant occurs from the activity of larvae mining the roots. Larvae laid in

stems mine pith as they travel to the roots, damaging vascular tissue and disrupting transport of photosynthetic products (photosynthates) to the roots for storage. Mining of the roots reduces the available supply of photosynthates to the plant which can eventually lead to plant mortality. Mining also weakens root structure making them brittle and more susceptible to mechanical damage if disturbed. However, plant impact is governed by the number of larvae present per root and root age. One or two larvae in the roots of a plant only a few years old can significantly impact that plant while the same number of larvae in an older root may produce little impact. It is expected that these insects alone or working in concert with *Galerucella calmariensis* and *G. pusilla*, two leaf feeders also released on purple loosestrife, will reduce this plant to more stable levels in North American wetlands.



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The Nuts and Bolts of Natural Enemy Rearing and Release

Raising Galerucella Beetles Indoors

The following is a stepwise procedure for the indoor rearing and subsequent release of *Galerucella* spp. beetles for biological control of purple loosestrife (*Lythrum salicaria*) in Michigan.

Overview

Using this procedure, purple loosestrife rootcrowns will be potted and placed in a tray with water. Each pot will have a tomato cage placed into it and a fine mesh bag that serves as a cage pulled over the top. After 4 to 6 weeks of plant growth, place 20–25 *Galerucella* beetle adults into the cage. The beetles will produce another generation, with the new adults ready for release in about 6 to 8 weeks. With this setup it is expected that 500 to 1000 new beetles per pot will be produced for observation and release into a purple loosestrife infested wetland.

A fairly typical timeline for rearing and release of *Galerucella* beetles, follows:

EVENT	APPROX. DATE
Pot rootcrowns	March 10
Place beetles into cages	April 15
Release beetles into wetland	June 1

Getting Started

The first step is to assemble the materials necessary for the rearing process. A list of supplies needed for the beetle rearing kit and their approximate cost is in the box above.

You will be able to purchase all of your supplies from a local garden center or nursery (consider asking for donated materials) except, perhaps, for the sleeve cage. You can create your own with two yards of mesh fabric and drawstfùngs. Your cost will vary depending on where materials are purchased and whether you are able to substitute used for new materials.

Indoor Beetle Rearing Kit

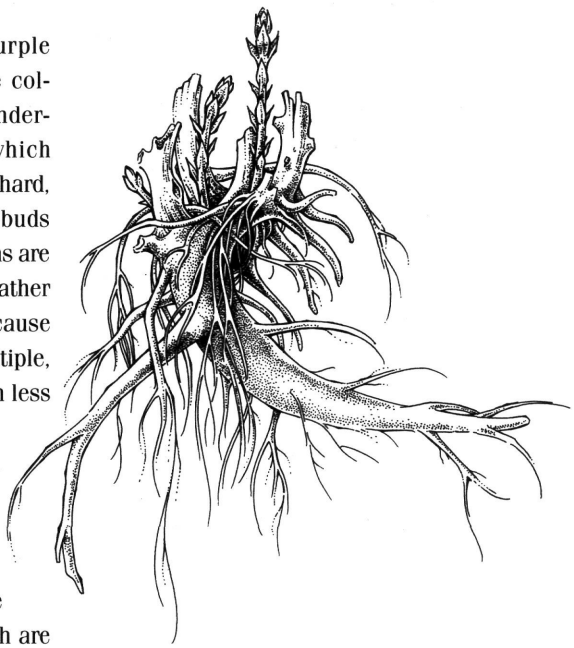
ITEM	COST	QUANTITY
Wire tomato cage (42 in.)	$2.50	1 ea.
Potting mix, non-soil type (20 qt.)	$5.00	1 ea.
Osmocote™ slow release fertilizer (2½ lb.)	$6.00	2 Tbs.
Four gallon pot	$6.00	2 ea.
Bottom tray	$4.00	2 ea.
Sleeve cage	–	1 ea.
Pole marker (PVC pipe)	50¢/ft.	6–8 ft.

Collecting Purple Loosestrife Rootcrowns

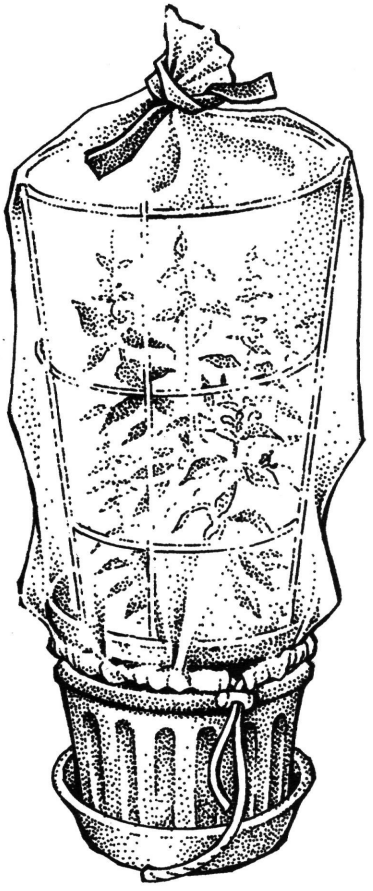
Prior to growing plants, purple loosestrife rootcrowns must be collected. The rootcrown is the underground portion of the plant which survives the winter. It consists of hard, woody storage roots and stem buds which arise from them. Rootcrowns are used to establish plants indoors rather than establishing from seed because the rootcrowns will produce multiple, hardy stems in less time and with less effort.

