Garlic Mustard Biological Control

Developing Biological Control Insects, Working Towards Field Release

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Executive Summary

Use of biocontrol agents to control garlic mustard would provide long-term control of this invasive biennial weed. Potential control agents of garlic mustard have been identified and include four European species of the weevil, *Ceutorhynchus* that attack different parts of the garlic mustard plant. Of these weevils, *C. scrobicollis* is a root mining weevil, *C. roberti* and *C. alliariae* are stem miners and *C. constrictus* larvae develop in seeds of garlic mustard.

Garlic mustard rosettes are most vulnerable to mortality during the winter, when they transition to bolting and flowering plants (Davis et al. 2006). Winter mortality can reduce rosettes populations by 7 to 45% in Minnesota (Van Riper et al. 2010). Of the four *Ceutrohynchus* species, Davis et al. predict that *C. scrobicollis* would be the most effective biological control agent because it attacks garlic mustard rosettes during the vulnerable overwintering period. Evans et al. (2012) predict that *C. scrobicollis* alone can control garlic mustard at some sites.

Problems encountered with rearing biocontrol insects can become a major obstacle to developing a weed biological control program (De Clerck-Floate et al. 2008). In order to develop *C. scrobicollis* as a biocontrol agent for garlic mustard, it was necessary to design reliable and consistent methods to rear the weevils. The purpose of the following studies were to develop mustard propagation methods and *C. scrobicollis* rearing protocols in our High Containment facility the University of Minnesota in anticipation of permission to release *C .scrobicollis* into the field for the biocontrol of garlic mustard. Experiments were conducted to develop the most efficient and consistently reliable methods to rear *C. scrobicollis* from garlic mustard plants.

We have successfully reared *Ceutorhynchus scrobicollis* on caged garlic mustard plants in a growth chamber by alternating growth chamber temperatures and photoperiods to mimic natural conditions in its native range. In Germany, *C. scrobicollis* produces one generation per year and F-1 adults emerge in late May. In containment, a new generation of adults emerged an average of 106 or 110 days after parent weevils were placed on plants for 2011-2012 and 2012-2013 respectively. After emergence, F-1 adults were allowed to feed on garlic mustard rosettes for two to four weeks before they were placed in a summer aestivation period. Simulating a three-month summer aestivation period, followed by a week of fall, and three weeks of winter resulted in optimum levels of oviposition. After receiving shipments of *C. scrobicollis* from Europe, it will be necessary to rear a minimum of one generation in a containment facility to ensure that the endoparasitoid, *Perilitus conseutor*, is not introduced along with adult *C. scrobcollis*. We describe the method we developed to rear parasitoid-free *C. scrobicollis.*

Garlic mustard biological control: rearing the crown-boring weevil,

***Ceutorhynchus scrobicollis* in containment.**

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The purpose of this paper is to describe garlic mustard propagation methods and *C. scrobicollis* rearing protocols developed in our High Containment facility the University of Minnesota in anticipation of permission to release this crown-boring weevil in North America for the biocontrol of garlic mustard. We have successfully reared *Ceutorhynchus scrobicollis* on caged garlic mustard plants in a growth chamber by alternating growth chamber temperatures and photoperiods to mimic natural conditions in its native range. In Germany, *C. scrobicollis* produces one generation per year and F-1 adults emerge in late May. In containment, a new generation of adults emerged an average of 106 or 110 days after parent weevils were placed on plants for 2011-2012 and 2012-2013 respectively. After emergence, F-1 adults were allowed to feed on garlic mustard rosettes for two to four weeks before they were placed in a summer aestivation period. Simulating a three-month summer aestivation period, followed by a week of

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fall, and three weeks of winter resulted in optimum levels of oviposition. After receiving shipments of *C. scrobicollis* from Europe, it will be necessary to rear a minimum of one generation in a containment facility to ensure that the endoparasitoid, *Perilitus conseutor*, is not introduced along with adult *C. scrobcollis*. We describe the method we developed to rear parasitoid-free *C. scrobicollis.*

