

## **M.L. 2013 Project Abstract**

For the Period Ending June 30, 2016

**PROJECT TITLE:** Biological Control of Garlic Mustard

**PROJECT MANAGER:** Laura Van Riper

**AFFILIATION:** Minnesota Department of Natural Resources

**MAILING ADDRESS:** 500 Lafayette Rd., Box 25

**CITY/STATE/ZIP:** St. Paul MN 55155-4025

**PHONE:** (651) 259-5090

**E-MAIL:** [laura.vanriper@state.mn.us](mailto:laura.vanriper@state.mn.us)

**WEBSITE:** [www.mndnr.gov/invasives](http://www.mndnr.gov/invasives)

**FUNDING SOURCE:** Environment and Natural Resources Trust Fund

**LEGAL CITATION:** M.L. 2013, Chp. 52, Sec. 2, Subd. 06e

**APPROPRIATION AMOUNT:** \$140,000

### **Overall Project Outcome and Results**

This project advanced the goal of having effective biological control insects for garlic mustard (*Alliaria petiolata*). Host-specificity testing focused on the potential biocontrol insects *Ceutorhynchus scrobicollis* (a crown feeding weevil) and *C. constrictus* (a seed-feeding weevil). Monitoring garlic mustard populations in Minnesota provided information on garlic mustard populations in the absence of biocontrol. *C. scrobicollis* host specificity testing was completed for 15 plant species. Based on these results, *C. scrobicollis* has the host specificity to be a successful biocontrol agent of garlic mustard. The researchers wrote a petition for release which summarizes the 18 years of *C. scrobicollis* host specificity testing. The petition was submitted to the USDA-APHIS Technical Advisory Group in June 2016. Rearing protocols, release methods, and biocontrol manuals were developed for *C. scrobicollis*. *C. constrictus* host specificity testing was completed for 19 plant species. The results show that *C. constrictus* continues to show the host specificity to be a successful biocontrol agent for garlic mustard. Approximately 30 more species need to be tested and then a petition for release of *C. constrictus* can be submitted to the USDA. Garlic mustard is a biennial and long-term monitoring shows that its populations can fluctuate widely from year to year. When the plots were established in 2005 and 2006, garlic mustard was present in 100% of the plots. Garlic mustard is still present in 88% of the plots as of June 2016. Garlic mustard is currently experiencing very little herbivory in Minnesota with an average amount of leaf removed due to herbivory ranging from 0.6 to 4.5% in 2014 - 2016. It is expected that after biological control release, garlic mustard cover and density will decrease and shoot heights and silique production of individual plants will decrease as well.

### **Project Results Use and Dissemination**

- Dr. Jeanie Katovich and Dr. Roger Becker presented a poster on this project at the Upper Midwest Invasive Species Conference in Duluth, MN held October 20-22, 2014.
- Dr. Jeanie Katovich gave a presentation on the project to the "Invasive Plant Management" class at the University of Minnesota – Twin Cities during the spring 2015 and 2016 semesters.
- Dr. Roger Becker gave a presentation titled "Petition to release *Ceutorhynchus scrobicollis* for biological control of garlic mustard (*Alliaria petiolata*)" at the 2015 Midwest Invasive Plant Network Invasive Plant Symposium (part of the North Central Weed Science Society annual meeting) in Indianapolis, IN on December 9, 2015.

- Dr. Jeanie Katovich gave a presentation on the project to Northeast Region US Forest Service researchers and staff at a meeting in Roseville, MN on March, 3, 2016.
- Dr. Jeanie Katovich presented the host-specificity data for *Ceutorhynchus scrobicollis* to the USDA APHIS Technical Advisory Group at their annual meeting in Greenbelt Maryland on April 6, 2016.
- Monitoring data has been shared with a consortium of researchers led by Dr. Bernd Blossey of Cornell University. This group will work together to produce a peer-reviewed publication on the results of garlic mustard monitoring plots in the Midwest and Northeast regions of the United States. The paper is currently being written.
- The petition for release for *C. scrobicollis* was submitted to the USDA APHIS Technical Advisory Group.
  - Title: A Petition for the Introduction, Experimental Release and Open-Field Release of the Root-Mining Weevil *Ceutorhynchus scrobicollis* (Coleoptera: Curculionidae) for the Biological Control of *Alliaria petiolata* (Garlic Mustard) in North America
  - Authors: Laura Van Riper, Esther Gerber, Harriet L. Hinz, Ghislaine Cortat, Elizabeth Katovich, Roger Becker, Mary Marek-Spartz
  - Date submitted: June 21, 2016
- Dr. Laura Van Riper will give the presentation “Perspectives on Garlic Mustard Biocontrol in the Midwest” at the Upper Midwest Invasive Species Conference in La Crosse, WI held October 17-19, 2016.



# Environment and Natural Resources Trust Fund (ENRTF) M.L. 2013 Work Plan Final Report

---

**Date of Status Update Report: 8/15/2016**

**Date of Next Status Update Report: Final Report**

**Date of Work Plan Approval: June 11, 2013**

**Project Completion Date: June 30, 2016**

---

**PROJECT TITLE: Biological Control of Garlic Mustard**

**Project Manager:** Laura Van Riper

**Affiliation:** Minnesota Department of Natural Resources

**Mailing Address:** 500 Lafayette Rd., Box 25

**City/State/Zip Code:** St. Paul MN 55155-4025

**Telephone Number:** (651) 259-5090

**Email Address:** [laura.vanriper@state.mn.us](mailto:laura.vanriper@state.mn.us)

**Web Address:** [www.mndnr.gov/invasives](http://www.mndnr.gov/invasives)

---

**Location:** Statewide

---

**Total ENRTF Project Budget: \$140,000**

**ENRTF Appropriation: \$140,000**

**Amount Spent: \$140,000**

---

**Balance: \$0**

---

**Legal Citation:** M.L. 2013, Chp. 52, Sec. 2, Subd. 06e

**Appropriation Language:**

\$140,000 the first year is from the trust fund to the commissioner of natural resources in cooperation with the University of Minnesota to continue the implementation of biological control for invasive garlic mustard plants. This appropriation is available until June 30, 2016, by which time the project must be completed and final products delivered.

