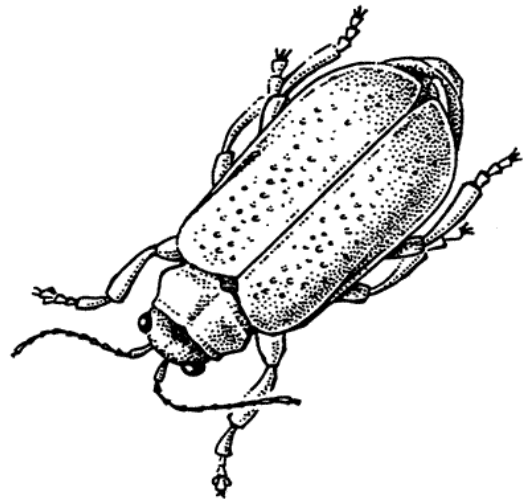
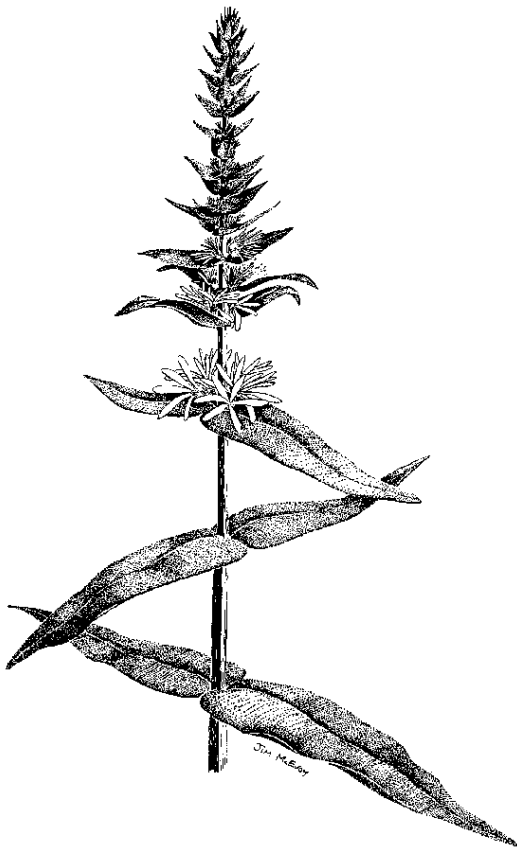


Biological Control of Purple Loosestrife

Galerucella Rearing Guide



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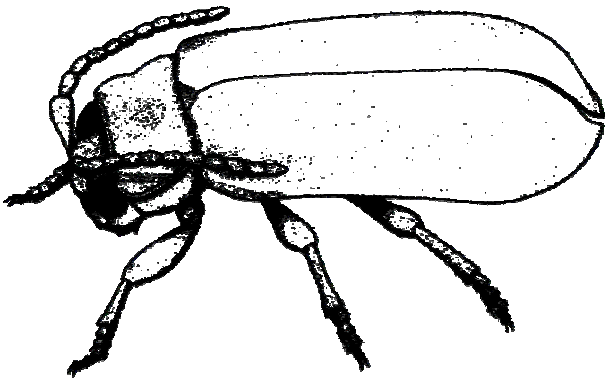


York County Soil & Water Conservation
District

Beetle Rearing Protocol

This guide will explain the fine points of propagating *Galerucella* beetles, a biological control agent for purple loosestrife. Releasing biocontrol beetles throughout the range of loosestrife will allow native plants to re-establish themselves on invaded wetlands, restoring the biological viability of these important ecosystems.

Several quality references for rearing *Galerucella* beetles exist online which are both helpful and easily accessible. We prepared this document to provide our conservation partners with a rearing guide specific to southern Maine, in terms of beetle and loosestrife life-cycle timetable. In addition, many guides assume beetle breeding-stock are being purchased; this guide describes how to collect local beetles from previously established populations in the wild. We have learned some specific tricks of the trade through our experience in southern Maine over the last several years and hope these will prove helpful in the practical application of beetle propagation.



