

## 2012 *Galerucella* Beetle Rearing Report and Guide

### PRE-PLANNING NOTES

We did not do too much preplanning for this year's project, focusing mainly on acquiring materials. We purchased potting soil, insect aspirators, an extra kiddie pool, tomato cages and mosquito netting in early March. Additional planning could involve marking loosestrife collection sites in the early fall, determining release sites, coordinating with volunteers, conservation partners, and potential beetle ranchers. Assess the materials you have left over from last year. If you need to sew more plant nets, winter is a great time to do it, and volunteers are often willing to help. The nets we used in 2012 were sewn by a local Girl Scout troop. Even though we did not make nets ourselves, instructions for how to do so are included in this report.

### STEP 1: COLLECTING PURPLE LOOSESTRIFE STOCK

**Materials:** GPS, rounded shovel, boots or waders, heavy duty trash bags (optional)

We collected our purple loosestrife root stock in multiple stages to get a feel for the time required. We ultimately collected all of our loosestrife from the wetlands south of the FEMA gate on Assabet River NWR, at 0298409, 4697810 ± 9 m UTM. We chose the Assabet site because it offered an adequate number of plants and because beetles have not been released there in the past. *Galerucella* beetles overwinter in the duff at the base of the plants, so digging up root balls at sites that already have robust beetle populations could harm many beetles.

**\*Note:** We spent about half a day searching for a suitable loosestrife population from which to collect roots. To save time in the future, we recommend marking plants with flagging tape in the late summer or early fall while they are still in bloom, and noting their location. Even if you do not mark and record the GPS coordinates of your chosen site the summer in advance, record the GPS coordinates when you do collect the roots so you will have a data point for future collection. Based upon 2012 purple loosestrife photo monitoring data, the wet meadow at Water Row harbors a robust loosestrife population. Beetles have not been released at this site since 2006, so future harvesting of loosestrife root balls here may not have much of a negative impact on any existing beetle populations.

We collected our first 10 pots worth of root balls on March 16 and set them up in one plastic 5' diameter child's wading pool. We collected an additional 20 pots worth on March 19, and 7 more on March 20, setting them up in additional pools (see Step 2 for further details). You can begin collecting root balls as soon as possible after the spring thaw. The more time your plants have to grow before you add the beetles the better.

Select plants that have at least four to six dead stems to ensure that the root stock will be large enough. Depending on how early you are able to collect the roots balls, you may see small new loosestrife shoots. This early in the year, they will be small, scaly-looking reddish nubbins. Look for these to

determine which root balls are still viable. Use the shovel to cut around the base of the plant, several inches from the stem. When digging up the plants, we took care to include at least a softball-sized root ball. We wanted the plants to have a large enough root stock to ensure their success, but also needed them to fit in the pots. You can always trim down the roots with garden clippers, so feel free to be more generous with the size of the root ball. After we released the roots from the soil, we left the dead stems attached and swished the roots in the water to rinse off as much muck as possible to make transporting them easier.

**\*Note:** We were transporting our plants a very short distance and it was overcast and raining lightly, so we just put them in the bed of a truck. If it is a very hot and/or sunny day and you have to transport the plants some distance to the rearing facility site, we recommend making arrangements to keep the roots wet so that they do not become stressed. One option is to place the plants in opaque black trash bags and twist the bags shut.

## **STEP 2: SETTING UP THE ARTIFICIAL WETLANDS**

**Materials:** 5' diameter plastic kiddie pools, 10" diameter x 12" tall plastic pots (make sure they have drain holes in the bottom), hose with sprayer attachment, slow-release fertilizer (eg, Osmocote), potting soil, plant clippers

**\*Notes on site selection:** Make sure this location is okay *before* you fill the pools with water and the pots! The plants need plenty of sun, so place the pools somewhere that gets full sun. The ground should also be as absolutely level as possible – even a slight slope can lead to water level differences that may become problematic. Keep in mind that the pools will be in place for several months, so any grass growing beneath them will die. The facility will also be quite visible, so make sure your location has the approval of the land owners. If your facility is in a publicly accessible area or somewhere that people who are not involved with the project will see it, we highly recommend posting a sign or poster that explains what you are doing. Access to an outdoor faucet or hose hookup is not necessary, but *extremely* beneficial. If you don't have access to a hose, you will need to wash your root balls indoors and refill your pools with buckets. This will be extremely tedious, time consuming, and probably messy.