Nomenclature: garlic mustard, *Alliaria petiolata, Ceutorhynchus scrobicollis*, *Perilitus conseutor*, rearing

Garlic mustard (*Alliaria petiolata)* is an invasive biennial and native to Europe, where it has historically been valued for its medicinal and herbal properties (Grieve 1971). It was first recorded in North America in 1868 (Nuzzo 1993). Since its introduction, this invasive plant has spread to the northeast, mid-west, and western United States (Nuzzo 1993). Garlic mustard is now recorded in 38 states in the U.S. and 6 Canadian provinces (plants.usda.gov) and has the potential for wider distribution (Welk et al. 2002). Garlic mustard is also listed as a noxious weed in eight states in the U.S. (plants.usda.gov).

Garlic mustard seedlings germinate early in the spring, which allows them to maximize soil nutrient and light capture before tree canopy closure, while native species are still dormant (Myers and Anderson 2003, Engelhardt and Anderson 2011). Overwintered, second-year rosettes bolt and flower by May in the Upper Midwest (Katovich, personal observation). With the capacity for abundant seed production, garlic mustard can rapidly colonize mesic forests to produce dense stands (Meekins and McCarthy 2002) and become more competitive than other woody understory species (Meekins and McCarthy 1999) which may reduce native plant diversity (Stinson et al. 2008). Garlic mustard plants also produce phytotoxins (Vaughn and Berhow 1999) that are exuded from root tissue and can directly reduce the growth of native tree seedlings, native grasses and herbs (Stinson 2007). In addition, the invasion of garlic mustard into native plant communities can disrupt the mutual associations between native tree seedlings and arbuscular mycorrhizal or ectomycorrhizal fungi (Roberts and Anderson 2001, Stinson et al. 2006, Wolfe et al. 2008, Anderson et al. 2010) that are critical for tree growth and survival.

Implementation of biological control would provide affordable long-term management of garlic mustard. Currently, four *Ceutorhynchus* (Curculionidae) species are under investigation as potential biological control insects. One species, *Ceutorhynchus scrobicollis* is a crown-

mining weevil, two are shoot miners and one species is a seed feeder. Extensive host specificity testing on the crown-miner, *C. scrobicollis,* has been completed at CABI Bioscience in Switzerland and at a Level 2 High Security Containment Facility at the University of Minnesota (Gerber et al. 2009). Results of these tests indicate that *C. scrobicollis* is a highly specific herbivore. Host range test results have been submitted to Technical Advisory Group (TAG) for Biocontrol of Weeds for approval for field release of *C. scrobicollis* and are under review.

***Ceutorhynchus scrobicollis* biology**. In Europe, *C. scrobicollis* is a common insect found on garlic mustard and field attack rates can reach 100% (Gerber et al 2007). In the field *C. scrobicollis* produces one generation per year. Oviposition begins in September, continues throughout the winter and ends in mid-April (Gerber et al. 2009). Oviposition ceases if the mean daily temperature drops below 5 C (Gerber et al. 2009.) so fewer eggs are laid in December and January. Females lay eggs directly under the leaf epidemnis, in leaf petioles and in root or crown tissue. Larvae progress through three instars, which can be distinguished by the diameter of the head-capsule. In Switzerland, first instar larvae are initially found in late September and by early November, third instar larvae are present. All three instars overwinter in garlic mustard roots and crowns. By late April, larvae exit garlic mustard roots and crowns to pupate in the soil. New adults emerge from early May to mid-June, feed briefly on garlic mustard leaves, then aestivate for the remainder of the summer (Gerber et al. 2009). Feeding and larval tunneling by *C. scrobicollis* on garlic mustard rosettes can cause whole-plant mortality. Alternatively, primary rosette shoots can be killed, releasing crown buds from apical dominance, which results in growth of secondary rosette shoots (Gerber et al. 2007).