Biological Control of Purple Loosestrife

Four species of insect have been approved for the biological control of purple loosestrife (*Lythrum salicaria*) in the United States. These species include a root-mining weevil (*Hylobius transversocittatus*), two flower-feeding weevils (*Nanophyes marmoratus* and *N. brevis*) and two species of phytophagous (leaf-feeding) beetles (*Galerucella californiensis* and *G. pusilla*). The *Galerucella* beetles, because of their prolific breeding and voracious appetite,

have proven to be the easiest to establish and the most effective biocontrol agent. The two *Galerucella* are very similar species and appear to occupy the same ecological niche in their native Eurasian wetlands. *G. californiensis* may be better adapted to cooler climates, and seems to be the more abundant species at release sites in New England and Great Lake states (McAvoy and Kok 2004).

Galerucella have been rigorously tested for host-specificity to purple loosestrife and examined for potential threats to native and ornamental plants in North America. Various testing agencies (USDA ARS, Cornell University) have concluded *Galerucella* pose no perceived threat to native and ornamental plants. *Galerucella* have been observed to nibble on other plant species in specific and infrequent occasions; i.e. the *Galerucella* population may balloon when first established and outgrow available loosestrife. In this case beetles nibble on

other plants while dispersing, yet non-target feeding is insignificant and limited to one season as the population comes in balance with loosestrife in subsequent years (Blossey et al 1994, Kaufman and Landis 2000). *Galerucella* are unable to complete their lifecycle on anything other than *Lythrum salicaria*. The beetles are thus limited by the presence of loosestrife, and once loosestrife supply is exhausted the beetles must disperse to new patches or perish.

Collecting Loosestrife Host

You will need to grow a lush crop of purple loosestrife in order to feed your beetles. Selecting sites for loosestrife collection is best done in the late summer while purple flowers are conspicuous; you will dig rootstock the following spring. Also, hauling loosestrife root crowns out of a wetland is cumbersome, so choose an easily accessible site.

The loosestrife you grow must be vigorous enough to support several hundred feeding beetles. Collect from healthy stands of multi-stemmed loosestrife, isolated from previous beetle release. Plan site locations for loosestrife harvest, beetle collection, and beetle release in advance.

The first step in beetle rearing is to prepare the necessary equipment—the rootstock you will collect must spend a minimum time out of soil, so have everything ready at your growing site. Before jumping into the swamp to collect loosestrife, assemble the following items at your rearing site:

- plastic wading/kiddie pools (~5.5 to 6.0 ft diameter; each pool holds 12-14 pots)
- 3-gallon plastic nursery pots

- potting soil (e.g. Pro-Mix™. One 3.8 ft³ bale fills about 22 pots)
- slow-release fertilizer pellets (e.g. Osmocote 17:6:10)

Your loosestrife will need 3-5 weeks of growth before acquiring the bulk necessary for supporting the beetles' appetite. Therefore, it is critical to collect early as possible (after spring thaw), gather vigorous root crowns, and provide special care to delicate young buds. If necessary, leave flagging around a few loosestrife clumps the summer before digging to aid identification. In spring, previous year's



dead loosestrife stems and flower spikes reveal the location of underlying rootstock; a plant with 6-8 stems will have an appropriate sized root crown (as large as will fit in your pots). Larger roots can be broken apart, and while large roots are ideal, 2-3 smaller roots can be combined to fill a single pot. Use a round-pointed shovel around the root, apply leverage to loosen the plant, then reach into the muck (don't worry, you'll

get dirty anyway) and heave out that loosestrife!

Cut off any dead stems and if standing water is nearby, wash some muck clear to facilitate handling. You will need to *fully* wash roots of all muck before potting as to leave loosestrife seeds onsite and remove predatory insects that would otherwise consume you beetles. Preferably roots are washed with a garden hose near the collection site—this makes handling much cleaner and easier. Rootstock must remain moist until potted. You might park a truck near the pulling site, so that roots can be transported in a half-filled wading pool.