When we set up our pools, we had not yet determined how much sun the location got throughout the day. Our facility was at the Assabet River NWR visitor center, and we did not determine an ideal spot in regards to visitor visibility and usage. As a result, we had to move the pools several times. This was time consuming and a bit tedious. As mentioned in the note above, make sure to assess your chosen location prior to setting up the pools.

Our final location for the pools was near the green generator box by the side parking lot at the Assabet River NWR.

**\*Note:** The plants seemed to grow adequately, but still did not receive sunlight until close to 10:00 am, even in June. In the future, we recommend placing the plants where they will receive

the maximum possible amount of sun to ensure adequate foliage growth to support the beetles and larvae.

When we arrived at Assabet, we thoroughly washed each root ball with the external faucet and a sprayer hose attachment. Some protocols state to scrub the roots, but we simply blasted them thoroughly with the hose, making sure to remove any extraneous roots and organic matter. The idea is to remove as many variables as possible in the loosestrife's growing environment, including other plants and organisms.

Arrange the wading pools where and how you want them for your facility. As noted above, try to keep the pools as level as possible. Cut several small drain holes in the sides of the pools several inches up from the bottom. We were reusing pools from past beetle rearing projects, so the holes were already cut. The drain holes are to prevent the pots from becoming flooded during a large rain event.

**\*Note:** Make sure to position the pools such that at least one drain hole is on the downslope side of the pool! In moving our pools several times, we lost track of this, did not check, and arranged the pools with the drain on the upslope side. The pools only had one drain hole cut in them, and our site had a slope of about 1-1.5 inches across the pools. We experienced some heavy rains, and the combination of the drain on the higher end and the degree of slope resulted in nearly 8" of water in the deep ends of the pools. With that much water, the pots floated and tipped. Even if they did not tip, the water levels in the pools were high enough so that water did not percolate through the soil and out into the pools, and all pots had an inch or two of standing water in them. This happened early in the season for us and so we had not added beetles yet. If this happens when there are larvae and pupae present, you run the risk of drowning many of your beetles' progeny.

Before planting the root balls, we sized them with a pot to make sure they would fit. The roots are very woody, so they may not flex enough to bend into the pot. If you encounter any roots that are too long, simply cut them shorter with garden clippers.

We filled the pots about 2/3 with potting soil and gently mixed in one (1) tablespoon of Osmocote slow-release fertilizer for each 10-inch diameter pot. We then added the root balls and topped off the pots with unfertilized potting soil. The fertilizer quantity was per the manufacturer's instruction, so the amount you add to your pots may differ.

**\*Note:** We opted to not put fertilizer in the top several inches of soil because the larvae pupate in the soil, and we did not want to run the risk of harming them. *However*, about a month after setting up the facility (April 17), we sprinkled one (1) teaspoon of fertilizer on top of the soil in each pot to boost our loosestrifes' growth. We chose to do this because our plants seemed to be lagging behind wild loosestrife growth, and we thought that enough time would pass between fertilizer application and pupation that it would not present a problem to the pupae. Osmocote typically provides three months of slow release feeding, and two months elapsed between when we applied the extra fertilizer and when we estimate our larvae began pupating. We did not notice any negative impacts on our final beetle numbers, but recommend just

mixing slightly more fertilizer than the manufacturer's instruction throughout all soil in the pot when you first plant the roots.

**\*Note:** We consistently used about  $1/3 \text{ ft}^3$  of soil per pot. This may vary depending on the size of your pots and the moisture content of the soil. Err on the side of caution and, budget allowing, be generous with your soil estimate when you purchase it. The soil won't spoil, so you can always store extra for use the following year. We purchased our soil from a garden center that stored the pallets outside, so a number of the bags were quite wet. If you do have left over soil, do your best to let it dry out within the bag to discourage fungal growth. The plastic bags are often vented, so lay them out in the sun and turn and flip them periodically.

