

Technology Development for the Biological Control of Invasive Native and Non-Native Plants. Fiscal Year 2012.

Project Title: Biological control of garlic mustard: host specificity of a seed-feeding weevil, *Ceutorhynchus constrictus*.

Forest Health Protection Technical Working Group Contact:

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Project Principal Investigator:

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Co-Investigators:

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Amount Requested: \$75,000

Project Goals: The goal of this project is to develop the seed-feeding weevil, *Ceutorhynchus constrictus* as a biological control agent for garlic mustard (*Alliaria petiolata*).

Objective 1: Complete host specificity testing of *C. constrictus* on Brassicaceae species native to North America.

Objective 2: Prepare petition to submit host-range testing results for *C. constrictus* to the Technical Advisory Group for Biocontrol of Weeds.

Project Justification/Urgency: Garlic mustard is an entrenched, invasive plant and is native to Europe. It poses a threat to native herbaceous and woody plants in the forest understory (Blossey et al. 2001, Cavers et al. 1979). Garlic mustard has spread to 38 states and 6 Canadian provinces, has the potential for a wider distribution (Welk et al. 2002) and was rated as the top invasive plant species in 20 Northeastern area states in a recent Forest Service survey (na.fs.fed.us/fhp/invasive_plants/pdfs/analysis.pdf). Garlic mustard is also listed as a noxious weed in eight states, including Minnesota (Plants.usda.gov). This invasive significantly impacts forested riparian habitats and can be found along high priority landscapes such as the Mississippi River corridor.

Due to abundant seed production, garlic mustard is able to rapidly colonize mesic forests to produce dense stands (Meekins and McCarthy 2002) and is more competitive than other woody understory species (Meekins and McCarthy 1999). Garlic mustard produces phytotoxins (Vaughn and Berhow 1999) that are exuded from root tissue and can directly inhibit growth of native hardwoods such as red maple, sugar maple and white ash, or disrupt the mutual associations between native tree seedlings and mycorrhizal fungi that are critical for tree growth and survival (Roberts and Anderson 2001, Stinson et al. 2006, Wolfe et al. 2008).

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 by CABI Europe - Switzerland. These agents 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.

Host-range testing has been completed for *C. scrobicollis* and the results have been submitted to the Technical Advisory Group for the Biological Control of Weeds (TAG). This crown-mining weevil is predicted to have the most significant impact on garlic mustard demography (Davis et al. 2006). However, releases of single control agents are unlikely to control garlic mustard across its full range. A combination of agents that simultaneously reduce rosette survival and seed production will be required to suppress the most vigorous garlic mustard populations (Davis et al. 2006).

The seed feeder, *C. constrictus*, can reduce seed production on individual garlic mustard plants by up to 60.5% (Gerber et al. 2011). *Ceutorhynchus constrictus* females oviposit in developing garlic mustard pods in early summer. Larvae feed on the seeds and when mature, leave the pods to pupate in the soil. Adult weevils do not emerge from the soil until the following spring.

Host specificity testing of the species is well advanced and so far revealed a very narrow host range for this species. At this moment, additional host-range testing of 12 Brassicaceae species is required before a petition for field release can be compiled and submitted to TAG. These tests include no-choice oviposition and larval development tests as well as field single-choice tests. Host-range tests need to be conducted at both the University of Minnesota quarantine facility and at CABI Europe – Switzerland, since *C. constrictus* is native to Europe and all field single-choice tests must be completed in Europe. In addition, our colleagues in Switzerland have three test species available that we were unable to grow under quarantine conditions.

Difficulties were encountered by CABI in obtaining and growing some North American native Brassicaceae species on the proposed TAG host-range test list. These species were collected and propagated at the University of Minnesota to complete tests for the crown-miner *C. scrobicollis*. These native Brassicaceae species are still available at University of Minnesota. Our request for funding would allow us to complete the required host range testing for *C. constrictus* in quarantine on these additional plant species, a very cost-effective approach considering the difficulties in propagating and growing some of these native plants.