Loosestrife harvest alternative:

Harvest roots in fall after first hard frost and store over winter in plastic bags. This option is limited to those with a cold room facility (~40°F); the key is keeping roots moist and free of light exposure

Loosestrife Potting & Maintenance

Now pot the loosestrife. Fully dampen potting soil in a wading pool and fill pots ¼ full with soil. Fertilize at rate on label (~50g per pot), add more soil so pots are ½ full, and mix. Insert a root crown and fill pots to within 1-2" from the top, making sure roots are well covered. Place 12-14 pots of loosestrife in each pool. The quantity of feeding material you grow will limit the sustainable number of beetles in each pot, so don't be stingy with the rootstock.

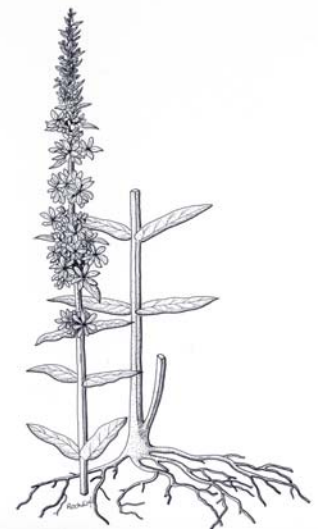
Loosestrife pools should be in full sunlight; arrange in rows so that clothesline can be strung overhead. To

prevent flooding during rain, it is important to cut two small drainage holes on the side of each pool, just above the half-full waterline. Keep enough water in the pools so the soil is moist but never water pots directly.

To stimulate lateral buds and produce bushy plants, carefully pinch back the tip of each shoot when 12" tall. Your loosestrife should be *at least* 18" tall before beetles are introduced. Plants lacking size will be consumed before the beetles complete their lifecycle, and you'll be committed to the time-consuming task of supplementing your beetles' appetite with handfuls of hand-picked loosestrife from a wetland.

Beetle Collection & Care

The tedious work is over and now you can enjoy the hunt of *Galerucella* beetles. Collect beetles only from well established 'source' populations—previous release sites where beetles have become firmly established—as removing beetles from sites with small, growing populations will inhibit establishment. Have landowner permission, and if participating in a cooperative community effort, have approval from the program coordinator before taking beetles from a location. Beetle collection is a good opportunity to employ the help of volunteers; gathering insects provides quality environmental involvement for



young people and brings adults back to their childhood. Remember, your loosestrife should be growing well and at least 18" high before being subjected to the herbivorous burden of beetles. Also, have the following equipment in place (Figure 1) before gathering beetles:

- steel sign posts (4 per pool row)
- clothesline
- net sleeves

The net sleeves will keep predators at bay and your beetles penned in. The nets should not restrict loosestrife growth, however, so support them with clothes line. Suspending nets from two lines will provide ample growing space; use a post-driver to place 2 posts at each end of a pool row (steel street sign posts work well). Tie lines 5 feet above the ground; if the line spans more than two pools a forked tree branch may be used as a prop to prevent line sag. See Appendix for net sleeve construction.

Small scale rearing: When only growing several pots of loosestrife, use a plastic dish pan to sub-irrigate and a tomato cage to support each net sleeve.

Be creative in attaching nets to loosestrife pots and the clothesline, just remember your nets should be easily opened and resealed (so beetles and possibly loosestrife cuttings can be conveniently added). Fancy nets might have pull cords at each end. Alternatively, secure each net to its pot with two sturdy rubber bands, and seal the top by folding the netting over itself and securing to the line with large steel binder clips. Duct tape is best avoided as instillation is burdensome, pool water and rain may compromise adhesion, and

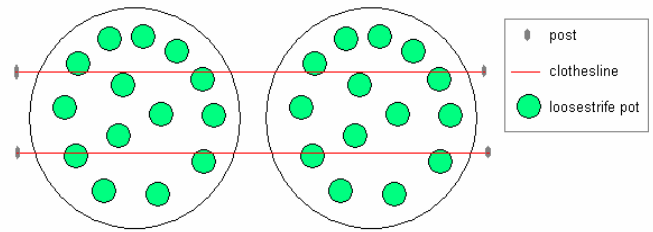


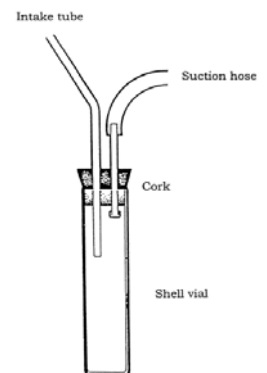
Figure 1. Loosestrife pool layout

nets may be damaged when disassembled in fall.