## **I. PROJECT TITLE: Biological Control of Garlic Mustard**

### **II. PROJECT STATEMENT:**

Garlic mustard is a non-native invasive plant species that is severely threatening native plant communities and degrading wildlife habitat in forest and riparian zones. The Minnesota Department of Natural Resources considers garlic mustard the highest priority species for the development of long-term management solutions, such as biological control. Garlic mustard is a prohibited noxious weed in Minnesota making management mandatory. Current management is cost and labor intensive. Biological control would provide long-term control. Successful biocontrol is now being achieved in Minnesota on two previous high priority species, purple loosestrife and leafy spurge, due in part to past ENRTF recommended funding.

The development and implementation of a biocontrol insect is a long-term commitment. Garlic mustard biological control efforts are currently ongoing and have been funded in part by the ENRTF (2003, 2005, 2007, 2010). This research is crucial in 1) determining if there are suitable insects that can be used to reduce impacts caused by garlic mustard and 2) implement introduction of insects to control garlic mustard and assess their establishment and success.

A petition to release the root-feeding weevil, *Ceutorhynchus scrobicollis*, for biological control of garlic mustard was submitted to the USDA-APHIS Technical Advisory Group (TAG) in September, 2011. If TAG recommends release and APHIS concurs, then releases of *C. scrobicollis* could start within the year. Host-specificity research for a second biological control agent, a seed-feeding weevil, *Ceutorhynchus constrictus*, is approaching completion. Multiple biological control insects can provide greater control than one species. The goal of this project is to release multiple biological control insects and monitor their effectiveness. The combination of the crown-mining weevil, *C. scrobicollis*, and the seed-feeding weevil, *C. constrictus* will provide the greatest potential for successful biological control of garlic mustard in Minnesota.

The desired outcomes of this project are 1) reduced negative impacts from garlic mustard on forest species; 2) long-term, self-sustaining control of garlic mustard; and 3) reduction in management time and cost for garlic mustard.

### **III. PROJECT STATUS UPDATES:**

#### **Project Status as of Dec. 31, 2013:**

A petition to release the root-feeding weevil, *Ceutorhynchus scrobicollis*, for biological control of garlic mustard was submitted to the USDA-APHIS Technical Advisory Group (TAG) in September, 2011. We were anticipating TAG approval for release, and then releases of *C. scrobicollis* could start within the year. However, TAG rejected the petition in June of 2013, requiring additional testing of threatened and endangered mustard species. Therefore, we will need to do additional host-specificity testing for *C. scrobicollis*. We are working with TAG to develop a plant test list of the additional mustard species.

A contract with the University of Minnesota has been written for the work in the work plan. Signatures are currently being obtained on the contract.

**Project Status as of June 30, 2014:** On Oct. 1, 2013 we submitted a proposed list of test plants to the USDA-APHIS Technical Advisory Group (TAG). TAG indicated they would respond within 4 months. In March 2014, TAG indicated the review was in the final stages. TAG has not responded to our follow-up email on May 5, 2014. We would like approval and agreement from TAG on a test plant list. In the meantime, the University of Minnesota began host-specificity testing on threatened and endangered mustards for which they can obtain seeds. The contract with the U of MN was fully signed and executed on 12-30-2013. The first invoice from the University of Minnesota will be received after June 30, 2014.

**Project Status as of Dec. 31, 2014:** On November 26, 2014, the USDA-APHIS Technical Advisory Group (TAG) responded to the proposed test plant list submitted on Oct. 1, 2013. Ten reviewers accepted the proposed test plant list. One reviewer recommended the list with reservations. One reviewer did not recommend the list and recommended an additional 8 species for host-specificity testing beyond the additional 16 proposed. This reviewer criticizes that western mustard species were tested instead of species with distributions in the eastern US, the area primarily infested by garlic mustard. In contrast, the January 2009 TAG response recommended testing western species.

The University of Minnesota continued host specificity testing of *Ceutorhynchus scrobicollis*. As of December 2014, they have completed oviposition, larval development and single-choice tests on all but three species on the proposed test plant list. They are continuing their efforts to rear and maintain a colony of *C. scrobicollis* in their containment facility. The University of Minnesota also continued conducting *Ceutorhynchus constrictus* host range tests. During the summer of 2014, sequential no-choice tests were conducted on six Brassicaceae species. No eggs were found in any of the tested native mustard species. Data was collected on the garlic mustard monitoring plots in Minnesota during October 2014.

**Project Status as of June 30, 2015:**

On November 26, 2014, the USDA-APHIS Technical Advisory Group (TAG) responded to the proposed test plant list submitted on Oct. 1, 2013. In response to the TAG comments, researchers at the University of Minnesota and CABI Bioscience, Switzerland, prepared a document with a rationale for testing/not testing these additional 8 species. The document was submitted to TAG in January, 2015 and researchers agreed to test 2 of the 8 suggested species; *Draba ramosissima* and *Boechera perstellata* (an endangered species that will be tested if the University can obtain seeds). A conference call among researchers, USDA-APHIS, USDA-Forest Service and the head of TAG was scheduled for February 27, 2015. The purpose of the call was to negotiate what additional species to test. All groups were represented in the conference call, with the exception of the head of TAG. Consequently, negotiations concerning additional species to test was not possible. Researchers are still waiting for response from TAG, but will continue to test these 2 additional species.

The University of Minnesota continues host specificity testing for *Ceutorhynchus scrobicollis* and *Ceutorhynchus constrictus*. Data was collected on the garlic mustard monitoring plots in Minnesota during June 2015.