At the University of Minnesota, our Level 2 High Security Containment Facility is currently the only location in the country where work on these *Ceutorhynchus* spp. is being conducted. At our facility, we have been rearing and conducting host specificity testing with *Ceutorhynchus* species for the past eight years. Our interdisciplinary team of entomologists, weed scientists and plant

ecologists have over twenty years of experience in weed biological control development and implementation. This, in combination with fact that we are the only group in the country to currently work on these *Ceutorhynchus* spp. in quarantine, renders us uniquely qualified to implement this project.

Methods Approach: Host specificity testing for *C. constrictus* will be conducted in our quarantine facility at the University of Minnesota, St. Paul, MN and sub-contracted work will be conducted at CABI-Europe Switzerland. We will follow the procedures developed by CABI (Gerber et al. 2001). The hypothesis we are testing is that these biocontrol insects will select only garlic mustard for oviposition, feeding and larval development and not other plant species. To test this hypothesis, we will conduct non-target screening through no-choice and single-choice oviposition tests, larval development tests and field single-choice screening.

Objective 1: Complete host specificity testing of *C. constrictus* on Brassicaceae species native to North America.

Sequential no-choice oviposition test: To see whether any of the test species are accepted for oviposition under no-choice conditions, one pair of *C. constrictus* will be placed on a caged inflorescence of test plants for 2-3 days. After each exposure period on a test plant, weevils will be provided with garlic mustard to ensure that they continue to lay eggs. Seeds will be dissected and inspected for presence of eggs. If eggs are deposited in test plants, then the species will be subsequently tested in single-choice oviposition and no-choice larval development tests.

Single-choice oviposition test: One pair of *C. constrictus* will be offered an inflorescence from a non-target test plant and garlic mustard simultaneously. After 2-3 days, seeds will be dissected for presence of eggs. If eggs are present on test plants, then the species will be subsequently tested in no-choice larval development tests.

No-choice larval development test: Two females and one male will be placed in gauze bags tied around seed-bearing inflorescences of potted test or garlic mustard (control) plants for 5-7 days. After infestation, individual pods will be removed from each plant and dissected for eggs. If eggs are found, the inflorescence will be re-covered so larval development could be monitored in the remaining pods. Transparent plastic vials will be attached to the gauze bag with an elastic band and the inflorescence bent so that larvae leaving the pods will fall directly into the vials. Vials will be regularly checked for emerging larvae. Emerged larvae will be placed into sifted soil to complete development.

Field screening single-choice or open field tests: All test plant species that supported larval development of *C. constrictus* will be exposed under more natural conditions at CABI Europe – Switzerland. For single-choice field tests, caged potted test and garlic mustard plants will be placed together into screen cages (2 x 2 x 1.6 m). Mating pairs of *C. constrictus* will be placed into each screen cage. After one- to-two weeks, plants will be removed and dissected for eggs. For open field tests, potted test and garlic mustard plants will be placed in the field and mating pairs of *C. constrictus* will be released in the vicinity of plants. After one- to two-weeks, plants will be removed and dissected for eggs.

A minimum of five replications will be conducted for each test and each test plant species. It is expected that these tests could be completed within two years.

Objective 2: Prepare petition to submit host-range testing results for *C. constrictus* to the Technical Advisory Group for Biocontrol of Weeds.

A petition for field release of *C. constrictus* will be prepared in cooperation with investigators at CABI-Europe Switzerland and submitted to TAG.

Expected Products and Outcomes: The expected product of the proposed research is to complete host range testing for the seed-feeding weevil, *C. constrictus*, on non-target test plants and submit a petition to TAG for review. If approved by TAG and APHIS-PPQ, this can lead to the release of *C. constrictus* for use on garlic mustard. This action can reduce the time before wide-spread control of garlic mustard can be achieved and reduce the impact of this invasive plant on forest habitat.

Timetable: We expect to complete the host-range testing by April, 2014 and complete and submit a petition to TAG by September 30, 2014. Progress reports will be submitted as required.

Vita for Roger Becker

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Appointment: Extension 75% / Research 25%

Specific Areas of Responsibility: Weed management strategies in annual and perennial systems in disturbed and undisturbed habitats. Current projects include management of purple loosestrife in wetlands; garlic mustard and buckthorn in woodlands; and Canada thistle in native prairies, pastures and right-of-ways. Also prairie establishment, weed management in forages and in processing vegetables, and the environmental impacts of herbicide and non-herbicide weed management systems.