Try to remove any predatory insects before installing net sleeves. Gently shake and brush off plants from bottom to top to remove insects hiding beneath leaves. Gently shower with a garden hose if available.

Galerucella beetles will emerge from hibernation around the same time crab apples and lilacs bloom (late May for southern Maine). During warm weather, collect beetles in cool mornings when they are less active, however, avoid collecting during inclement weather as beetles will seek shelter. Two of the more successful collecting techniques are described below:

Aspirator - An aspirator consists of a tube and attached container and allows you to selectively 'vacuum' beetles off a plant. To avoid the tedious process of sorting beetles after collection, bring lots of small plastic containers into the field (e.g. film cans, prescription bottles); create airholes with a small nail. Every time your aspirator fills with the number of beetles you will place in a pot (10-15 beetles) empty your aspirator into a film can. Now all you have to do is drop one container into each net sleeve. Aspirators can be purchased for about \$12.00, or see Appendix to construct your own.

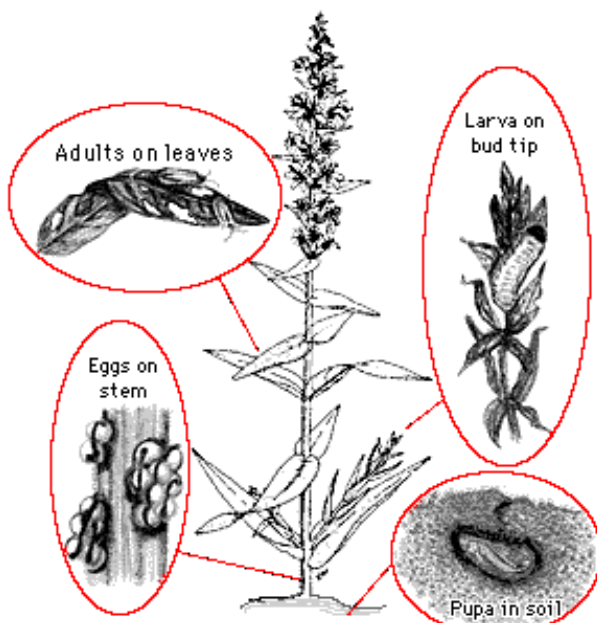


Funnel - To avoid predators, the *Galerucella* beetle's defense mechanism is to drop. Take advantage of this by holding a large funnel or piece of paperboard under a plant while shaking. Most beetles on the plant will drop and you can funnel them into a container. Carefully remove any non-target insects from your catch, also, avoid hot weather as many beetles may take flight before you cap the beetle container. Funnel capture may perform best when beetle density is high and many beetles can be quickly collected from each plant.

Heat will quickly exhaust your beetles so keep them cool! Place scraps of loosestrife in each container and transport in a lightly-chilled cooler with icepacks. Never leave beetle containers in direct sunlight but avoid over-cooling during transport as this may delay breeding.

Galerucella Life Cycle

A detailed familiarity with the *Galerucella* reproductive cycle will contribute to a successful project, and enable you to creatively overcome



unexpected challenges. Time requirement for each development stage is highly correlated to temperature and weather (McAvoy and Kok 2004); development from egg to adult takes 30-40 days.

Eggs- Beetles begin to mate soon after emerging from hibernation in the soil and leaf litter. Adult beetles create "shothole" style damage, chewing many roundish holes through leaves (Figure 2). Each female lays eggs over a period of 3-5 weeks, averaging 10 eggs per day. The spherical eggs are visible on loosestrife leaves and stems as small white clusters of 2-12 frass (excrement) covered eggs. The oviposition period lasts from May to mid June, with limited egg production until the end of July. Humidity is important for egg hatching and will be provided by water in pools. Adults die soon after laying eggs.