Parasitoids. In Europe, *Perilitus conseutor* (Hymenoptera, Braconidae) has been identified as an endoparasitoid of *C. scrobicollis* adults (Haeselbarth, unpublished?). *Ceutorhynchus*

scrobicollis appears to be the only host of *P. conseutor*. Field collected *C. scrobicollis* adults can have parasitism rates of up to 20% (Gerber et al. 2009). In Europe, *P. conseutor* pupae leave their hosts in May and adult parasitoids emerge by late May to mid-June. It is thought that *P. conseutor* adults attack *C. scrobicollis* in the spring or fall (Gerber et al. 2009). Presence of *P. conseutor* in field collected *C. scrobicollis* means that a minimum of one generation of *C. scrobicollis* should be reared in a containment facility to prevent release of this endoparasitoid into North America.

Garlic mustard rosettes are most vulnerable to mortality during the winter, when they transition to bolting and flowering plants (Davis et al. 2006). Winter mortality can reduce rosettes populations by 7 to 45% in Minnesota (Van Riper et al. 2010). Of the four *Ceutrohynchus* species, Davis et al. predict that *C. scrobicollis* would be the most effective biological control agent because it attacks garlic mustard rosettes during the vulnerable overwintering period. Evans et al. (2012) predict that *C. scrobicollis* alone can control garlic mustard at some sites.

Problems encountered with rearing biocontrol insects can become a major obstacle to developing a weed biological control program (De Clerck-Floate et al. 2008). In order to develop *C. scrobicollis* as a biocontrol agent for garlic mustard, it was necessary to design reliable and consistent methods to rear the weevils . The purpose of this paper is to describe garlic mustard propagation methods and *C. scrobicollis* rearing protocols developed in our High Containment facility the University of Minnesota in anticipation of permission to release *C. scrobicollis* into the field for the biocontrol of garlic mustard. Experiments were conducted to develop the most efficient and consistently reliable methods to rear *C. scrobicollis* from garlic

mustard plants. All studies were conducted in growth chambers inside a containment facility where space was limited so not all experiments were repeated in time and space.

MATERIALS AND METHODS

Garlic mustard propagation. Garlic mustard seeds were collected from Silver View Park, in Mounds View, MN (Lat: 45 - 06' 22" N, Long: 093 – 13' 00" W). Seeds were cleaned and stored at 4 C. Seeds were germinated using two methods. The first method consisted of planting seeds in plug trays filled with a standard potting mix. The trays were placed outside in November and lightly mulched with straw (E. Gerber, personal communication). In early spring, the mulch was removed when the seedlings started to germinate. The second germination method consisted of stratifying seeds by placing them in a plastic petri dish between layers of sterilized moist sand. Petri dishes were sealed with Para-film and placed in a refrigerator at 4 C (Baskin and Baskin 1992). After 4 months, seeds were removed and planted in a plug tray filled with a standard potting mix.

Seedlings were transplanted into 3.8 l pots containing a well-drained commercial rice hull growing mix (BM7; 35% bark, 20% rice hulls; Berger Peat Moss, 121 RR1, Saint-Modeste, Quebec, Canada). Depending on the season, plants were grown outside in a shaded area, or grown in a greenhouse with a 16/8 h photoperiod and a temperature of 18 to 21 C. Plants were fertilized with a slow-release fertilizer containing macro- and micro-nutrients. Plants were a minimum of three months old when they were used for *C. scrobicollis* rearing. Care was taken not to overwater plants as this promoted root and foliar diseases.

Aphids were a major problem encountered when propagating garlic mustard in the greenhouse. Secondary pests included diamondback moth *(Plutella xylostella*). Pesticides were not applied for insect control because they could cause adversely affect *C. scrobicollis*. For this reason, garlic mustard potted plants were reared inside large screen cages in the greenhouse. These cages consisted of 2.4 m x 0.9 m frames built from PVC pipe designed to fit inside a greenhouse bench. "No-see-um" polyester netting was used to construct the screen cages that were placed over the PVC frames. The edges of the cages were secured underneath the frames. Ladybugs (*Hippodamia convergens*) were purchased and placed into the screen cages for insect control.