**Amendment Request (09/28/2015):**

We are requesting a re-budget increasing the budget for Activity 1 while decreasing Activity 2 and an adjustment of goals for this project due to the additional testing required by the USDA APHIS Technical Advisory Group (TAG) since this grant was awarded. This project was initially budgeted for \$97,000 in Activity 1 and \$43,000 in Activity 2. Activity 1 is "Developing rearing protocols and complete required host specificity testing for garlic mustard biological control agents in Minnesota". Activity 2 is "Introduce and evaluate garlic mustard biocontrol agents in Minnesota". All funds are in one contract with the University of Minnesota. We are requesting to shift \$7,419 (5.3% of the total budget) from Activity 2 to Activity 1. TAG approval is required before we can release biological control insects. The requirement by APHIS for additional testing since this grant was awarded requires more salary and quarantine costs in Activity 1, and prevents us from being able to release any agents during this grant (Activity 2) though we continue monitoring preparatory work in Activity 2 in anticipation of release after this grant is closed. Approved by LCCMR 10-12-2015.

**Project Status as of Dec. 31, 2015:**

The University of Minnesota completed *Ceutorhynchus scrobicollis* host specificity testing for *Eutrema penlandii*, *Draba ramosissima* and *Boechera perstellata*. No feeding or eggs were found in any replications in the tests. The researchers are currently updating the original 2008 TAG Petition for *C. scrobicollis* and hope to re-submit the Petition to TAG in early 2016. Host specificity tests for the seed feeder *Ceutorhynchus constrictus* were conducted on seven Brassicaceae species. No eggs were found in any of the tested native mustard. Since no

eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary. To date, no *C. constrictus* eggs or larvae have been found in any seed capsules of Brassicaceae plants tested at the University of Minnesota.

Garlic mustard monitoring was conducted at the long-term monitoring sites during the month of October 2015. By the fall of 2015, population densities of rosettes were still significant at Cottage Grove, Fort Snelling, and Warner Nature Center, with 8.5, 12.8, and 30.7 rosettes per m<sup>2</sup>, respectively. Plots were established at sites where garlic mustard had become a serious problem and may have been established, in some cases, in the vicinity of the initial invasion. Since the beginning of this monitoring effort, garlic mustard has moved on the landscape at some locations, and population densities have become most severe in areas where garlic mustard habitat may be more suitable for invasion beyond the monitoring quadrats. Data from the monitoring sites will help determine if populations recover at some locations following the three consecutive years of late-season drought that may have caused significant mortality of garlic mustard, depleting the seedbank and reducing invasion pressure in 2014 and 2015.

### **Overall Project Outcomes and Results**

This project advanced the goal of having effective biological control insects for garlic mustard (*Alliaria petiolata*). Host-specificity testing focused on the potential biocontrol insects *Ceutorhynchus scrobicollis* (a crown feeding weevil) and *C. constrictus* (a seed-feeding weevil). Monitoring garlic mustard populations in Minnesota provided information on garlic mustard populations in the absence of biocontrol. *C. scrobicollis* host specificity testing was completed for 15 plant species. Based on these results, *C. scrobicollis* has the host specificity to be a successful biocontrol agent of garlic mustard. The researchers wrote a petition for release which summarizes the 18 years of *C. scrobicollis* host specificity testing. The petition was submitted to the USDA-APHIS Technical Advisory Group in June 2016. Rearing protocols, release methods, and biocontrol manuals were developed for *C. scrobicollis*. *C. constrictus* host specificity testing was completed for 19 plant species. The results show that *C. constrictus* continues to show the host specificity to be a successful biocontrol agent for garlic mustard. Approximately 30 more species need to be tested and then a petition for release of *C. constrictus* can be submitted to the USDA. Garlic mustard is a biennial and long-term monitoring shows that its populations can fluctuate widely from year to year. When the plots were established in 2005 and 2006, garlic mustard was present in 100% of the plots. Garlic mustard is still present in 88% of the plots as of June 2016. Garlic mustard is currently experiencing very little herbivory in Minnesota with an average amount of leaf removed due to herbivory ranging from 0.6 to 4.5% in 2014 - 2016. It is expected that after biological control release, garlic mustard cover and density will decrease and shoot heights and silique production of individual plants will decrease as well.

## **IV. PROJECT ACTIVITIES AND OUTCOMES:**

**ACTIVITY 1:** Develop rearing protocols and complete required host specificity testing for garlic mustard biological control agents in Minnesota

**Description:** Research activities will include development of rearing protocols, release methods, and manuals for biological control insects. When biocontrol insects are approved, partners must be instructed how to raise the insects so they may release on their target sites.

The USDA Technical Advisory Group has requested additional host-specificity testing for *C. scrobicollis*. Completing testing for *C. scrobicollis* and submitting a petition for release will be a priority. The additional work on *C. scrobicollis* means that there are less resources available for work on *C. constrictus*, *C. roberti*, and *C. alliariae*.

Host-specificity testing of *C. constrictus* is near completion due to previous research in Europe. Research in this proposal will focus on completing the required host specificity testing of *C. constrictus* on Brassicaceae species native to North America, in order to submit a request for approval of *C. constrictus* for release when testing is complete.

**Summary Budget Information for Activity 1:**

**ENRTF Budget: \$ 104,419**  
**Amount Spent: \$ 104,419**  
**Balance: \$ 0**

**Activity Completion Date:**

Outcome	Completion Date	Budget
1. Develop rearing protocols and manual for <i>C. scrobicollis</i>	06/30/14	\$15,000
2. Complete host specificity of <i>C. scrobicollis</i> and <i>C. constrictus</i>	12/31/14	\$85,419
3. If results warrant, write proposal to TAG for release of <i>C. scrobicollis</i>	06/30/15	\$4,000

\*Estimated amount per task, amounts may vary per task, but total budget will stay at \$104,419.