Rootcrowns may be collected in the late fall after purple loosestrife topgrowth has died, or early in the spring prior to new growth. Collect from mature stands of purple loosestrife which are two years or more old. Mature stands have larger rootcrowns which contain more stored energy for plant growth.

Locate groups of dead stems from the most recent year's growth and dig around these "clumps" to dislodge them from the soil. A tile spade, one with a long, narrow blade, works well for this



Purple Loosestrife rootcrown



type of digging but any spade will work. Use the spade to break apart large rootcrowns into sizes that will fit into the pots being used. These will be roughly eight inches in diameter. Using the spade, knock away loose soil from the rootcrowns and cut away all but about six inches of the dead stem topgrowth. It is not necessary to clean all soil and debris from the rootcrowns. Live, healthy rootcrowns have a tan to brown color, are pink to whitish on the inside, and are somewhat flexible. Rootcrowns which are dead will be charcoal-black in appearance and brittle. Sometimes stem buds can be found sprouting from the rootcrowns, ensuring a healthy rootcrown has been chosen.

Rootcrowns collected in the spring may be potted immediately, or stored outdoors for a short time. Keep the rootcrowns moist until ready for planting. Collection of rootcrowns will need to take place about 12 weeks prior to the date you plan to release new beetles. Fall-collected rootcrowns may be stored outdoors. If collecting in the fall, gather twice as many rootcrowns as you will need in order to compensate for winterkill and other factors that can damage the rootcrowns. Moisten the pile, then cover it with a tarp to keep rootcrowns moist and out of light. Do not cover with straw as this attracts nesting animals that may feed on the rootcrowns during the winter months.

Potting the Rootcrowns

Set up an extra pot or two to ensure that you have a healthy plant to provide to the beetles. Fill the four-gallon pots halfway with potting mix and sprinkle in one tablespoon of Osmocote™ fertilizer. This slow-release fertilizer will gradually provide the plants with needed nutrients. Place one large, or several smaller, rootcrowns into the center of each pot and fill the remainder with potting mix. Pot the rootcrowns oriented in the same direction they were growing in the wetland, i.e., stems directed upwards, and cover with potting mix. Ideally, the rootcrown(s) put into each pot will result in six or more healthy stems per pot. *Do not press the potting mix into the pot.* The beetle larvae move into the top inch of the soil to pupate and this region should remain loose.

Put the potted plant into the bottom tray. Make certain that the pot has holes in the bottom to allow water uptake. For the initial wetting of the potting media, water the tops of the pots to settle the media around the rootcrowns. Add more potting media if needed. *After this initial wetting, water plants by filling the bottom tray only.* The bottom tray should be kept filled with water.

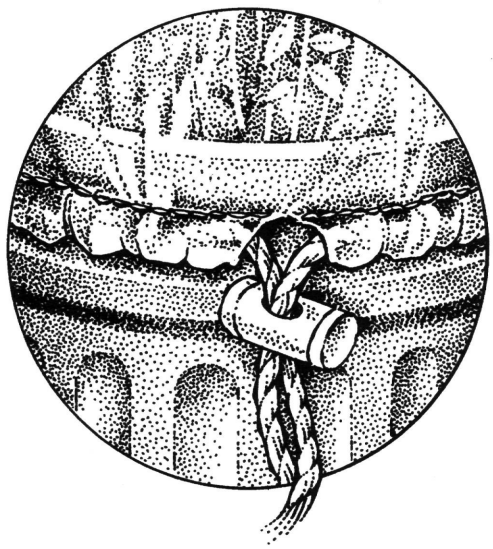
Place a tomato cage into the pot, pushing it all the way to the bottom. As the stems grow, direct them into the center ring of the tomato cage. Later, you will pull a sleeve cage over the tomato cage. Leave the cage off for now, until the plant has reached at least 12 inches in height. This is to maximize the amount of light reaching the plant.

Plant Placement

Place the potted plants where they will receive the most sunlight, preferably in a south-facing window. Use a greenhouse if one is available. A grow light may be a necessary addition in classrooms where sunlight is minimal. Temperatures between 75° to 85° Fahrenheit are ideal for the plants and beetles. Consider the use of a heat lamp if your room is too cold.

Maintaining the Plant

Make sure that the plants have adequate water at all times. After the initial wetting of the potting media, water plants by filling the bottom tray only. *Do not water the top of the pots again,* as this may cause a crust to form on the potting media surface, which could interfere with pupation. As the plants



grow larger they will require daily watering.

When stems reach about 12 inches in height pinch off about half an inch of the tip of each stem (apical meristem) to stimulate lateral shoot development. This will produce a bushier plant with lots of preferred larval feeding sites. One to two weeks after pruning, the plants should be ready to receive the *Galerucella* beetles.