***Ceutorhynchus scrobicollis* rearing and collection of F-1 adults.** All *C. scrobicollis* were reared in growth chambers (Model GR-48, Environmental Growth Chambers, 510 E. Washington Street, Chagrin Falls, OH, 44022; Model E8, Conviron, 572 S. Fifth Street, Pembina, ND, 58271) inside our biosafety level 2 containment facility. *Ceutorhynchus scrobicollis* were reared on individual potted garlic mustard plants with a screen cage placed over the top. Cages were supported with two wires loops stuck inside the pot and secured with elastic around the pot (Gerber 2009). Cages were made of "no-see-um" polyester netting. Greenhouse or field grown plants were used for rearing and were propagated as described previously. Ladybugs were also released into individual screen cages placed over potted plants in the containment facility for aphid control. Plants were placed on plastic saucers and sub-irrigated as needed and care was taken not to overwater.

In the fall, *C. scrobicollis* adults were field collected in the vicinity of Berlin, Germany and shipped to Minnesota. Shipment sizes varied, but were a minimum of 27, the number of individual weevils required to capture 99% of the diversity at the Berlin collection site (Rauth et

al. 2011). Shipments were opened in the University of Minnesota containment facility. Adult males and females were marked different colors with a paint pen to easily differentiate between sexes and to distinguish between F-1 adults and their parents (E. Gerber, personal communication). Weevils were allowed to feed on caged plants for a minimum of two weeks after the arrival of a shipment before they are were used for rearing.

For rearing, three to five pairs of adults were placed on each caged garlic mustard plant. All plants were numbered and the number of males and females added and removed on each plant was recorded. Plants were placed in a growth chamber simulating winter conditions of 15/14 C day/night temperature regime with a 9.5 h photoperiod (Table 1) since *Ceutorhynchus scrobicollis* laid the maximum number of eggs at 15 C (Gerber 2002). The temperature and photoperiod were similar to average winter temperatures and daylength at Berlin, Germany. In growth chambers, both incandescent and florescent lighting was used to provide an average light intensity of 170 µmol m^{-2} s^{-1}, similar to the shaded conditions in the outdoor propagating area.

After emerging, F-1 adults were removed from caged plants and placed on new garlic mustard plants for a minimum of two weeks in "spring" conditions (Table 2). Adults were then placed into "summer" for aestivation. The number and date of F-1 adult collection was also recorded for each plant.

Newly emerged, F-1 adults were collected with a funnel apparatus which covers a potted garlic mustard plant (Figure 1). To assemble the funnel apparatus, a polypropylene funnel, 150 mm x 137 mm (top diameter x height) was spray painted completely black, except for the spout. The inside of the funnel was scored so that adult weevils could crawl up into the funnel. A 5 mm wide piece of foam pipe insulation was placed into a clear plastic tube to secure a garlic mustard

leaf in place. The tight-fitting plastic tube was attached to the top of the funnel to collect emerging adults.

A freshly harvested garlic mustard leaf was placed inside the tube and the tube was attached to the funnel. Any green garlic mustard leaves or stems were removed from the pot, the funnel was placed inside the pot, and a screen cage was placed over the funnel apparatus and was secured with elastic. Plants were returned to the growth chamber. During the adult emergence period, plants were checked every 4 to 6 days and adults were removed and placed onto new garlic mustard plants to feed. A new garlic mustard leaf was placed into the tube and the funnel apparatus was again placed over the plant.