Larvae- Eggs hatch after 7-10 days into tiny dark colored larvae (Figure 3). Young larvae feed in leaf buds where they are well hidden from predators and will molt through 3 instars. Most larval growth and resulting plant damage occurs in the 3rd instar; these conspicuous orange larvae feed openly on leaves' photosynthetic tissue, creating "window pane" type damage where only the leaf's transparent cuticle membrane remains unconsumed, framed by leaf veins. When larvae density is high the entire plant may be stripped, except the main stalk and stems.

Pupae- After feeding for 2-3 weeks, 3rd instar larvae descend loosestrife stems to pupate in the top 1/2" of soil or leaf litter layer. Excessive soil saturation or drying during pupation will increase mortality; also, larvae and

especially pupating beetles are susceptible to a fungus. Allowing pool water-level to drop during pupation will probably alleviate fungal mortality, yet maintain at least an inch of water in pools to prevent drying loosestrife. Pupation lasts 2-3 weeks, after which the new generation of adult beetles will emerge.



Figure 2. *Galerucella* feeding damage. Adult 'shothole' damage, top. Larvae 'windowpane' damage, bottom.

Adults- Newly emerged beetles are HUNGRY and you will want to release them *as soon as possible*. Also, if the loosestrife host was entirely consumed during the larvae stage, offer freshly clipped loosestrife to maintain adults until released. Adults are vulnerable to exhaustion during hot weather; emergers will forgo eating and

aimlessly congregate in the top of net sleeves on sunny days. The delicate new adults lack the darker coloration seen on overwintered beetles and cannot fly for 24 to 36 hours after emergence.

Occasionally, newly emerged beetles will mate and produce a small, second generation. Such an occurrence was documented at a release site in Brattleboro VT, yet is probably rare in this region.

The idea that females produce eggs over a limited time span should be taken into account when collecting beetles. For example, beetles collected in late May have a high 'egg potential'; each female may be expected to lay about 300 eggs (10 eggs/day * 30 days). Whereas females collected in early June have already expended half of their laying period should only be expected to lay about 150 eggs on your loosestrife.

Beetle Release

Now that *Galerucella* have multiplied in your predator-free constructed wetlands, release them into the wild! Transport the entire netted pots to the field, place next to a large clump of loosestrife, and remove the net. Nets are usually covered with beetles, so

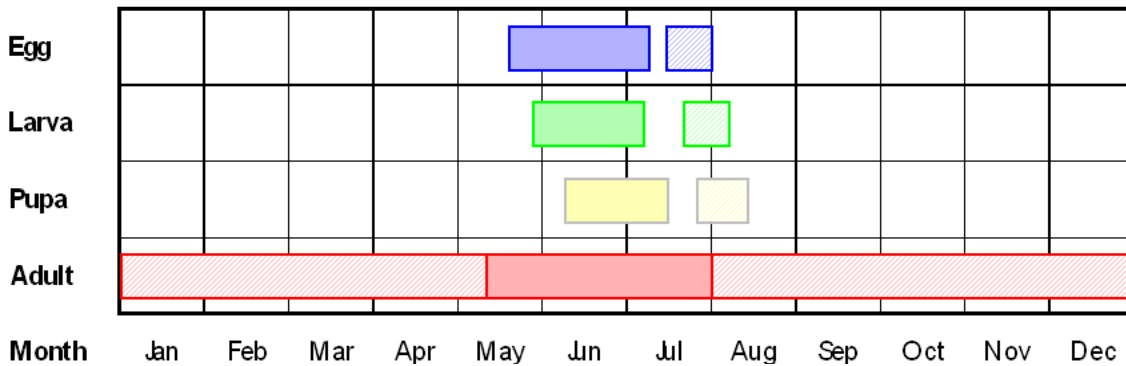


Figure 3. Lifecycle of *Galerucella californiensis* and *G. pusilla*. Solid colored bars represent the length of activity for each of the life stages. Short patterned bars for the upper three stages represent potential second generation activity.

turn the sleeve inside out and gently lay atop wild loosestrife while placing your other pots. Break stems from surrounding loosestrife to create a bridge from pot to wild plants.