Choose the healthiest looking plants to infest with the beetles. You may need to transfer the tomato cage to the other pot. Wrap tape around the welds on the tomato cage to protect the sleeve cage from tearing as it is pulled over the tomato cage. Use string or a rubberband to tie off the end of the sleeve cage that has no drawstring, this is the top. Slide the sleeve cage over the tomato cage and down onto the upper lip of the pot. Most pots have a groove along the upper lip which works well to hold the drawstring in place. Secure the drawstring tight around the pot. It is very important to have a tight fit between the cage and the pot.

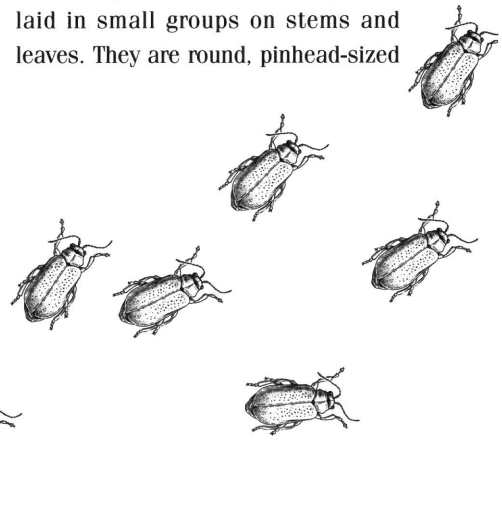
Infesting Purple Loosestrife with Beetles

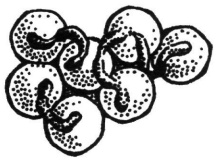
Twenty to twenty-five *Galerucella* beetle adults will arrive via overnight delivery and must be promptly placed onto plants. The beetles are very good at "hiding," so half or fewer of the beetles will be readily visible. They will be sent with enough purple loosestrife foliage to keep them fed during their trip. Keep the shipping container with beetles inside out of direct sunlight until ready to infest plants. *Do not expose beetles to temperature or humidity extremes while in the shipping container.*

Untie the string at the top of the sleeve cage, shake the beetles out of the container and into the cage. Securely close the cage top. Check that the drawstring at the bottom of the sleeve cage is tight against the pot. If the drawstring does not fit securely against the pot use a large rubberband or heavy tape to secure it. The sleeve cage must be secured to prevent it from slipping down away from the pot, which would allow beetles and larvae to escape, or predators to enter the cage. Periodically check that the bottom of the sleeve cage is secured tightly against the pot.

Galerucella Development

The *Galerucella* beetle adults will begin to feed on the foliage of the plant soon after placing them in the cage. Holes in leaves are an indication of adult feeding. Males and females will begin mating soon after feeding. It may take up to two weeks before the females begin to lay eggs. The eggs are laid in small groups on stems and leaves. They are round, pinhead-sized



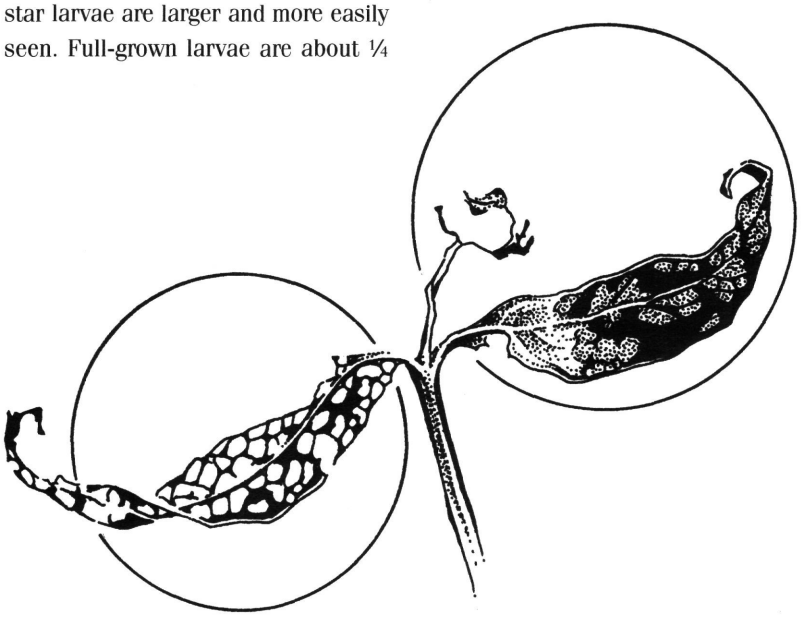


Galerucella eggs

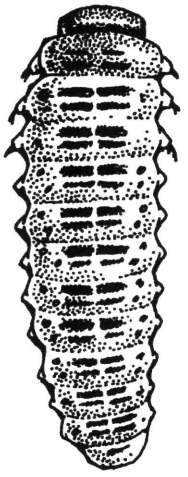
and white to cream-colored with a thin black, stringy deposit across the top of each mass. The black material is excrement that the female deposits. Egg masses are small (0.5–1 mm diameter) and can be very difficult to see through the screen, but keep looking carefully and you will eventually become quite good at spotting them. Females lay eggs for several weeks.