**Activity Status as of Dec. 31, 2013:**

A contract with the University of Minnesota has been written for the work in Activity 1. Signatures are currently being obtained on the contract.

In June 2013, the USDA APHIS Technical Advisory Group (TAG) requested additional testing of threatened and endangered mustard species and *C. scrobicollis*. Therefore, we will need to do additional host-specificity testing for *C. scrobicollis* and are working with TAG on developing this species list. At the annual meeting of the Technical Advisory Group for Biological Control Agents of Weeds (TAG) group in June, 2013, it was recommended that we include additional Threatened and Endangered (T and E) plants on the Federal list of Threatened and Endangered Species in our test plant list for the potential crown-boring biocontrol insect, *Ceutorhynchus scrobicollis*. There are currently 35 T and E and 7 candidate species in the Brassicaceae family that are listed by the USFWS. To further define the host specificity of *C. scrobicollis*, 7 T and E, one candidate and 6 surrogate species have been identified for further testing. The surrogate species represent T and E species which cannot be tested directly since seed are not available. When we selected surrogates for testing, taxonomically related species were chosen with similar life histories, habitats or ranges as the listed species. With the addition of these Brassicaceae species, we will have tested T and E, candidate species or surrogates from all of the Brassicaceae genera on the USFWS Federal List of Threatened and Endangered Species.

In October, 2013, we submitted a “Proposed Supplemental Test Plant List” based on reviewers’ concerns arising from our TAG petition, as well as comments received from the June 2013 TAG meeting. We anticipate a TAG response to our supplemental test plant list in the spring of 2014.

**Activity Status as of June 30, 2014:**

***Ceutorhynchus scrobicollis*:** The contract with the U of MN was fully signed and executed on 12-30-2013. As of June 18, 2014, we await a response from TAG on the proposed test plant list for *C. scrobicollis*. The proposed test plant list contains 16 species. The University of Minnesota began host-specificity testing on threatened and endangered mustards for which they can obtain seeds. As of June 2014, they have completed oviposition, larval development and single-choice tests on all but three species. They have had difficulty propagating the remaining three species, but are continuing their efforts. These species are *Cochlearia officinalis*, *Leavenworthia torulosa*, and *Warea* spp. They are still looking for a *Eutrema* spp. seed source.

***Ceutorhynchus constrictus*:** The University of Minnesota continues to conduct oviposition tests with the seed-feeding weevil, *C. constrictus*. During the spring of 2014, they tested plants from the following Brassicaceae species; *Hesperidanthus linearifolia*, *Physaria douglasii* ssp. *tuplashensis*, *Erysimum menziesii*, canola, *Lunaria annua*, and *Iodanthus pinnatifidus*.

**Activity Status as of Dec. 31, 2014:**

***Ceutorhynchus scrobicollis*:** As of December 2014, the University of Minnesota has completed oviposition, larval development and single-choice tests on all but three species identified in the additional test plant list submitted to the USDA APHIS Technical Advisory Group. They have had difficulty propagating the remaining three species, but are continuing their efforts. These species are *Cochlearia officinalis*, *Leavenworthia torulosa*, and *Warea* species. They are still looking for a *Eutrema* species seed source. Eggs were found in all species tested, except for *Erysimum menziesii* and *Thelypodium milleflorum*. In larval development tests, live larvae were found in *Streptanthus glandulosus* ssp. *niger*, but no adults were recorded.

On November 26, 2014, the USDA-APHIS Technical Advisory Group (TAG) responded to the proposed test plant list submitted on Oct. 1, 2013. Ten reviewers accepted the proposed test plant list. One reviewer recommended the list with reservations. One reviewer did not recommend the list and recommended an additional 8 species for host-specificity testing beyond the additional 16 proposed. The researchers are now reviewing the TAG response.

***Ceutorhynchus constrictus*:** During the summer of 2014, sequential no-choice tests were conducted on six Brassicaceae species at the University of Minnesota. No eggs were found in any of the tested native mustard species and an average of 1.9 eggs were found in each garlic mustard silique (seed pod). Larvae were collected from each garlic mustard plant and counted. Larvae were placed into growth chambers and a refrigerator to simulate outdoor conditions so that the larvae could complete their life cycle and emerge into adults. Since no eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary.

**Activity Status as of June 30, 2015:**

***Ceutorhynchus scrobicollis*:** As of June 30, 2015, the University of Minnesota has completed oviposition, larval development and single-choice tests on all but four species identified in the additional test plant list submitted to the USDA APHIS Technical Advisory Group in October 2013. New seed sources have been obtained for three of these species; *Cochlearia officinalis*, *Leavenworthia torulosa*, and *Eutrema pendlandii*. Testing will commence as soon as possible. Researchers are still trying to germinate seeds of *Warea* and obtain a new seed source for *Sibara filifolia*. A shipment of *C. scrobicollis* from Switzerland was received in March, 2015. Adults have been used to supplement our rearing colony. *Ceutorhynchus scrobicollis* rearing is continuing.

On November 26, 2014, the USDA-APHIS Technical Advisory Group (TAG) responded to the proposed test plant list submitted on Oct. 1, 2013. In response to the TAG comments, researchers at the University of Minnesota and CABI Bioscience, Switzerland, prepared a document with a rationale for testing/not testing these additional 8 species. Researchers are still waiting for response from TAG, but will continue to test these 2 additional species.

***Ceutorhynchus constrictus*:** Thirty adults were collected from rearing containers. These adults emerged from larvae collected last summer from our containment colony. A shipment of *C. constrictus* was received in the containment facility in April, 2015. We are currently conducting oviposition tests with native Brassicaceae with the reared and newly imported *C. constrictus*.