We placed the filled pots in the empty kiddie pools, and then added water. We added an additional pot to a pool that already had its several inches of water in it, and even at  $2/3$  full with soil, the pot floated and a lot of water flowed in through the holes on the bottom of the pot, flushing soil and fertilizer into the pool. Don't do this, it's messy! We collected loosestrife root balls on three different days as mentioned above, and ultimately set them up in four different pools. Three pools were 5' in diameter, and each held 11 pots. The remaining pool was 3' in diameter and held four pots.

After all the pots were arranged, we aimed to fill each of the 5' diameter pools with water to a depth of 3" at their shallowest point. Our pools had to be on slightly sloped land, so we split the difference so that the downslope side of the pool was not significantly deeper than 3", and the upslope side no shallower than 2". We also chose to experiment with the 3' diameter pool, filling it to a depth of 4". Bernd Blossey notes that the larvae and pupae need certain levels of humidity to development. He also found that the eggs had a higher hatch rate and that loosestrife leaves appeared to be more palatable on plants and in areas that were inundated with water. We did not want to flood our plants and risk drowning any pupae, so we instead chose to increase the water level in order to hopefully provide a more humid microclimate and more saturated soil due to water pressure and capillary action in the soil.

When the pools were all filled, we watered each pot thoroughly from above – a hose attachment with a "shower" feature works great for this. We watered the plants from above to really saturate the soil. Other protocols state to not water from the top at all, but the reason given is to avoid drowning your beetles, larvae, and pupae. We continued to water our pots from above up until when we added beetles. The primary reason we watered our plants so much was to avoid transplant shock. Particularly as purple loosestrife is a wetland plant, we wanted it to feel as at home as possible.

### **STEP 3: CONTINUED MONITORING OF THE PLANTS**

**Materials:** Hose, ruler, data sheet

We began our continued monitoring of the pools by checking on them every day. We had not done any initial observations of sun exposure for our pool location, so we had to move our pools several times. For future projects, we recommend marking your desired location and observing when the area is in full

sun in the morning, and how late into the evening it receives sun as well. Keep in mind that there will be some leeway with this as the days become longer, so do not become too concerned if you cannot find a location that does not receive an excess of eight hours of sunlight in March.

We completed a data sheet upon each visit noting the observer, time of day, any action we took (such as adding water), and any additional comments. We measured the water levels in each pool at multiple points to get a rough estimate of overall water depth, and added water with the hose accordingly. If it was raining we did not shower the pots with water, but if it was not we gently watered them all to soak the soil. We also noted any growth, new growth, and the progress of the plants. We recorded the length of the longest buds, and once the plants had put forth considerable shoots, we recorded the height of the plants.

As plants reached 12 inches in height, we began pinching off the apical meristems. This encourages lateral bud growth, resulting in fuller and bushier plants. The plants will continue to grow taller and the additional leaves and branching stems will provide more food for beetles and larvae.

In order to ensure that your plants are large enough to support beetles, protocols recommend waiting until your plants are at least 18" tall before adding beetles to them. Some state that it may take 3-5 weeks for your plants to reach this height. We had to wait 8-9 weeks before our plants were large and robust enough to support the beetles. Many plants already had 1-2 inches of growth within a week after we collected them, but on March 26-27 we experienced a hard frost. The pools all had at least ¼ inch of ice on them, and many buds had frost bite. We also had stretches of cool and rainy weather. These conditions likely delayed our plants' growth – we noted growth of nearly 1 inch per day during periods of sunny weather, so water and nutrients were likely not the cause of growth delays.

**\*Note:** To protect against frost, cover the buds in the early evening before it gets dark. You may cover the entire pool with a large cloth, like an old bed sheet or piece of burlap. It's important to cover the plants before it gets dark so that you may still trap adequate heat, and also important to uncover them the next morning so that they do not overheat.