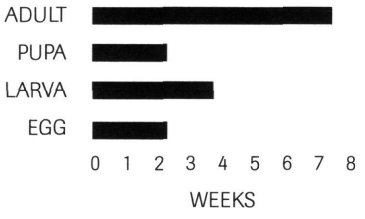
Eggs hatch within two weeks. Larvae are yellow to orange and have black stripes across the width of the body. The larvae have three growth stages, called instars. The first two instars are very small and feed concealed in the growing points of the plant. You will probably not seem them, but damage to the tips will be noticable. Third instar larvae are larger and more easily seen. Full-grown larvae are about ¼ inch long. The first indication that late second and third instar larvae are present will be a characteristic type of leaf-feeding. These larger larvae eat the upper layer of the leaf, leaving the lower layer behind. This "window-paning" of the leaves occurs near the top of the plant first and progresses downward over the entire plant. Fine, dustlike, black excrement (frass) collecting on upper leaf surfaces or sleeve cage folds is another indication of feeding activity.

The larvae take about three weeks to become full-grown. Last instar larvae crawl to the soil and burrow just under the surface where they change into a pupa. This is the growth stage between the larva and the adult. Pupae do not feed and are not mobile. The pupal stage lasts about two weeks, after which the new adult beetles crawl out of the soil and begin to look for food. The diagram below shows approximate development times for each life stage. These are highly variable, depending on temperature and food-quality.



Galerucella larva





The newly emerged adults gather at the top of the sleeve cage. These new beetles are lighter colored than the older beetles that were originally placed on the plants. The combination of extensive defoliation, new beetles at the top of the cage, and few larvae remaining on the plant indicate that the beetles are ready for release into a wetland. From the time that beetles are put into the cages to the time that the

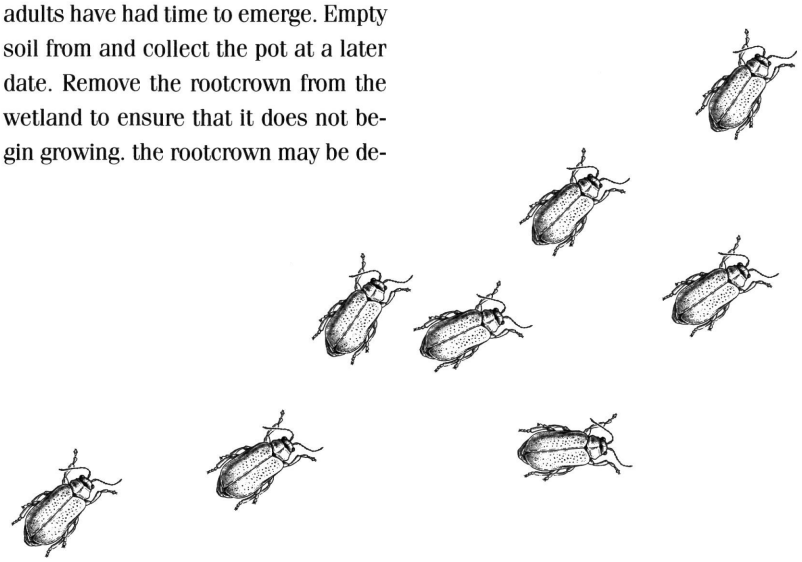
new generation of beetles are ready to be released into a wetland will be from 6 to 8 weeks. Do not wait for all of the new beetle adults to emerge. In most cases, the plant will be heavily defoliated by the larvae and there will not be enough food to sustain the adults for very long. If the plant is completely stripped of its foliage and many larvae (more than 50) are still present it may be necessary to supplement feeding with purple loosestrife from your "extra plant" or foliage collected from a wetland. If collecting from a wetland be sure that the foliage has not been treated with any insecticide (against mosquitoes, for example). Place the freshly cut shoots in a container of water and place in contact with the stripped plants. Larvae will move to the new shoots to feed.

Making the Release

Take the entire pot with tomato and sleeve cage to the release area. It may be difficult to transport the potted plant intact in the pot due to limited vehicle space. In this case, loosen the sleeve cage from around the pot and cut the stems off just above the soil line. Cover the four legs of the tomato cage with tape to prevent them from ripping the sleeve cage and pull the drawstring closed at the bottom of the cage. Cover the pot with a cloth, garbage bag, etc. and secure it around the pot with string or tape. Take the cage with plants inside and the pot to the release area.

Within the release site, choose a location which is not readily visible to vandals who may disturb the pot or release area. Also, beetles prefer areas that receive full sunshine, so avoid areas that will be shaded for a long period each day. Place the potted plant with the sleeve cage still in place next to purple loosestrife plants already growing in the release area. Set the plant on the ground where it will remain upright so that the unemerged insects in the soil will be able to safely complete development. Remove the sleeve and tomato cage from the pot. Invert the sleeve cage and shake the beetles off of it. If there are larvae inside of the cage, gently place them onto purple loosestrife plants. Beetles and remaining larvae will move to new plants to continue feeding and development. Leave the intact pot, plant and soil behind for several weeks, until all of the adults have had time to emerge. Empty soil from and collect the pot at a later date. Remove the rootcrown from the wetland to ensure that it does not begin growing. the rootcrown may be destroyed by leaving it where it can dry out completely.