**Activity Status as of Dec. 31, 2015:**

***Ceutorhynchus scrobicollis*:** The University of Minnesota continues to rear and maintain a colony of *C. scrobicollis* in containment and they are preparing a manuscript for publication describing *C. scrobicollis* rearing in containment. Host specificity tested was completed for *Eutrema penlandii* (USFWS Threatened Species), *Draba ramosissima* and *Boechera perstellata* (USFWS Endangered Species). No feeding or eggs were found in any replications in the tests. The researchers are currently updating the original 2008 TAG Petition for *C. scrobicollis* and hope to re-submit the Petition to TAG in early 2016.

***Ceutorhynchus constrictus*:** During the summer of 2015, sequential no-choice tests were conducted on seven Brassicaceae species. No eggs were found in any of the tested native mustard species and an average of 1.8



eggs were found in each garlic mustard silique (seed pod). Larvae were collected from each garlic mustard plant and counted when found. Larvae were placed into growth chambers and a refrigerator to simulate outdoor conditions so that the larvae could complete their life cycle and emerge into adults. Since no eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary. To date, no *C. constrictus* eggs or larvae have been found in any seed capsules of Brassicaceae plants tested at the University of Minnesota.

#### **Final Report Summary:**

##### ***Ceutorhynchus scrobicollis*:**

Host specificity testing was completed for the potential garlic mustard biocontrol insect *Ceutorhynchus scrobicollis*. Under this grant period, testing with *C. scrobicollis* was completed for the following 15 plant species: *Braya alpina*, *Cochlearia officinalis*, *Draba ramosissima*, *Boechera perstellata*, *Boechera hoffmannii*\*, *Erysimum menziesii*, *Eutrema edwardsii* (*penlandii*), *Hesperidanthus linearifolia*, *Lepidium barnebyanum*\*, *Paysonia densipila*, *Physaria douglasii* ssp. *tuplashensis*, *Rorippa subumbellata*\*, *Streptanthus glandulosus* subsp. *niger*, *Thelypodium laciniatum*, *Thelypodium milleflorum*.

\*denotes species tested at CABI Bioscience-Switzerland.

Tests include oviposition, larval development and single-choice tests. Researchers completed oviposition, larval development and single-choice tests on all but three species. They had difficulty propagating the remaining three species, *Leavenworthia torulosa*, *Sibara filifolia* and *Warea* spp., and are working to overcome these obstacles.

In sequential no-choice oviposition tests, eggs were not found in *Erysimum menziesii*, *Thelypodium milleflorum*, *Cochlearia officinalis*, *Draba ramosissima*, *Boechera perstellata*, or *Eutrema penlandii* so these species were not tested further. In larval development tests, live larvae were found in *Streptanthus glandulosus* ssp. *niger*, but no adults were recorded. In single-choice tests of *Streptanthus glandulosus* ssp. *niger*, 81% of eggs were found in garlic mustard and only 19% in *Streptanthus glandulosus* ssp. *niger*. A single dead larvae was found in one plant of *Physaria douglasii* ssp. *tuplashensis* but no eggs were found in this species in single-choice oviposition tests. Based on these results, *C. scrobicollis* continues to show the host specificity to be a successful biocontrol agent of garlic mustard.

The goal of the host-specificity testing is to submit a new petition to the USDA-APHIS Technical Advisory Group (TAG) for the release of *C. scrobicollis* as a biological control agent for garlic mustard in North America. In June 2013, in response to a previous petition for the release of *C. scrobicollis*, TAG requested testing of additional threatened and endangered mustard species. In October 2013, the researchers submitted a "Proposed Supplemental Test Plant List" with an additional 16 species based on reviewers' concerns and recommendations. In November 2014, TAG responded to the proposed test plant list with additional species to be tested. In February 2015, the researchers sent a letter to the TAG chair detailing which of the additional reviewer suggested species they think are appropriate to add. In July 2015, the TAG chair replied that the researchers should test the species they think are appropriate and then submit the petition. The researchers then completed the remaining host-specificity tests and analyzed the results. The researchers wrote the document "A Petition for the Introduction, Experimental Release and Open-Field Release of the Root-Mining Weevil *Ceutorhynchus scrobicollis* (Coleoptera: Curculionidae) for the Biological Control of *Alliaria petiolata* (Garlic Mustard) in North America" which summarizes the 18 years of *C. scrobicollis* host specificity testing and provides additional information on garlic mustard biocontrol for the TAG reviewers. The petition was submitted to the USDA-APHIS Technical Advisory Group in June 2016.

Rearing protocols, release methods, and biocontrol manuals were developed for *C. scrobicollis*. The rearing manual will be submitted to the LCCMR as part of the final report. A publication entitled: [Biology and Biological Control of Garlic Mustard](#) was published in November, 2013. The manual describes the biology of garlic mustard

and potential biocontrol agents, and the biological control component of an integrated garlic mustard management program.

***Ceutorhynchus constrictus*:**

The University of Minnesota conducted host-specificity tests with the seed-feeding weevil, *Ceutorhynchus constrictus*. During the summers of 2014, 2015 and 2016, no-choice oviposition tests were conducted on six, seven, and six Brassicaceae species respectively. No eggs were found in any of the tested native mustard species and an average of 1.6 eggs were found in each garlic mustard silique (seed pod). Larvae were collected from each garlic mustard plant and counted when found. Larvae were placed into growth chambers and a refrigerator to simulate outdoor conditions so that the larvae could complete their life cycle and emerge into adults. Since no eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary. Our results show that *C. constrictus* continues to show the host specificity to be a successful biocontrol agent for garlic mustard.

In 2014, the University of Minnesota performed sequential no-choice tests on the following Brassicaceae species: *Hesperidanthus linearifolia*, *Physaria douglasii* ssp. *tuplashensis*, *Erysimum menziesii*, canola, *Lunaria annua*, and *Iodanthus pinnatifidus*. No eggs were found in any of the tested native mustard species and an average of 1.9 eggs were found in each garlic mustard silique (seed pod). Since no eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary.