Figure 1. Funnel apparatus used to collect F-1 *C. scrobicollis.*

A study was designed to determine the percent recovery of *C. scrobicollis* adults from garlic mustard plants with the funnel apparatus. Ten F-1 adults were placed on a potted garlic mustard plant with all leaves removed. A funnel with a fresh garlic mustard leaf was placed over the pot. Numbers of adults collected in the vial were recorded every four- to- six days until adults were no longer collected in vials. At each sampling date, weevils collected in the vial

were removed and a new leaf was placed in the vial. The experiment was repeated five times with a single plant as a replication.

Estimates of the optimum number of weeks required to collect all weevils from caged plants was determined for F-1 adults reared in 2012-2013. Only plants where all parent adults were removed after 14 to 20 days were included in the estimates of optimum length of collection time.

Soil medium for optimum *Ceutorhynchus scrobicollis* emergence. In the growth chambers, we encountered problems with *C. scrobicollis* adult emergence. Since garlic mustard crowns had numerous larvae and extensive larval tunneling, we hypothesized that few pupae were surviving in the soil to emerge into adults. We speculated that the larvae did not have the correct soil needed to create their soil pupal cases, or alternatively, the soil mix remained too moist. For this reason, a study was conducted to determine the best soil mix to ensure pupa survival and maximize adult emergence. Two treatments tested were 1) standard rice hull potting mix used to propagate garlic mustard and 2) addition of approximately 4 cm of a standard greenhouse soil mix (silt loam:sand:manure:peat, 1:1:1:1, v/v/v/v) covering the soil of the potted garlic mustard plant. Each treatment was replicated 11 times and randomly assigned to a single caged plant as a replicate. Three pairs of marked *C. scrobicollis* adults were placed on each plant for approximately two weeks and were then removed. Plants were maintained in a growth chamber as described previously until adult emergence. Number of adults emerging from each plant was recorded.

Continuous winter vs. winter/spring adult emergence study. In their native range, *C. scrobicollis* larvae exit from garlic mustard crowns in April and adults emerge from the soil from mid-May to late June. In growth chambers, F-1 adults emerged during periods of continuous

winter. To determine whether adults reared in containment would emerge earlier when placed in winter/spring instead of continuous winter conditions, the following study was conducted and consisted of two treatments. In the first treatment, caged plants with insects were placed into winter conditions in a growth chamber for 2 months followed by 2 months of spring conditions (Table 1). For the second treatment, caged plants with insects were kept in continuous winter conditions for 4 months (Table 2). Four to five pairs of weevils were added to plants and F-1 adults were reared as described previously. The experiment was replicated four times, with each replication consisting of one caged plant with weevils added.

Length of summer aestivation treatments to optimize *C. scrobicollis* rearing in a containment facility. In Europe, *C. scrobicollis* adults emerging in the spring require a summer aestivation period before adult females are able to oviposit (Gerber 2009). After a summer of aestivation, adults begin to feed and lay eggs in September. We wanted to determine the minimum length of summer aestivation required for adult females to reach maturity and oviposit when reared in growth chambers in containment.

A study was designed to determine the length of aestivation required by *C. scrobicollis* before they would feed and oviposit. F-1 adults were placed onto garlic mustard plants, allowed to feed a minimum of two weeks and then placed into the summer aestivation treatments (Table 1) of three months (standard treatment), two months or one month. After the aestivation treatment, all caged plants were placed in fall conditions for one week, followed by winter conditions for three or seven weeks (Table 2). After the winter treatment, adults were removed from garlic mustard plants and placed into an oviposition test.

For the oviposition test, two females and one male (unless otherwise noted) were placed in a glass jar containing a garlic mustard leaf inserted into a piece of saturated florist foam. After

2 to 3 days, leaves and petioles were dissected and checked for eggs. The number of eggs present per leaf was recorded. A minimum of four replications were completed, with each jar as a replication. Treatment means were separated with a Least Significant Difference test at the 0.05 level of significance.