In addition, record the exact location of the release site with a diagram and with measurements from permanent landmarks (trees, docks, etc.). It is very important to have multiple means of relocating the release site since vandals, seven-foot-tall loosestrife, ice or heavy snow can easily remove or obscure other markers you will place. Use PVC pipe to mark the release area. A 6-to-8-foot length of PVC pipe can usually be sunk 2 to 4 feet into the wetland. This will ensure that the same spot may be located in the fall and in subsequent years to monitor the progress of your release.



The Nuts and Bolts of Natural Enemy Rearing and Release

Outdoor Rearing of *Galerucella* Beetles

The following is a stepwise procedure for the outdoor rearing and release of *Galerucella* spp. beetles for biological control of purple loosestrife (*Lythrum salicaria*) in Michigan.

Overview

Using this procedure, purple loosestrife rootcrowns will be potted and placed in a children's wading pool with water. Each pot will have a tomato cage placed into it and a fine mesh bag that serves as a cage pulled over the top. After 4 to 6 weeks of plant growth, you will receive *Galerucella* beetle adults to place into each cage (approximately 25 per cage). The beetles will produce another generation, with new adults ready for release in about 6 to 8 weeks. It is expected that 500 to 1000 new beetles will be produced from each pot. With 10 pots in each wading pool, 5000 to 10,000 or more adult beetles will be produced for release into a purple loosestrife infested wetland.

A typical timeline for rearing and release of *Galerucella* beetles is given below. Your dates will vary depending on your location and weather.

EVENT	APPROX. DATE
Pot rootcrowns	April 5
Place beetles into cages	May 10
Release beetles into wetland	June 30

Getting Started

The first step will be to assemble the materials necessary for the rearing process. A list of supplies needed for the beetle rearing kit and their approximate cost is listed in the box above.

Outdoor Beetle Rearing Kit

ITEM	COST	QUANTITY
Wading pool (6-foot diameter)	$15.00	1 ea.
Wire tomato cage (42 in.)	$2.50	15 ea.
Soilless mix (Baccto™ High-Porosity Professional Planting Mix, 40 qt., or similar product)	$4.00	2 ea.
Osmocote™ fertilizer 14-14-14 NPK (2½ lb.)	$12.00	1 ea.
Four gallon pot	$1.00	15 ea.
Six-foot metal fence posts (T-post)	$2.00	4 ea.
Heavy duty string	$1.50	1 ea.
Sleeve cage	–	12 ea.
Aspirator	–	1 ea.
PVC pipe (8 ft.)	50¢/ft.	4 ft.

Suppliers

You may be able to purchase all of your supplies from a local garden center or nursery (consider asking for donated materials). Alternately, you may wish to order your supplies. Costs can vary considerably depending on the supplier. Be sure to call suppliers for current pricing, ordering restrictions, shipping costs. Some suppliers have minimum purchase orders. To take advantage of bulk pricing some supplies must be purchased in whole units, e.g., pots in bundles of 50. The following companies are listed as suggested sources of supplies only and this does not constitute an endorsement.

- Hortmark, 302 S. Main, P.O. Box 127, Capac, MI 48014; tel (800) 482-4800 or (810) 395-2075
- Burton Flower & Garden Supply, 4570 Commercial Ave., Suite B, Portage, MI 49001; tel (800) 482-4800 or (616) 324-3750

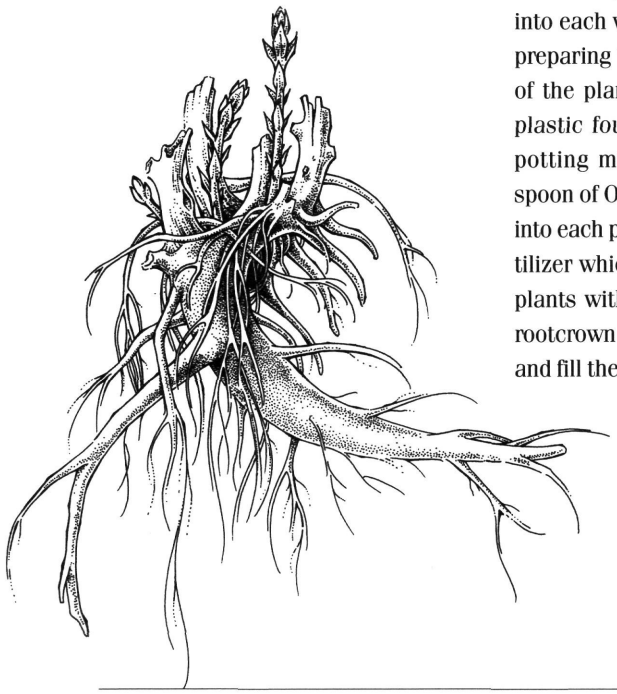
Collecting Purple Loosestrife Rootcrowns

Prior to growing plants, purple loosestrife rootcrowns must be collected. The rootcrown is the portion of the plant which survives the winter. It consists of hard, woody storage roots and stem buds which arise from them. Rootcrowns are used to establish plants rather than establishing from seed because the rootcrowns will produce multiple, hardy stems in much less time and with less effort.