Education:

<u>Degree</u>	<u>Major</u>	<u>College/University</u>	<u>Years</u>
B.S.	Agronomy	Iowa State University	1972 - 1976
M.S.	Botany-Plant Physiology	Iowa State University	1976 - 1978
Ph.D.	Agronomy-Crop Production and Physiology	Iowa State University	1978 - 1982

Professional Positions:

<u>Employer</u>	<u>Dates</u>	<u>Position</u>
Iowa State University	1978-1982	Extension Associate
Monsanto Company	1982-1985	Product Development Representative
Monsanto Company	1985-1987	Product Development Associate
University of Minnesota	1987-1993	Assistant Professor
University of Minnesota	1993-1999	Associate Professor
University of Minnesota	1999- present	Professor

Recent Referred Publications:

- Katovich E.J.S., R.L. Becker, D.W. Ragsdale, and L.C. Skinner. 2008. Growth and Phenology of Three Lythraceae Species in Relation to Feeding by *Galerucella californiensis* and *Galerucella pusilla*: Predicting Ecological Host Range from Laboratory Host Range Testing. *Inv. Plant Sci Management*. 1(2):207-215.
- Sheaffer C.C., Undersander, D.J., and R.L. Becker. 2007. Comparing Roundup Ready and Conventional Systems of Alfalfa Establishment. © 2007 Plant Management Network. Published 24 July 24, 2007. 7 p. <http://www.plantmanagementnetwork.org/sub/fg/research/2007/alfalfa/>
- Bollman, J.D., C.M. Boerboom, R.L. Becker, and V.A. Fritz. 2008. Efficacy and tolerance to HPPD-inhibiting herbicides in sweet corn. *Weed Technol*. 22:666-674.
- Van Riper, L.C., R.L. Becker, L.C. Skinner. 2010. Population biology of garlic mustard (*Alliaria petiolata*) in Minnesota hardwood forests. *Inv. Plt. Sci Mgt*. 3:48-59.

Elizabeth J. Katovich

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Education:

1981	Purdue University, West Lafayette, IN B.S. in Plant Protection
1984	University of Arkansas, Fayetteville, AR M.S. in Agronomy
1988	University of Minnesota, St. Paul, MN Ph.D. in Agronomy/Weed Science

Employment:

1998-present	Senior Scientist, Department of Agronomy and Plant Genetics, University of Minnesota.
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Relevant Publications:

- Strefeler, M. S., E. Darmo, R. L. Becker and E. J. Katovich. 1996a. Isozyme characterization of genetic diversity in Minnesota Populations of *L. salicaria*, *Lythrum salicaria* (Lythraceae). *Amer. J. of Bot.* *Amer. J. Bot.* 83:265-273.
- Strefeler, M. S., E. Darmo, R. L. Becker and E. J. Katovich. 1996b. Isozyme variations in cultivars of *L. salicaria* (*Lythrum* sp.). *Breeding and Germplasm Resources.* *Hort. Sci.* 31:279-282.
- Katovich, E.J.S., R. L. Becker, C. C. Sheaffer, and J. L. Halgerson. 1998. Seasonal fluctuations of carbohydrate levels in roots and crowns of purple loosestrife (*Lythrum salicaria*). *Weed Sci.* 46:540-544.
- Katovich, E. J. S., A. J. Milles, D. W. Ragsdale and R. L. Becker. 1999. Feeding and oviposition preference of *Galerucella* spp. in *Lythrum*. X International Symp. Biol Control of Weeds. p. 99.
- Katovich, E. J. S., R. L. Becker and D. W. Ragsdale. 1999. Effect of *Galerucella* spp. on survival of purple loosestrife (*Lythrum salicaria*) roots and crowns. *Weed Sci.* 47:360-365.
- Katovich, E.J.S., D. W. Ragsdale, L. C. Skinner, and R. L. Becker. 2001. Effect of *Galerucella* spp. feeding on seed production in purple loosestrife. *Weed Sci.* 49:190-194.
- Katovich, E. J. S., R. L. Becker and J. L. Byron. 2003. Winter survival of late emerging purple loosestrife (*Lythrum salicaria*). *Weed Sci.* 51:563-568.
- Katovich, E.J. S., R. L. Becker, D. W. Ragsdale and L. C. Skinner. 2008. Growth and phenology of three Lythraceae species in relation to feeding by *Galerucella californiensis* and *Galerucella pusilla*: predicting ecological host range from laboratory host range testing. *Invasive Plant Sci. and Management.* 1:207-215.
- Gerber, E., Cortat, C., Hinz, H.L., Blossey, B., Katovich, E., Skinner, L., 2009. Biology and host specificity of *Ceutorhynchus scrobicollis* (Curculionidae; Coleoptera), a root-crown mining weevil proposed as biological control agent against *Alliaria petiolata* in North America. *Biocontrol Science and Technology*, 19 (2), 117-138.