Rearing parasitoid-free *Ceutorhynchus scrobicollis* The endoparasitoid, *P. conseutor*, emerges from *C. scrobicollis* adults during the spring or fall (Gerber 2009). A procedure was developed to temporally separate *C. scrobicollis* and *P. conseutor*. To accomplish this, ovipositing *C. scrobicollis* females and males were placed on garlic mustard plants kept in winter conditions. Since *P. conseutor* adults emerge in the spring, this allowed females to oviposit during a period when parasitoids do not emerge from parasitized *C. scrobicollis*, thereby producing a new generation of *C. scrobicollis* which did not have the chance to become parasitized by *P. conseutor*.

Adult *C. scrobicollis* were sexed and marked with a paint pen to easily distinguish between males and females and one generation from the next. Marked adults were placed on caged garlic mustard plants which were then placed into a growth chamber in winter conditions (Table 1). Adults were removed 14 to 20 days later, the length of time required for *C. scrobicollis* eggs to hatch at 15 C (Katovich, unpublished data). We removed adults before eggs hatched as some *Perilitus* spp. are able to parasitize larva (Heimpel, personal communication). To remove adults, the cage was removed and any weevils found near the crown or in the adjacent soil layer were collected. Next, the topsoil and leaf litter were sifted through a screen and soil was collected in a white plastic dishpan. All individual adults were hand collected from the sieved soil and numbers collected were compared to number of adults added to each plant. If all adults were collected, any subsequent offspring were considered to be “parasitoid free”. If all

adults were not collected from a cage plant, then any offspring were not considered parasitoid-free.

RESULTS

***Ceutorhynchus scrobicollis* rearing and collection of F-1 adults in a containment facility.** In containment, rosette aboveground vegetation often dies and turns brown after *C. scrobicollis* larval mining of roots and crowns. Frequently, new lateral shoots will arise from crown buds after larvae have left the crowns to pupate in the soil. After F-1 adults emerge from the soil, they often feed on these lateral shoots, an indicator of when to start collecting F-1 adults. F-1 adults are also found frequently crawling around on screen cages.

In 2011-2012, adults emerged after an average of 106 days (n=78, SE = 1.9) ranging from 77 to 144 days (Table 2). An average of 4.4, F-1 adults emerged from each plant (n=78, SE=0.4) with a range of 1 to 16 adults per plant. In 2012-2013, adults emerged an average of 110 days (n=115 SE=1.5) ranging from 75 to 162 days (Table 2). An average of 4.7 adults emerged per plant (n=115 SE= 0.5), with a range of 1 to 31 adults per plant.

Ceutorhynchus scrobicollis adults emerged over a period of time. Checking and removing F-1 adults from caged plants is a labor intensive process. It is useful to know how long a time period is necessary to maximize collection of F-1 adults while minimizing length of the collection period. During 2012-2013, all F-1 adults had emerged after an average of 11 days ($\bar{x} = 10.9$), from the time the first adult was found on a plant (N=73, SE=1.4, Min=1 day, Max=40 days). One week after the first F-1 adult was found on each individual plant, all F-1 adults had been collected from 34/73 caged plants (47%) (Figures 2a and 2b). After three and four weeks, 74% and 86% of plants had all F-1 adults collected, respectively After three weeks,

it might not warrant the labor commitment to collect the remaining F-1 weevils from caged plants.

In our weevil recovery experiment, where 10 F-1 adults were placed in a pot covered by a funnel apparatus, an average of 78% adults were recovered over a three week period. Although not all F-1 adults were collected in the funnel apparatus, it is still a more efficient collection method than the alternative of hand sifting through the soil and leaf litter of each individual plant.

Soil medium for optimum *Ceutorhynchus scrobicollis* emergence. Adults emerged from the soil an average of 95 days after initial placement on plants. The addition of the standard greenhouse soil mix resulted in an average of 10, F-1 adults emerging from pots verses 2, F-1 adults from pots with potting mix alone. Although these results were not significant at the 0.05 level, we now routinely add the standard greenhouse soil to the top of the potting mixture when rearing *C. scrobicollis.* Adding a well-drained soil to the top of the pots while sub-irrigating could allow the larvae to pupate in a drier, warmer soil mix. *Ceutorhynchus scrobicollis* larvae form soil pupal chambers, so larvae may prefer the greenhouse soil mix for their pupal chambers.