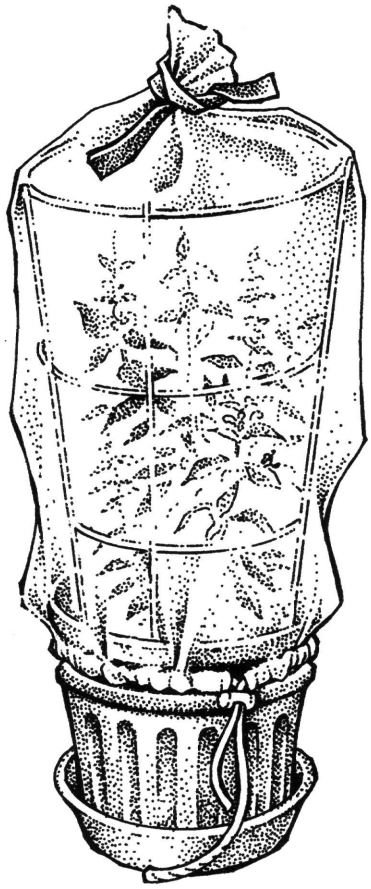
Rootcrowns may be collected in the late fall after purple loosestrife topgrowth has died, or early in the spring prior to new growth. Collect from mature stands of purple loosestrife which are two years or more old. Mature stands have larger rootcrowns which contain more stored energy for plant growth.

Locate groups of dead stems from the most recent year's growth and dig

around these "clumps" to dislodge them from the soil. A tile spade, one with a long, narrow blade, works well for this type of digging but any spade will work. Use the spade to break apart large rootcrowns into sizes that will fit into the pots being used. These will be roughly eight inches in diameter. Using the spade, knock away loose soil from the rootcrowns and cut away all but about six inches of the dead stem topgrowth. It is not necessary to clean all soil and debris from the rootcrowns. Live, healthy rootcrowns have a tan to brown color, are pink to whitish on the inside and are somewhat flexible. Rootcrowns which are dead will be charcoal-black in appearance and brittle. Sometimes buds can be found sprouting from the rootcrowns, ensuring a healthy rootcrown has been chosen.



Rootcrowns collected in the spring may be potted immediately, or stored outdoors for a short time. Keep the rootcrowns moist until ready for planting. Collection of rootcrowns will need to take place about 12 weeks prior to the date you plan to release new beetles. Fall-collected rootcrowns may be stored outdoors. If collecting in the fall, gather twice as many rootcrowns as you will need in order to compensate for winterkill and other factors that can damage the rootcrowns. Moisten the pile, then cover it with a tarp to help keep rootcrowns moist and out of the light. Do not cover with straw as this attracts nesting animals that may feed on the rootcrowns during the winter months.



Potting the Rootcrowns

Although you will be placing 10 pots into each wading pool, we recommend preparing 15 pots. This is in case some of the plants fail to grow. Fill the 15 plastic four-gallon pots halfway with potting mix and sprinkle one tablespoon of Osmocote or similar fertilizer into each pot. This is a slow-release fertilizer which will gradually provide the plants with needed nutrients. Place a rootcrown into the center of each pot and fill the remainder with potting mix. Pot the rootcrowns oriented in the same direction they were growing in the wetland, i.e., stems directed upwards and cover with potting mix. Multiple stems will grow from each rootcrown. Ideally, the rootcrown put into each pot will result in six or more healthy stems per pot. Several smaller rootcrowns can be combined within a pot to produce this number of stems. *Do not press the potting mix into the pot.* The beetle larvae move into the top inch of the soil to pupate and this region should remain loose.

For the initial wetting of the potting media, water the tops of the pots to settle the media around the rootcrowns. Add more potting media to pots that need it. The soil line should be within

an inch of the top of the pots. After this initial wetting, water plants by filling the pool only.

Place a tomato cage into each of the 15 pots, pushing them all the way to the bottom. Wrap tape around the welds on each tomato cage to protect the sleeve cage from tearing. Use string or a rubberband to tie off the end of the sleeve that has no drawstring, this is the top. Slide a sleeve cage over the tomato cage and down onto the upper lip of the pot. Most pots will have a groove along the upper lip which works well to hold the drawstring in place. Place the drawstring in the groove and pull it tight around the pot. It is very important to have a tight fit between the cage and the pot. Putting the sleeve cage on at this point is important to help keep aphids and predators from infesting the plants prior to beetle rearing. At any point prior to receiving beetles you may exchange a healthy plant for one that is not growing vigorously.

Place the wading pool outdoors on level ground in an area that gets full sunlight. Do not place the pool in an area where air movement is greatly restricted. Evenly space the pots within the pool to allow air to move freely between the cages. Put potted plants into the wading pool and add water to the pool. *Do not water the top of the pots again,* as this may cause a crust to form on the potting media surface, disrupting larval movement and pupation in the soil.

Pots should be supported to remain upright in a wind when the plants become taller. The tops of the sleeve cages can be tied to any handy overhead support such as a clothes line. Alternatively, drive four fenceposts into the soil in a square pattern around the pool. Tie heavy string or light wire to the posts and connect them in an X-pattern. Use string or a clothespin to attach the top of each sleeve cage to one of the lines to provide support.