It's a good idea to check on the growth rates of wild purple loosestrife at your collection site to compare to the growth rates of your potted plants. Doing so will allow you to troubleshoot growth problems without losing much time. If your plants seem to be growing significantly slower than the wild plants, try to figure out what may be causing it. Are they getting enough sun? Is the soil remaining saturated? We checked on wild purple loosestrife on April 10, three weeks after we had planted our last batch of root balls and found that the wild loosestrife was taller, though the stems were thinner. Even though tall, thin spindly stems are indicative of inadequate light as the plants are essentially stretching themselves to reach as much light as they can. Our plants being more robust, albeit shorter, than wild loosestrife was therefore a good sign but we still wanted to do the most we could to ensure their best possible growth. Consequently, a week later on April 17 we added the additional fertilizer to our pots.

As our plants became more established, and provided we did not have any heavy rain events or periods of excessive heat, we began checking on the facility every other day rather than every day.

We compared our potted loosestrife growth to wild again on April 30, and at this time observed emerged beetles in the wild as well. At this point our loosestrife plants had reached 12 inches in height and were still slightly shorter than wild loosestrife, but remained leafier.

We waited two days before we started pinching off the apical buds to let a few more plants reach the 12-inch mark, but waiting is not really necessary – you may begin pinching the apical buds off of your plants as soon as they are 12 inches tall.

Once our plants grew to 12 inches, it took two weeks before half of them reached 18 inches tall. This time is likely extremely variable and very dependent on the weather – our plants grew 4 inches in the five days immediately before we added the first batch of beetles.

#### **STEP 4: PREPARING YOUR PLANTS FOR THE BEETLES**

**Materials:** Tomato cages (48" tall), net or mesh sewn into tubes (see Step 4a below for instructions), twine

**Before** you add beetles to your potted loosestrife, you must first set up your net housing. Some protocols give instruction for building around all of the pools but we opted to enclose plants individually. Netting each plant by itself is easier for small-scale facilities and makes release much easier as well – simply transport the entire netted plant to your release site.

We set up the tomato cages around our plants the morning of the day that we collected beetles, but you can place the tomato cages around the plants at any time. Placing them a little earlier in the growing season may even ease the process as you will be able to guide the loosestrife stems into the confines of the cage as they grow, rather than trying to stuff them in after they are 1.5 – 2 feet tall. However, to make sure your plants are getting as much light as they possibly can while they grow, we recommend waiting until shortly before adding the beetles to pull the mesh sleeves over the cage. The day before you collect beetles or earlier that same day, gently pull the mesh tubes over the tomato cages. Leave the draw string at the top of the cage. If you are putting the nets on the cages at the same time that you are putting the cages over the plants, it can be easier to net the cages before putting them in the pots. Try both methods to see which works better for you. Pull the netting down over the top rim of each pot and tie it firmly in place with twine around the pot. Some protocols also suggest using heavy duty or extra large rubber bands to secure the bottom of the nets. We chose to just use twine because it seemed as though getting rubber bands around the pots after they were filled with dirt, 18 inch tall plants, and four foot tall tomato cages would be a hassle. Now you're ready to add some beetles!

#### **Step 4a: Making Mesh Tubes**

**Materials:** appropriate yardage of fine-weave mesh (no-see-um netting, a fine-weave tulle will work as well), sewing supplies, plastic drawstring locks, measuring tape

To make the tubes, you will cut a rectangle of the mesh fabric. We started with fabric that was 42" wide x 78" tall. Double check these measurements with the dimensions of your tomato cages. The fabric rectangle will essentially wrap around the tomato cage, so the **width** should be a few inches longer than the circumference of widest point of the cage (at the very top). Allot 1" for the seam. The **height** of the fabric should be enough that the tube extends several inches below the top rim of the pots and can also be drawn closed at the top of the cage. Allot at least 2" at the top for the drawstring seam, a little more is always fine if the fabric is long enough.