In 2015, the University of Minnesota conducted no-choice oviposition tests on the following seven Brassicaceae species: *Matthiola incana*, *Thelypodium laciniatum*, *Thelypodium milleflorum*, *Draba ramosissima*, *Paysonia densipila*, *Iberis sempervirens*, *Lunaria annua*. No eggs were found in any of the tested native mustard species and an average of 1.8 eggs were found in each garlic mustard silique. Since no eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary.

In 2016, the University of Minnesota conducted no-choice oviposition tests on the following six Brassicaceae species: *Thelypodium milleflorum* (required additional replications), *Iberis sempervirens* (required additional replications), *Cochlearia officinalis*, *Cardamine diphylla*, *Physaria bellii*, *Physaria acutifolia*. No eggs were found in any of the tested native mustard species and an average of 1.2 eggs were found in each garlic mustard silique. Since no eggs were found in test plant species, no-choice larval development tests and single-choice tests were not necessary.

To date, no *C. constrictus* eggs or larvae have been found in any seed capsules of Brassicaceae plants tested at the University of Minnesota. This indicates that *C. constrictus* would likely be a host-specific biocontrol agent. Approximately 30 more species need to be tested and then a petition for release of *C. constrictus* can be submitted to the USDA-APHIS Technical Advisory Group.

**ACTIVITY 2:** Introduce and evaluate garlic mustard biological control agents in Minnesota

**Description:** A national, standardized garlic mustard monitoring protocol is in place. Twelve permanent monitoring sites were established in Minnesota and data has been collected on garlic mustard and native plant populations at these sites since 2005. When biocontrol agents are approved they will be released at these monitoring sites, and at other release sites as prioritized based on availability of agents, suitability of the site, and past cooperator participation supporting this effort. Pre-release plant monitoring data will be essential to understand garlic mustard population dynamics and to determine if the biological control agents are effective once released. Data is collected from the monitoring plots in June and October in each year.

**Summary Budget Information for Activity 2:**

**ENRTF Budget: \$35,581**  
**Amount Spent: \$35,581**

Balance: \$0

**Activity Completion Date:**

Outcome	Completion Date	Budget
1. Collect October 2013 monitoring data and analyze results	12/31/13	\$6,000
2. Collect June and October 2014 monitoring data and analyze results	12/31/14	\$14,000
3. Collect June and October 2015 monitoring data and analyze results	12/31/15	\$12,581
4. Collect June 2016 monitoring data.	06/30/16	\$3,000

\*Estimated amount per task, amounts may vary per task, but total budget will stay at \$35,581.

**Activity Status as of Dec. 31, 2013:**

A contract with the University of Minnesota has been written for the work in Activity 2. Signatures are currently being obtained on the contract.

**Activity Status as of June 30, 2014:**

The contract with the U of MN was fully signed and executed on 12-30-2013. Staff are identified to do monitoring work. October 2013 monitoring work results will be covered as part of the final report for the 2010 ENRTF project "Biological Control of European Buckthorn and Garlic Mustard" which was extended through June 30, 2014.

**Activity Status as of Dec. 31, 2014:** Garlic mustard monitoring was conducted at the monitoring sites during the month of October 2014. In October, data was collected on garlic mustard cover, density, insect damage to garlic mustard, cover of other plant species, and leaf litter cover and depth.

**Activity Status as of June 30, 2015:** Garlic mustard monitoring was conducted at the monitoring sites during the month of June 2015. In June, data was collected on garlic mustard cover, density, height and number of siliques of second year plants, insect damage to garlic mustard, cover of other plant species, and leaf litter cover and depth.

**Activity Status as of Dec. 31, 2015:**

Garlic mustard monitoring was conducted at the monitoring sites during the month of October 2015. In October, data was collected on garlic mustard cover, density, insect damage to garlic mustard, cover of other plant species, and leaf litter cover and depth. The analysis of monitoring data shows that garlic mustard population densities varied dramatically from year to year due to environment and due to the biennial nature of garlic mustard. Populations in subsequent years are highly dependent on seed rain from adults to replenish the seed bank. Seedling or rosette mortality can dramatically alter population densities and decrease the number of adults the following year. These seed driven population densities were highly dependent on precipitation and are greatly impacted by drought, particularly widespread in Minnesota in the latter half of the 2011, 12, and 13 growing seasons. Averaged across all locations, adult population densities varied from a high of 48 plants per m<sup>2</sup> in June of 2008 to a low of 5.1 per m<sup>2</sup> by June of 2014. Average population densities of adults trended to rebound in 2015 with a population density of 9.2 plants per m<sup>2</sup>. Competition for light with seedlings of desire native species likely is most intense in the spring with garlic mustard seedlings and adults comprising just under 50% cover when the trial began, which has declined to around 10% by 2014 and 2015.

By the fall of 2015, population densities of rosettes were still significant at Cottage Grove, Fort Snelling, and Warner Nature Center, with 8.5, 12.8, and 30.7 rosettes per m<sup>2</sup>, respectively. Plots were established at sites where garlic mustard had become a serious problem and may have been established, in some cases, in the vicinity of the initial invasion. Since the beginning of this monitoring effort, garlic mustard has moved on the landscape at some locations, and population densities have become most severe in areas where garlic mustard habitat may be more suitable for invasion beyond the monitoring quadrats. Researchers will be watching with interest to see if populations recover at some locations following the three consecutive years of late-season

drought that may have caused significant mortality of garlic mustard, depleting the seedbank and reducing invasion pressure in 2014 and 2015.