Maintaining the Plants

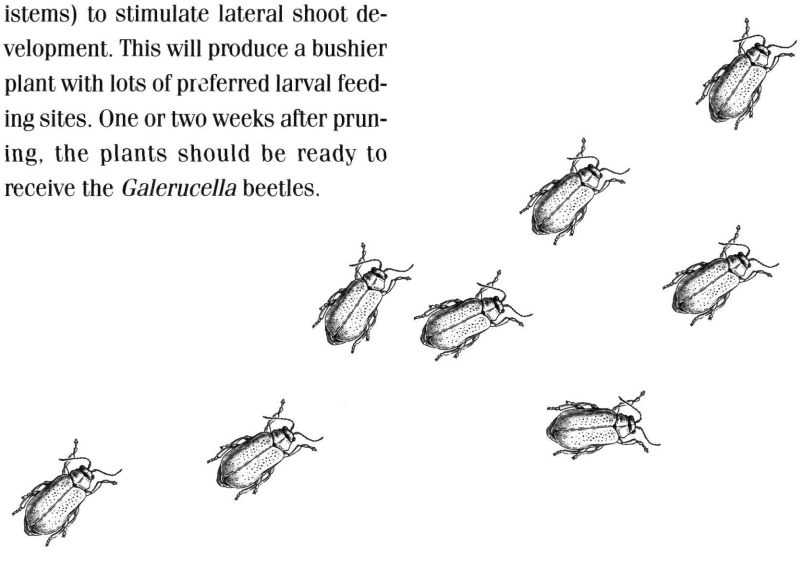
Make sure that plants have adequate water at all times. Keep about three inches of water in the pool. Too much water gives the pots enough buoyancy to easily tip over. An overflow hole should be cut into the side the pool 5 inches from the bottom to prevent tip-overs when heavy rainfalls occur. Periodically check that each sleeve cage is secured tightly around each pot.

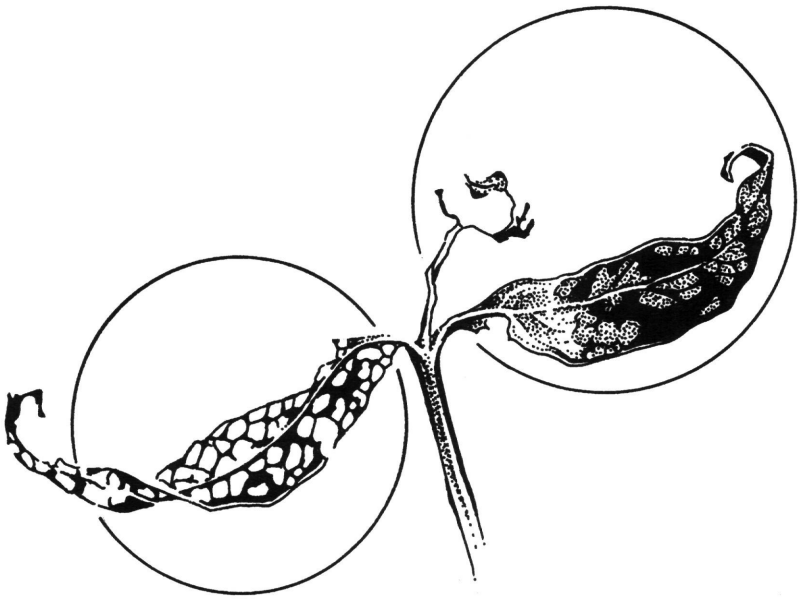
When plants reach about 12 inches in height, pinch off about half an inch of the tip of each stem (apical meristems) to stimulate lateral shoot development. This will produce a bushier plant with lots of preferred larval feeding sites. One or two weeks after pruning, the plants should be ready to receive the *Galerucella* beetles.

Infesting Plants with Beetles

Galerucella beetle adults will arrive via overnight delivery and must be promptly placed onto plants. They will be sent 250 per container with enough purple loosestrife foliage to keep them fed during their trip. Keep the shipping container with beetles inside, out of direct sunlight until ready to infest plants. *Do not expose beetles to temperature or humidity extremes while in the shipping container.*

Use the aspirator that has been provided to remove 20 to 25 beetles from the bulk container for each potted plant. Cover the bulk container when not aspirating to prevent escapes. Some of the beetles will fly away when the container is opened. To prevent this from happening, remove a sleeve cage from one of the extra plants, lay it flat and place the container inside. Aspirate beetles from the shipping container and any escapes from the inside of the sleeve cage. Untie the string at the top





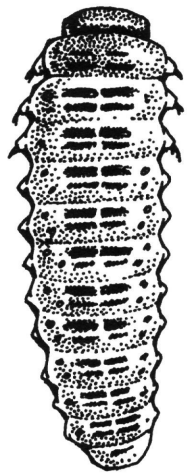
of the sleeve cage you want to infest with beetles and shake them out of the aspirator vial into the cage. Securely close the cage top. Repeat for each pot. The beetles are very good at "hiding," so half or fewer of the beetles will be readily visible. If there are extra beetles they may be divided up among the pots or placed on your extra plants.