Galerucella are 'social' beetles; they release aggregation pheromones and are much more productive per capita when density is high (Grevstad and Herzig 1997, Blossey and Hunt 1999). Help fulfill their social needs by clumping several pots at each release site. 1,000-3,000 beetles per site (3-6 pots) is recommended to ensure a sufficient release for population establishment. Large loosestrife infestation may benefit from multiple release sites. Ideally, release is done on cooler days when beetles are less likely to fly and disperse; avoid release during windy or inclement weather. Beetle populations seem to establish in a much shorter time (1-3 years) from large releases compared to performing small releases (7+ years) as to cover more wetlands. This is possibly a result of *Galerucella's* proclivity to intensify breeding at high densities and the appetite of beetle predators becoming satiated and non-limiting to population growth (Hunt-Joshi et al 2005).

When release is complete, encourage any beetles remaining on nets into their new home. It is important to leave your loosestrife potted and soil undisturbed—many pupae are likely still developing and will emerge shortly. Pots can be collected as the growing season ends.

Release Site Selection

Experience has shown *Galerucella* to establish quickly at some locations, while ecological conditions

cause populations to linger at low levels at other release sites. Indeed, sites selected for release should provide suitable habitat for *Galerucella*, and ideally, offer an avenue of beetle dispersal to loosestrife throughout the landscape. Site selection is particularly important when establishing a 'source' population for future beetle collection.

Loosestrife stands that are prone to flooding and standing water offer poor habitat for overwintering and pupating beetles, which may drown. On flooded sites, beetles are capable of pupating in the spongy, air-filled aerenchyma tissue in the base of loosestrife stems, yet such conditions seem incapable of supporting large beetle populations. Similarly, smaller populations can be sustained when *Galerucella* overwinter along the upland edge of such sites. Hydrology of superior release sites often produces wet meadows, where soil is moist but not saturated.

The aim of biocontrol involves the establishment of a biocontrol agent



throughout the landscape, aided by its natural ability of dispersal. Likewise, an ideal *Galerucella* release site is located on some corridor which facilitates the beetle's spread to regional loosestrife. Rivers, waterways, and interconnected wetland systems are good examples. Public roadways are another prime corridor for both loosestrife and beetle spread. Be conscious of releasing *Galerucella* where your efforts will produce greatest effect.

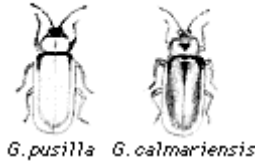
As beetle populations must exist above some minimum size to remain viable, release sites should include at least 100 loosestrife plants or cover ¼ acre area. If possible, strategically scatter release sites throughout the landscape as to cover a larger geographical area; *Galerucella's* natural dispersal will fill in any blanks.

Case Example

This rearing guide may explain how *Galerucella* propagation *could* be done, but not necessarily how you *will* get the job done. The following section will recount the 2006 *Galerucella* experience of the US Fish and Wildlife Service and the York Soil and Water Conservation District; it may be helpful to note the unique challenges faced by these organizations and how they were overcome.

Due to the seasonal nature of employment at Rachel Carson NWR, loosestrife could not be dug and potted until May 15th. This presented us with concerns that the loosestrife would not reach suitable size in time for beetle introduction. Also of concern was the handling of loosestrife roots, which by

Galerucella species



this time in the season each held delicate leaf shoots, about 6 inches long. Note: we mixed fertilizer pellets in the bottom of pots, rather than mixing

throughout, to prevent possible negative exposure to pupating beetles. One hundred fifty pots of rootstock were successfully potted with extra care, however, Maine weather in spring 2006 was unusually overcast and rainy, further delaying loosestrife growth.

Beetles were fully emerged from overwintering and actively breeding before June 4, yet collection was delayed so that reproducing adults would not overcome the small plants. However,

Fresh cut loosestrife: You may need to place cut loosestrife into your pots, either to introduce eggs/larvae or to feed your beetles; however, be aware of possible negative effects. Do not drive stems into the soil when beetles are pupating—you can check for orange pupae by gently exposing the top layer of soil/leaf litter. Other propagators recommend placing cut stems in jars of water for each pot. This will delay wilting and offer better beetle food, but was not done in this project due to time cost and the burden of duct tape sealed pots.