After you have cut out the rectangle of fabric, fold a couple inches over at one of the short ends (42" across). Sew a straight seam across, at least ½" from the fabric edge. This creates the sleeve that the drawstring will be pulled through.

Now fold the rectangle in half lengthwise, so you have a long skinny rectangle. Sew a straight seam about ½" from the **open** edge of the fabric, to create a long tube. Make sure to leave a small opening at the ends of the drawstring sleeve!

Cut a length of string that is long enough to go through the sleeve and have about 12" left over. Thread the string through the sleeve – tying one end to a safety pin and working the pin through the fabric works well. Now thread both ends of the string through a drawstring lock and tie the two ends together. If you opt to not use drawstring locks, just tie the two ends together after making sure you have enough length to tie around the cinched top of the net tube.

#### **Step 5: Collecting Beetles**

**Materials:** Insect aspirators, beetle receptacles (washed out small yogurt containers, film canisters, etc) rubber bands, extra mesh, cooler with ice

You will want to collect 10-15 beetles for every potted loosestrife you are growing. Before you collect the beetles though, it's important to keep in mind that they can be tricky to transfer to your netting loosestrife. They can be quite active and crawl out of your containers, and they will also fly away if given the opportunity. If possible, we highly recommend measuring out 10-15 beetles into small containers right when you collect them. That way you can simply drop the canister into the netting or gently tap the beetles from the container, rather than trying to count and pick out the appropriate number from a larger container as they crawl and fly away.

Film canisters or single-serve yogurt containers would be a great size for this purpose. Wash them thoroughly and then cut swatches of the mesh that will fit over the top. Affix the mesh with rubber bands. Once you get to the collection site, break off a few sprigs of loosestrife leaves for each container. This will help to shade the beetles, keep the humidity levels adequate, and provides a bit of a snack.

It's best to collect the beetles when the weather is clear, so they will be active and out on the leaves. If it is hot, however, make sure you have a way to shade your collection containers. You should also put some ice or icepacks in a cooler and place the beetles in the cooler once you have collected them. We collected beetles both by sucking them up through an insect aspirator and by tapping them into plastic bottles (soda and sports drink bottles are large enough) from the plants. The tapping method worked well to quickly collect many beetles, but created more difficulties when we were distributing the beetles onto our plants. The aspirator worked, but it was often difficult to suck the beetles all the way through the tube. We ultimately took the tubes off and just sucked beetles up through the bent straw on the aspirator, but even then some beetles got stuck inside the straw. Drawing air in too strongly or sharply also caused some beetles to ping off of the inside of the canister, which in turn killed them. We think that tapping the 10-15 beetles needed for each plant into smaller, individual containers would be the best method in future years.

If you do use an aspirator, transfer the beetles to either a large collection vessel or the smaller pre-sorted containers periodically. You shouldn't have more than 15-20 beetles in the aspirator canister at any given time.

If you are unable to sort the beetles as you collect them or before you place them on your plants, you can also chill them to slow them down. This will make counting out the 10-15 beetles needed per plant much easier because the beetles won't be flying away. You can chill the beetles either by keeping them on ice in a cooler or by placing them in a refrigerator for about 30 minutes.

Quickly and carefully distribute your beetles onto the plants. Keep the netting sleeve opening as small as possible as you tap the beetles out of their container to prevent them from flying away. A benefit to chilling the beetles prior to distributing them is that they will be much less active and much easier to place on the plants. Be careful to not freeze them or chill them too extensively, as you don't want to harm them.

**\*Note:** Just as it is important to wait until your plants are 18" or taller before adding the beetles, it is also important to make sure you only add as many beetles as your plant can sustain. We tried to just count the beetles as we put them in to the nets without chilling them first, but as a result several nets got more than the intended 10-15 beetles. We had to supplement nearly all of our plants with extra loosestrife cuttings before and especially after the new adults had emerged, as the larvae had completed defoliated the plant before they were even finished developing.

**\*Note:** Unless you pre-counted the adult beetles that you put in to each net, you very likely ended up with MORE than 10-15 beetles per plant. It's easy to underestimate the number of beetles you have because they are so small.