Be sure to secure the drawstring at the bottom of the sleeve cage tightly against the pot. If the drawstring does not fit securely against the pot use a large rubberband or heavy tape to secure it. The sleeve cage must be secured to prevent it from slipping down away from the pot, which would allow beetles and larvae to escape, or predators to enter the cage. Periodically check that the bottom of the sleeve cages are secured tightly against the pots.

Galerucella Development

The *Galerucella* beetle adults will begin to feed on the foliage of the plants soon after placing them in the cages. Holes in leaves are an indication of adult feeding. Males and females will begin mating soon after feeding. It may take up to two weeks before the females begin to lay eggs. The eggs are laid in small groups and may be attached to stems and leaves. They are round, pinhead-sized and white to cream-colored with a thin black, stringy deposit across the top of each mass. The black material is excrement that the female deposits. Females lay eggs for several weeks. Eggs can be very difficult to see through the screen, but keep looking carefully and you will eventually become quite good at spotting them.

Eggs hatch within two weeks. Larvae are yellow to orange and have black stripes across the width of the body. The larvae have three growth stages, called instars. The first two instars are very small and will probably not be noticeable through the sleeve cage. Third instar larvae are larger and more easily seen. Full-grown larvae are about ¼ inch long. The first indication that larvae are present will be a characteristic type of leaf-feeding. Larvae eat the upper layer of the leaf, leaving the lower layer behind. This "window-paning" of the leaves usually occurs near the top of the plant first and progresses downward over the entire plant. Fine, black, dust-like excrement (frass) collecting on leaf surfaces or sleeve cage folds, is another indication of feeding activity.



The larvae take about 3 weeks to become full-grown. Last instar larvae crawl to the soil and burrow just under the surface where they change into a pupa. This is the growth stage between the larva and the adult. Pupae do not feed and are not mobile. The pupal stage lasts about two weeks, after which the new adult beetles crawl out of the soil and begin to look for food. The diagram below shows approximate development times for each life stage. These are highly variable, depending on temperature and food-quality.

The newly emerged adults gather at

the top of the sleeve cage. These new beetles are lighter colored than the older beetles that were originally placed on the plants. The combination of extensive defoliation, new beetles at the top of the cage, and few larvae remaining on the plant indicate that the beetles are ready for release into a wetland. From the time that beetles are put into the cages to the time that the new generation of beetles are ready to be released into a wetland will be from 6 to 8 weeks. Do not wait for all of the new beetle adults to emerge. In most cases plants will be heavily defoliated by the larvae and there will not be enough food to sustain the adults for very long. If a plant is completely stripped of its foliage, and many larvae (more than 50) are still found on the plant, it may be necessary to supplement feeding with purple loosestrife shoots from another pot or collected from a wetland. If collecting from a wetland be sure that the foliage has not been treated with any insecticide (against mosquitoes, for example). Place the freshly cut shoots in a container of water and place in contact with the stripped plants. Larvae will move to the new shoots to feed.

Making the Release

Unless a pickup truck or van is available, it may be difficult to transport the potted plants intact with tomato and sleeve cages due to vehicle space restrictions. In this case, loosen the sleeve cage and cut the stems just above the soil line. Cover the four legs of each tomato cage with tape to prevent them from ripping the sleeve cage and pull the drawstring closed at the bottom of the cage. Cover the pot with a cloth, garbage bag, etc., and secure it around the pot with string or tape. Immediately take the cages and pots to the release area. Do not leave the beetles inside of a vehicle for a prolonged period.

Within each release site, choose a location which is not readily visible to vandals who may disturb the pots or release area. Also, beetles prefer areas that receive full sunshine, so avoid areas that will be shaded for a long period each day. Place potted plants with the sleeve cage still in place next to purple loosestrife plants already growing in the release area. Space the pots around an area of about 33 feet by 33 feet square (10×10 meters). Set the potted plants on the ground where they will remain upright so that the unemerged insects in the pots will be able to safely complete development and emerge. Remove the sleeve and tomato cage from the pot. Invert the sleeve cage and shake the beetles off of it. If there are larvae inside of the cages, gently place them onto purple loosestrife plants. The beetles and remaining larvae will move to new plants to continue feeding and development. Leave the pot, plant, and soil intact for several weeks, until all of the adults have had time to emerge. Empty soil from and collect the pots at a later date. Remove the rootcrown from the wetland to ensure that it does not begin growing. The rootcrown may be destroyed by leaving it where it can dry out completely.

Use PVC pipe to mark the four corners of the release area. A 6-to-8-foot length of PVC pipe can be sunk 2 to 4 feet into the ground. Use a permanent marker to label each PVC pipe with the direction of the corner represented, e.g., NE, SW, etc. to help you locate all four corners of the release area when the wetland vegetation becomes taller and more dense. This will ensure that the same spot may be located in the fall and in subsequent years to monitor the progress of your release. In addition, record the exact location of the release site with a diagram and with measurements from permanent landmarks (trees, docks, etc.). It is very important to have multiple means of relocating the release site since vandals, seven-foot-tall loosestrife, ice or heavy snow can easily remove or obscure other markers you will place.