Unwanted predators will certainly be introduced, try to screen out spiders, etc. Also, adding loosestrife stems may encourage fungus and mold. Varying degrees of fungal beetle mortality was observed in our pots, the presence of rotting vegetation from wilted cut stems was unlikely to ameliorate this. See "Fungus" box below.

not wanting to miss the limited window of breeding activity, several plants were sacrificed and inoculated with beetles—eggs could be collected from these pots and distributed to the other plants if need be.

By June 15, our loosestrife was sufficiently robust to support beetles. A cooperative collection day produced about 1,500 *Galerucella*—adequate to

Fungus: Beetles and larvae were observed to succumb to a certain fungus (possibly *Beauveria bassiana*; *Blossey and Hunt 1999*) in several pots. Afflicted adults, pupae, and larvae were overcome with fluffy, white, fungal hyphae. Four pots of the original 150 were entirely devoid of emerging beetles; an abundance of white hyphae on the soil revealed fungal-based mortality during pupation to be the cause. High humidity and stressful conditions will aggravate risk of fungus.

Effect of the fungus was minimal, however, a beetle-lethal pathogen could have potentially serious impacts on a rearing project. To reduce risk of fungus:

- Keep less water in pools after loosestrife is established, (but always have at least 1 inch).
- Avoid adding cut loosestrife stems if possible (adds rotting vegetation).
- Wash all equipment in mild bleach solution between seasons if fungus is detected.
- Never reuse plants or soil.

inoculate half of our pots.

Unfortunately, beetles activity had declined by June 15 and subsequent visits produced a limited catch. More beetles were needed to inoculate the pots, especially considering the adults had a reduced egg potential by this time of season.

We decided to inoculate the remaining pots with eggs, rather than wait to collect delicate larvae. Egg-laden loosestrife stems were cut from the source wetland (and from our sacrificial plants); after counting eggs on a sample of stems we determined a handful-size bouquet would provide the 1,000-2,000 eggs needed per pot. Stems were inserted deep into the potting soil in attempt to delay wilting. Stems wilted within a day or two anyway, nonetheless, egg hatch seemed to be successful and soon plants were covered with tiny larvae.



Figure 4. Ladybeetle adult, larvae, and eggs. Larvae are avid predators of *Galerucella*

Upon close observation, the presence of unwelcome predators was apparent in our small netted ecosystems. Matos and Obrycki (2006) found *Galerucella* is not suitable prey for

ladybeetles (*Coleomegilla maculata*), as they cannot complete development while eating *Galerucella* alone. However, ladybeetle larvae do very well consuming *Galerucella* eggs and larvae whether or not it provides them with a well balanced diet; they were squished with satisfying yellow bursts, without removing net sleeves.

Adults began to emerge from pupation by July 4, thirty days after breeding beetles were first introduced. Netted loosestrife was taken to local wetlands as emergers appeared, pots were retrieved in September after beetles had emerged. All beetles were released by July 14.

Due to our inconsistent approach of inoculating plants, netted loosestrife contained variable numbers of

larvae/beetles. All pots were full, yet many surpassed carrying capacity as larvae stripped loosestrife of all green material. The tiny larvae will squeeze through netting if their food supply is expended. The pots were supplemented with fresh cut loosestrife to prevent confused, hungry larvae from wandering out of nets and being eaten themselves.

Duct tape was used to secure pots to net sleeves, however, this may have been ill-advised considering the resulting inconvenience of adding fresh cut loosestrife. Also, larvae wishing to pupate wander down the net as well as stems to reach the soil, and many became adhered to the tape. Rubber bands may offer a better alternative.

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Biocontrol Resources

Vermont DEC: gives a detailed account of VT's efforts with loosestrife biocontrol:

http://www.anr.state.vt.us/dec/waterq/wetlands/docs/wl_loosestrife-report.pdf

Scientific article about *Galerucella* host specificity (Kaufman and Landis 2000). Beetle's effect on wild & ornamental plants besides purple loosestrife:

<http://www.landislab.ent.msu.edu/documents/PDF%20Pubs/Host%20Specificity%20Testing%20Galerucella.pdf>

Minnesota Coop. Extension website for *Galerucella* rearing:

<http://www.extension.umn.edu/distribution/horticulture/DG7080.html>

Two more sites with info/control techniques on loosestrife & other invasive plants