Length of summer aestivation treatments to optimize *C. scrobicollis* rearing in a containment facility. After one month of summer aestivation (plus one week of fall and three weeks of winter), all weevils were feeding on plants, but only a total of three eggs were found out of 5 replications (Table 3). After two months of aestivation, adults were also actively feeding, but only two eggs were found out of 5 replications. Following the 3 month aestivation period, a total of 69 eggs were found, an average of 13.8 eggs per leaf, a significantly higher number of eggs per leaf than the other aestivation periods. It should be noted that females laid a small number of eggs without receiving an aestivation period (data not shown).

When adults were re-tested after an additional month of winter (four total months of winter), an average of 4 to 7 eggs were found in each replication for the 1 and 2 month treatments respectively. Females increased the number of eggs they laid after one additional month of winter. However, results showed that total numbers of eggs per leaf were highest with the standard 3 month summer aestivation treatment, with a total length of time of 4 months (3 months of aestivation followed by 1 week fall plus 3 weeks of winter) before oviposition commenced. The three month aestivation period may be necessary for complete development of the females' ovaries prior to oviposition.

Continuous winter vs. winter/spring adult emergence study. This study was designed to determine whether adults reared in containment would emerge earlier when placed in winter/spring conditions instead of continuous winter conditions. We found no significant difference (0.05) in the number of adults emerging or days to emergence after two months of winter followed by two months of spring compared with four months of continuous winter. However, when caged plants were placed into the winter/spring treatment, adults emerged approximately one week earlier than with the continuous winter treatment. An average of 7 adults per plant emerged in the winter/spring treatment compared to 2 adults with the continuous winter treatment. Although not significant, placement of caged plants into spring conditions, following two months of winter, may slightly reduce the total F-1 emergence time.

Rearing parasitoid-free *Ceutorhynchus scrobicollis*. At the time of writing, we have not been able to obtain a specimen of the parasitoid, *P. conseutor*, so have not been able to identify any of the few parasitoids collected from caged plants. Thus, we have not been able to determine whether our protocol has been effective in eliminating *P. conseutor* from our F-1 weevils. Future plans include developing a protocol to determine effectiveness of our protocol. We suspect that

the majority of collected parasitoids are *Perilitus coccinellae*, a parasitoid of *H. convergens* but we cannot verify this.

Conclusions. *Ceutorhynchus scrobicollis* can be successfully reared on caged garlic mustard plants in a growth chamber by alternating growth chamber temperatures and photoperiods to mimic natural conditions in its native range. In Germany, *C. scrobicollis* produces one generation per year and F-1 adults emerge in late May. In containment, a new generation of adults emerged an average of 106 or 110 days after parent weevils were placed on plants in 2011-2012 and 2012-2013 respectively. After emergence, F-1 adults fed on garlic mustard rosettes for a minimum of two weeks before they were placed in a summer aestivation period. A three month summer aestivation period, followed by a week of fall, and three weeks of winter resulted in optimum levels of oviposition.

Optimally, we can produce a generation of *C. scrobicollis* every three to four months, generating the maximum quantity of weevils for release. Before release into North America, it will be necessary to rear a minimum of one generation of *C. scrobicollis* in a containment facility to ensure that the endoparasitoid, *P. conseutor*, is not released along with adult *C. scrobcollis*. We can successfully achieve this via a method whereby we deprive *P. conseutor* of an adult *C. scrobicollis* host, disrupting the life cycle of the parasitoid.

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Table 1. Growth chamber conditions used to rear *Ceutorhynchus scrobicollis* in the Biosafety Level 2 Containment Facility. University of Minnesota, St. Paul, MN. 2011-2013.