## Step 6: Continued Monitoring of Your Plants and Beetles

**Materials:** Hose, ruler, data sheet, camera (optional)

As you continue to check on your pools to monitor water levels and plant growth, also observe beetle activity and plant defoliation. As soon as you have added beetles to your plants, add water ONLY to the pools. Do not water the plants from above or shower the soil. Know the beetles' life cycle so you know what to expect and can plan accordingly.

**Seed adults and eggs:** Adults create "shothole" feeding damage on the purple loosestrife leaves. They feed by chewing small, roughly circular holes through the entire leaf (this is different from how larvae feed). Beetles begin mating shortly after emerged from hibernation, so if you are collecting beetles from the wild they likely will already have begun to lay eggs. We saw eggs the day after we collected the beetles. Each female beetle will lay eggs for about 3-5 weeks, from May through June. Some beetles may continue to lay through into July. Beetles lay an average of 10 eggs per day on the stems and leaves of the loosestrife plants. The eggs are whiteish, spherical, and about the size of a poppy seeds. You will see them in small clusters, each egg with frass (excrement) on it. Proper humidity is critical for the eggs to hatch, so you must make sure that there is several inches of water in the pools at all times. The adults will die shortly after they have finished laying their eggs.

**Larvae:** After 7-10 days the eggs will begin to hatch into tiny dark-colored larvae. The first instar larvae are very hard to see, and we found that we could not discern any on our plants at all. The newly hatched larvae will migrate to the top of the plant and feed in the leaf buds. You will know when you have first instar larvae because the top buds of your loosestrife plants will begin to shrivel, and there will be an increase in the dark, thin lines of excrement that the larvae leave behind. The larvae will molt through three instars, and most of the feeding damage to the plants happens during the third instar stage. Third instar larvae are yellowish-orange and relatively conspicuous. Larvae feed on the plant's photosynthetic material so they create "window pane" style feeding damage. You will notice that leaves will have patches of just the outer cuticle and leaf veins remaining. Heavily fed upon leaves will soon turn brown and shrivel. Third instar larvae can cause a significant amount of damage to the plants, and may completely strip your plants of foliage. Watch carefully for this because should it occur, you must supplement the nets with fresh cuttings of purple loosestrife.

**Pupae:** After about 2-3 weeks, the third instar larvae will descend into the top layer of soil to pupate. If the soil is too saturated, they will burrow into the base of the loosestrife stems to pupate. If the soil is much too wet or too dry, you may experience a higher pupae mortality rate. For this reason, it is critical to maintain water levels in the pools, but also critical to not water the soil directly. Larvae and pupae are also susceptible to fungus, so monitor the soil surface and lower plant stems for any fungus. Pupation lasts for about 2-3 weeks, after which the new adults will begin to emerge.

**New adults:** Once the new adults emerge, they will be very hungry! New adults are also very vulnerable, and can become exhausted by heat quickly. If they become overheated, they will stop eating and begin to congregate at the top of the nets. Newly emerged beetles cannot fly for 24-36 after they emerge, so they are at a higher risk from predators. Make plans to release them as soon as you notice adults beginning to emerge.

Include as much detail as possible in your observations of the plant conditions and what the beetles/eggs/larvae are up to. If you can, take photos to keep track of your project's progress. Photos are great for writing up a final report or presentation, and are a fantastic resource if others come to you for assistance in setting up their own beetle rearing facility, or if they are experiencing a specific problem with their facility.

Now that your beetles are in the nets, it's extremely important to look for predators!! The nets will not be perfect barriers, and the drawstring opening can allow a surprising number of unwelcome visitors, even when tightly fastened. Spiders are a threat to the adults and larvae, and lady bugs and their larvae are a particular threat to the beetle eggs and larvae. Play it safe and do your best to remove any outside critters that you find in or on your nets. Two green frogs took up residence in our pools and we made the decision to let them be, as they were more of a threat to the resident mosquito population than our beetles.