<http://www.invasive.org>

<http://www.invasiveplants.net>

<http://tncweeds.ucdavis.edu>

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- Page 3: loosestrife root (Michigan Sea Grant)
- Page 4: loosestrife (by Cindy Roche, <http://www.invasive.org>)
- Page 5: pool layout (R. Kenning, US FWS); aspirator (Martin, J.E.H. 1977. Collecting, preparing and preserving insects, mites, and spiders. The Insects and Arachnids of Canada, Part 1. Publ. 1643, Res. Br., Canada Dep. Agric., Ottawa, ON.)
- Page 6: life cycle (U Minnesota, Coop. Extension Service); leaf damage (Bernd Blossey, <http://www.invasive.org>)
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Appendix: Equipment Construction

Net Sleeves

Construct nets of fine mesh or no-see-um type material. A cylinder shape works well and gives ample growing space—cut a rectangle pattern of netting material and sew up one seam, leaving top and bottom unstitched. First, calculate the net dimensions corresponding to your net size:

$$\text{Circumference} = B * \text{Diameter}$$

If your 3-gallon pot has a top diameter of 9 5/8 inches, your net sleeve will have a circumference of $B * (9.625") = 30.2"$. A few extra inches (33") will make the seam and give your net some wiggle-room around the pot. A net height of 6 feet will provide enough extra material to overlap the pot and tie to the clothesline. Sew up the seam and you're done! Now repeat.

Aspirators

Aspirators can be purchased for about \$12.00 from a professional outdoor catalog (www.benmeadows.com, or www.forestry-suppliers.com). Or, recycle some pop bottles and make your own. Screw-on caps make plastic 12oz. bottles are ideal; the narrow tops allow you to empty into prescription bottles without losing any beetles.

Materials:

- plastic tubing (1/2" dia; 2 to 2.5 feet per aspirator)
- plastic bottles (narrow mouth, as with typical 12oz. is ideal)
- filter (gauze or some window screen)
- glue (JB Weld epoxy, hot glue, etc.)

Cut tubing to appropriate length then slice a hole for beetles to drop into the bottle. Note: cutting a small beetle-drop hole (rather than attaching two pieces of tube) makes your tubing sturdier and prevents air leaks where tubing joins bottle. Glue gauze/screen in place. Next carefully cut appropriate size holes in the plastic bottles, make for a snug fit. Insert tube through the bottle and glue in place (roughen plastic surfaces with sandpaper to increase bonding strength). Glue the tube-to-bottle joint liberally; this will be the weakest part of the aspirator and the first place air will leak.

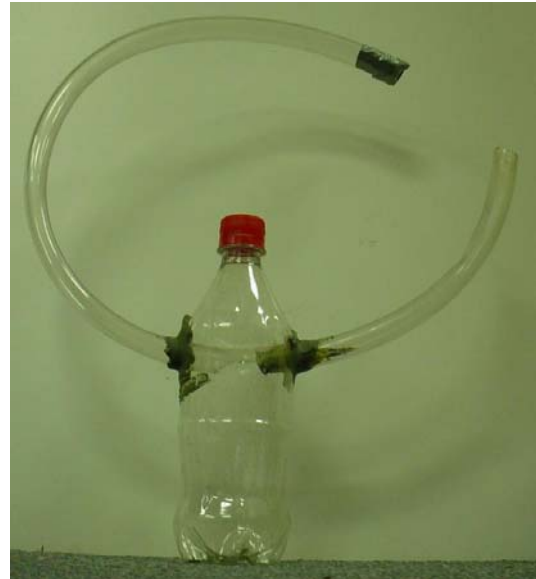
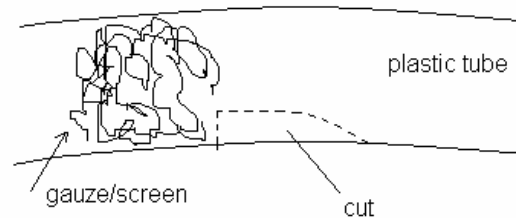


Figure 5. Homemade aspirator. Note: hose was too wide (3/4") for desired vacuum; tapered nozzle was constructed on intake hose with duct tape