### **Final Report Summary:**

Monitoring is a key tool to understand changes in garlic mustard populations over time as well as the impacts of garlic mustard biocontrol insects should they be released. As garlic mustard biocontrol agents were not approved for release during the project time period, we cannot report on their efficacy in the field. We can report on trends in garlic mustard populations at the monitoring sites. Garlic mustard is a biennial and long-term monitoring shows that its populations can fluctuate widely from year to year. To monitor garlic mustard populations we used a nationally standardized protocol in which data is collected on garlic mustard population density and cover, garlic mustard plant heights and silique (seed pod) production, insect damage to garlic mustard, the cover of the associated plant community, and litter cover. By 2006, twenty permanent 0.5m<sup>2</sup> monitoring plots were established at 12 sites throughout Minnesota. Data has been collected each June and October since that time. The Pine Bend Bluff site was dropped following data collection in the fall of 2012 due to the site being converted from a forest to a savannah.

This report summarizes garlic mustard monitoring from October 2013 to June 2016. When the plots were established garlic mustard was present in 100% of the plots. Garlic mustard is still present in 88% of the plots as of June 2016. Garlic mustard is currently experiencing very little herbivory in Minnesota with an average amount of leaf removed due to herbivory ranging from 0.6 to 4.5% in 2014 - 2016. As in previous years, herbivory in Minnesota was never observed at levels indicting significant herbivory.

Garlic mustard cover and density can vary considerably from year to year. At some sites, population changes in garlic mustard from year to year are due to the biennial nature of garlic mustard. These sites tend to be dominated by either the 1<sup>st</sup> or 2<sup>nd</sup> year plants in any given year. The other sites had more variable garlic mustard populations. The highest density of 133 adults per m<sup>2</sup> occurred at Willmar in June of 2016, the lowest, 0 adults per m<sup>2</sup> at Willmar in June 2012, 2014 and 2015, and Nerstrand in 2014. Averaged over the course of study, sites ranged from mean cover of seedlings and adults garlic mustard of a high of 42% cover at Ft. Snelling to a low percent cover of less than 18% at Baker and Cottage Grove. Percent cover of garlic mustard rosettes in the fall ranged from a high of 13.4% at Ft. Snelling to a low of 5% or less at Baker, Coon Rapids, and Pine Bend. The highest % cover of 85% for adults and seedlings occurred at Luce Line in June of 2016, the lowest, 1.4% at Willmar in June 2015

There was variation in garlic mustard adult plant height and silique (seed pod) production from year to year. The mean height of garlic mustard over the course of the study ranged from a high of 67.1 cm at Coon Rapids to the shortest mean of 33.3 cm at Willmar. Mean siliques (seed pods) per plant ranged from a high of 14.3 to a low of 3.5 siliques per stem at Willmar. It is expected that after biological control release, garlic mustard cover and density will decrease and shoot heights and silique production of individual plants will decrease as well. Our data provides a basis from which to evaluate the effectiveness of biological control agents if released. Absent the knowledge gained from this research, localized declines in garlic mustard populations could be erroneously attributed to biological control. Establishment following initial releases of insects could be jeopardized or ineffective if released on populations of garlic mustard that are on the move, still finding their most suitable niches on the landscape.

### **V. DISSEMINATION:**

**Description:** It is expected that the results of this project will be published in peer-reviewed scientific journals and also in special publications and newsletters. Results also will be presented at national, regional and state scientific meetings to peers in the field, as well as to resource managers and planners who will use the results of this project.

**Status as of Dec. 31, 2013:** No updates.

**Status as of June 30, 2014:** Dr. Jeanie Katovich and Dr. Roger Becker have submitted a poster on this project to be presented at the Upper Midwest Invasive Species Conference in Duluth, MN held October 20-22, 2014.

**Status as of Dec. 31, 2014:** Dr. Jeanie Katovich and Dr. Roger Becker presented a poster on this project at the Upper Midwest Invasive Species Conference in Duluth, MN held October 20-22, 2014.

**Status as of June 30, 2015:** Dr. Jeanie Katovich gave a presentation on the project to the “Invasive Plant Management” class at the University of Minnesota – Twin Cities during the spring 2015 semester.

**Status as of Dec. 31, 2015:** Dr. Roger Becker gave a presentation titled “Petition to release *Ceutorhynchus scrobicollis* for biological control of garlic mustard (*Alliaria petiolata*)” at the 2015 Midwest Invasive Plant Network Invasive Plant Symposium (part of the North Central Weed Science Society annual meeting) in Indianapolis, IN on December 9, 2015.

#### **Final Report Summary:**

- Dr. Jeanie Katovich and Dr. Roger Becker presented a poster on this project at the Upper Midwest Invasive Species Conference in Duluth, MN held October 20-22, 2014.
- Dr. Jeanie Katovich gave a presentation on the project to the “Invasive Plant Management” class at the University of Minnesota – Twin Cities during the spring 2015 and 2016 semesters.
- Dr. Roger Becker gave a presentation titled “Petition to release *Ceutorhynchus scrobicollis* for biological control of garlic mustard (*Alliaria petiolata*)” at the 2015 Midwest Invasive Plant Network Invasive Plant Symposium (part of the North Central Weed Science Society annual meeting) in Indianapolis, IN on December 9, 2015.
- Dr. Jeanie Katovich gave a presentation on the project to Northeast Region US Forest Service researchers and staff at a meeting in Roseville, MN on March, 3, 2016.
- Dr. Jeanie Katovich presented the host-specificity data for *Ceutorhynchus scrobicollis* to the USDA APHIS Technical Advisory Group at their annual meeting in Greenbelt Maryland on April 6, 2016.
- Monitoring data has been shared with a consortium of researchers led by Dr. Bernd Blossey of Cornell University. This group will work together to produce a peer-reviewed publication on the results of garlic mustard monitoring plots in the Midwest and Northeast regions of the United States. The paper is currently being written.
- The petition for release for *C. scrobicollis* was submitted to the USDA APHIS Technical Advisory Group.
  - Title: A Petition for the Introduction, Experimental Release and Open-Field Release of the Root-Mining Weevil *Ceutorhynchus scrobicollis* (Coleoptera: Curculionidae) for the Biological Control of *Alliaria petiolata* (Garlic Mustard) in North America
  - Authors: Laura Van Riper, Esther Gerber, Harriet L. Hinz, Ghislaine Cortat, Elizabeth Katovich, Roger Becker, Mary Marek-Spartz
  - Date submitted: June 21, 2016
- Dr. Laura Van Riper will give the presentation “Perspectives on Garlic Mustard Biocontrol in the Midwest” at the Upper Midwest Invasive Species Conference in La Crosse, WI held October 17-19, 2016.