Track the life cycle progress of your beetles with each visit. We found that color coding and marking a calendar helped immensely. Continue to observe the status of your plants' foliage. As the eggs hatch and the larvae develop, you will notice your plants becoming increasingly defoliated. About four weeks after we first observed eggs, some of our plants were extremely defoliated, and many more quickly followed suit.

We noticed larvae squeezing out through tiny holes and spaces in the mesh of the nets when their plants became defoliated, probably in search of food. We *very carefully* collected all the larvae that we could see and put them back into their nets on each visit. The larvae are very delicate, so take care when picking them up and moving them. We found that coaxing them onto the top of a pencil and then collected many on a spare loosestrife leaf worked well. Even though they are no longer on the plant, they are still driven to travel down in search of soil in which to pupate. We noticed many that had just continued to crawl down the outside of the nets and into the water. Look carefully for larvae at the water's surface, because many of ours were actually still alive.

**\*Note:** While you will likely have to supplement your plants with fresh clippings towards the end of your project, do your best to keep this to a minimum. The branches will wilt within one or two days, and the addition of rotting plant matter can introduce fungus to your facility. If you do find fungus in any of your pots, wash all contaminated equipment with a mild bleach solution between seasons.

## Step 7: Releasing Your Beetles

A couple of weeks before you expect your new adults to emerge, contact any partners you will be working with to release the beetles. You will need to release the beetles as soon as possible once you see the new generation emerging, and preparing everyone in advance will help make the process go much more smoothly. Be generous with your time estimates, just in case. Our new beetles emerged a week or two sooner than we anticipated, and we had to move very quickly to get them delivered to our partners and released.

To release your beetles, bring the entire netted plants out to your release site. Place the pots next to a relatively dense stand of purple loosestrife and carefully remove the nets. If possible, place the pots where they will be sitting in some water. There will still be pupae in the soil, and they require a certain level of soil moisture to develop. Beetles and larvae are often all over the nets, so examine them closely for both life stages. Turn the nets inside out and drape them across the tops of wild loosestrife plants while you uncover your other pots. After you have finished placing all of your pots, check the nets again and gently remove any larvae or beetles still remaining. Help your beetles disperse by breaking off stems from surrounding loosestrife and creating bridges from the pots to the wild loosestrife. You will likely still have pupae in the soil, so leave your pots out for at least several weeks until you can be sure that all larvae have made it to adulthood.

*Galerucella* beetles are social creatures, and so large releases of multiple pots in one area seem to be much more successful at establishing a healthy beetle population. They release aggregation pheromones and are much more productive when they exist in high densities. Release at least three – six pots at any given location to have a beetle density of about 1,000-3,000 per release site.

When selecting your release site, keep in mind that the beetles will be overwintering and pupating in the soil at the base of the plants. Beetle populations do not appear to be as productive in frequently flooded areas as pupae and overwintering adults tend to drown. In the event of flooding, beetles and larvae will burrow into the spongy tissue at the base of the plant stem both to overwinter and to pupate. They are not as successful when they are forced to do this, however, and beetle populations remain lower at sites that are frequently flooded. Ideal release sites remain wet but have limited standing water and are not prone to flooding, such as wet meadows.

\*Note: Before you release your beetles, make sure you have checked with the property owners about any permits or permissions needed to release a live organism into the wild.

**There are a number of different estimates for how many new generation beetles your plants will produce. Some conservative estimates say that each pot will only yield 500-1000 beetles, but others state 1000-2000 beetles. Use your discretion when estimating your own beetle numbers. If you can**

see your nets teeming with new beetles, then perhaps you can use a larger estimate. Even if your plants are completely defoliated and you have to supplement with wild cuttings, you may not see very many new adults. This could be because many are still pupating, but it could also be because the larvae stripped the plants and the new beetles did not have enough fresh loosestrife, and died before they could be released. In this instance, it would be best to use a more conservative estimate. Ranges are okay to use as well – there is really no way of knowing precisely how many beetles your pots will yield, due to the beetles varying life stages.