Simulated season	Temperature		Photoperiod[a] (h)	Dark period (h)	Relative humidity (%)
	Day	Night			
Fall	18	15	13.5	10.5	60-70
Winter	15	14	9.5	14.5	60-70
Spring	18	15	13.5	10.5	60-70
Summer	21	20	16.0	8.0	60-70

[a]Photoperiod and dark period total 24 hours.

Table 2. *Ceutorhynchus scrobicollis* F-1 emergence. Biosafety Level 2 Containment Facility. University of Minnesota, St. Paul, MN. 2011-2012, 2012-2013.

	F-1 emergence (days)		Total numbers of F-1 adults	
	2011-2012	2012-2013	2011-2012	2012-2103
N	78.0	115.0	78.0	115.0
$\bar{x}$	106.1	109.6	4.5	4.7
SE $\bar{x}$	1.9	1.5	0.4	0.5
Min	77.0	75.0	1.0	1.0
Max	144.0	162.0	16.0	31.0

Table 3. Number of *Ceutorhynchus scrobicollis* eggs present in garlic mustard shoots after adults were placed in one, two or three month aestivation periods. Biosafety Level 2 Containment Facility. University of Minnesota, St. Paul, MN 2012.

Length of aestivation (months)	Length of fall/winter (weeks)	Total months	Eggs (total)	Eggs per shoot (average)	Feeding
1	1/3	2	3	0.6	+
2	1/3	3	2	0.4	+
3	1/3	4	69	13.8	+
1	1/7	3	18	3.6	+
2	1/7	4	27 (only 4 reps)	7.0	+
LDS (0.05)				2.1	

Figure 2a. Number of plants with all *Ceutorhynchus scrobicollis* F-1 adults collected

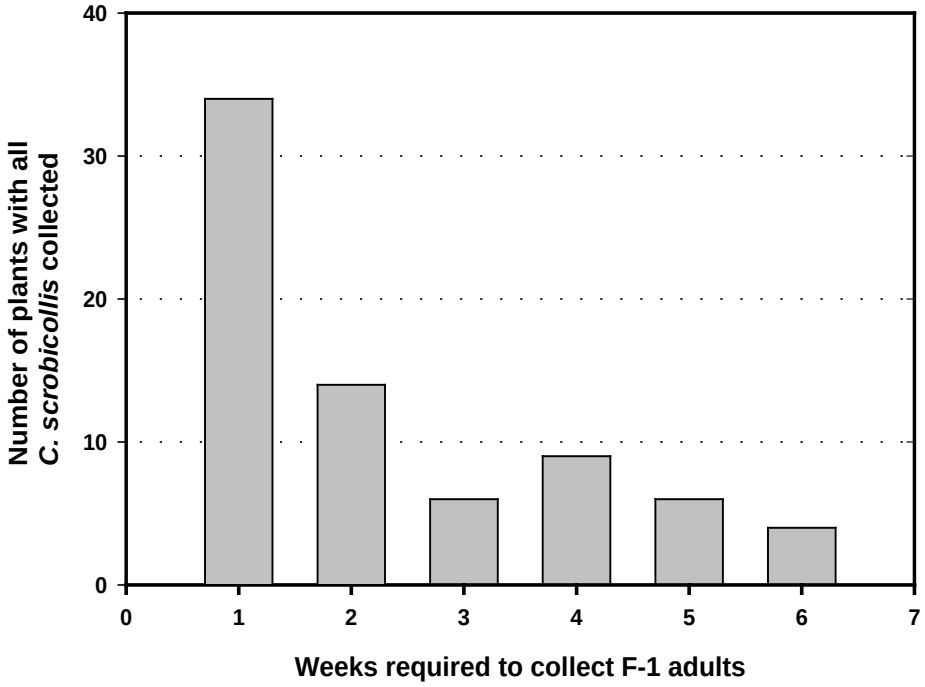

Figure 2b. Cumulative percent of plants with all *Ceutorhynchus scrobicollis* F-1 adults collected