## **VI. PROJECT BUDGET SUMMARY:**

### **A. ENRTF Budget:**

<b>Budget Category</b>	<b>\$ Amount</b>	<b>Explanation</b>
Professional/Technical/Service Contracts:	\$140,000	This project is in cooperation with the University of Minnesota. Work will be

		contracted out to the University of Minnesota as the University has the required BioSafety Level II facility which is necessary for host-specificity testing of insects not yet approved for release in the United States.
<b>TOTAL ENRTF BUDGET:</b>		<b>\$140,000</b>

**Explanation of Use of Classified Staff:** Not applicable.

**Explanation of Capital Expenditures Greater Than \$3,500:** Not applicable.

**Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:** Not applicable.

**Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation:** 1

**B. Other Funds:**

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
<b>Non-state</b>			
USDA Forest Service, cash support.	\$73,059	\$73,059	Funds for host specificity testing of <i>Ceutorhynchus</i> spp. This grant expires federal fiscal year 2013 (Sept. 2013).
USDA Forest Service, cash support.	\$75,000	\$75,000	Funds for host-specificity testing in the US and Europe. This grant expires after federal fiscal year 2014 (Sept. 2014).
<b>State</b>			
MN DNR, general fund, support	\$10,000	\$10,000	MN DNR project manager will assist in managing the contract, analyzing data, writing proposals for release, writing manuals for release and monitoring, identifying potential release sites, and collecting data in the field. MN DNR will provide fiscal oversight and direct and necessary costs related to this project.
<b>TOTAL OTHER FUNDS:</b>	<b>\$158,059</b>	<b>\$149,357</b>	

**VII. PROJECT STRATEGY:**

**A. Project Partners:** This project is in cooperation with the University of Minnesota. Dr. Roger Becker and Dr. Elizabeth J. Stamm Katovich from the University of Minnesota have expertise in garlic mustard biological control research and access to a Biosafety Level II facility for host-specificity testing. The \$140,000 from the ENRTF will go to a contract with the University of Minnesota.

**B. Project Impact and Long-term Strategy:** Development and implementation of biological control is a long-term endeavor. Research on garlic mustard biocontrol has taken more than ten years to date. Consistent funding over multi-year periods is critical to support researchers to collect, grow, and adequately test and study the plants and insects involved. The research on *C. scrobicollis* has brought us to the critical stage of gaining approval from USDA-APHIS to release and implement the first biological control of garlic mustard in North America. The research on *C. constrictus* is nearing completion and could provide greater and more consistent control of garlic mustard if our work results in its approval for release.

Future needs include follow-up on the release and monitoring of *C. scrobicollis* to ensure success and to understand its impacts on Minnesota ecosystems, and to implement release and monitoring of *C. constrictus* should we gain approval for release. Multiple biocontrol agents are found to give greater control of the target invasive plant. Release and distribution of the four weevil species (*C. scrobicollis*, *C. constrictus*, *C. roberti*, and *C. alliariae*) is the long-term strategy for control of the invasive plant garlic mustard.

**C. Spending History:**

Funding Source	M.L. 2007 or FY08	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13
ENRTF	\$135,000 Subd. 4(i)			\$150,000 Subd. 6(a)	
USDA Forest Service		\$115,000		\$72,000	\$62,760

**VIII. ACQUISITION/RESTORATION LIST:** Not applicable.

**IX. MAP(S):** Not applicable.

**X. RESEARCH ADDENDUM:** This project is part of a multi-phase effort and a peer review occurred through the LCCMR process as part of the first phase.

**XI. REPORTING REQUIREMENTS:**

Periodic work plan status update reports will be submitted not later than Dec. 31, 2013, June 30, 2014, Dec. 31, 2014, June 30, 2015, and Dec. 31, 2015. A final report and associated products will be submitted between June 30 and August 15, 2016 as requested by the LCCMR.

**Final Attachment A: Budget Detail for M.L. 2013 Environment and Natural Resources Trust Fund Projects**

<b>Project Title:</b> <i>Biological Control of Garlic Mustard</i>								
<b>Legal Citation:</b> <i>M.L. 2013, Chp. 52, Sec. 2, Subd. 06e</i>								
<b>Project Manager:</b> <i>Laura Van Riper</i>								
<b>M.L. 2013 ENRTF Appropriation:</b> \$ 140,000								
<b>Project Length and Completion Date:</b> <i>3 years, June 30, 2016</i>								
<b>Date of Update:</b> <i>8-15-2016</i>								
<b>ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET</b>	<b>Revised 10-12- 2015 Activity 1 Budget</b>	<b>Amount Spent</b>	<b>Balance</b>	<b>Revised 10-12- 2015 Activity 2 Budget</b>	<b>Amount Spent</b>	<b>Balance</b>	<b>TOTAL BUDGET</b>	<b>TOTAL BALANCE</b>
<b>BUDGET ITEM</b>								
<b>Professional/Technical/Service Contracts</b>								
University of Minnesota for garlic mustard research	\$104,419	\$104,419	\$0	\$35,581	\$35,581	\$0	\$140,000	\$0
<b>COLUMN TOTAL</b>	<b>\$104,419</b>	<b>\$104,419</b>	<b>\$0</b>	<b>\$35,581</b>	<b>\$35,581</b>	<b>\$0</b>	<b>\$140,000</b>	<b>\$0</b>