Budget

Biological control of garlic mustard: host specificity of the seed-feeding weevil, *Ceutorhynchus constrictus*

USDA FS Forest Health Tech. Enterprise System proposal.

FY 2012 - 2014

RFP Contact: Michelle S. Frank

	Item	Requested Funding	25% Min. In Kind Other-Source Funding	Source 25% Cost Sharing, \$18750
FY 2012				
Administration	Salary	\$11,000.00 ¹	\$7,074.00	Portion of the USDA Indirect Costs Not Collected by U of M
	Fringe benefits	\$4,374.55	\$1,061.00	Salary for PI (1%)
	Travel	\$10.00	\$90.00	Benefits for PI
Procurements	Services	\$14,759.00		
	Space rental	\$500.00		
	Supplies	\$300.45		
Year Totals		\$30,944.00	\$8,225.00	
FY 2013				
Administration	Salary	\$10,900.00 ¹	\$7,048.00	portion of the USDA Indirect Costs Not Collected by U of M
	Fringe benefits	\$4,366.94	\$1,061.00	Salary for PI (1%)
	Travel	\$10.00	\$90.00	Benefits for PI
Procurements	Services	\$14,759.00		
	Space rental	\$500.00		
	Supplies	\$278.06		
Year Totals		\$30,814.00	\$8,199.00	
FY 2014				
Administration	Salary	\$4,100.00 ¹	\$1,175.00	portion of the USDA Indirect Costs Not Collected by U of M
	Fringe benefits	\$1,659.61	\$1,061.00	Salary for PI (1%)
	Travel	\$0.00	\$90.00	Benefits for PI
Procurements	Services	\$7,482.00		
	Space rental	\$0.00		
	Supplies	\$0.39		
Year Totals		\$13,242.00	\$2,326.00	
Total		\$75,000.00	\$18,750.00	

¹Salary and fringe not provided by the University of Minnesota, rely on outside sources for funding

BUDGET REQUEST EXPLANATION: Annual budgets include salary costs for a 0.19 FTE scientist for Year 1 and Year 2 and a 0.07 FTE for Year 3 as well as one part-time summer student worker to conduct host range screening to implement the biocontrol of garlic mustard. Salary and fringe benefits are not provided by the University of Minnesota and rely on outside sources for funding. Salary for scientist FY 2012, \$10,500, student worker, \$500; salary for scientist FY 2013, 10,500, student worker, \$400; salary for scientist FY 2014 \$4000, student worker, \$100. Fringe benefits are included in overhead costs: 41.3% of \$10,500 = \$4,337 for Years 1 and 2, and of \$4000 = \$1652 for Year 3 for scientist. A part time worker at 7.61% of \$500, \$400, and \$100 salary are \$38, 30, and 8 for Year 1, 2, and 3, respectively. Travel requested will cover the costs for incidental travel associated with research materials acquisition. Supplies requested will cover supplies required to implement host range studies. General operating services include weekend watering services. Short term rents and leases include renting growth chamber space in the High Security Containment Facility on the University of Minnesota campus to conduct the host range studies with native mustards and well as field space rental charges for growing plants. This grant will be administered as a Cooperative Agreement with the US Forest Service with a 75/25 matching requirement. A sub-contract to CABI-Europe Switzerland for \$14,259 for Years 1 and 2, and \$7482 for year 3 is included. CABI Europe Switzerland is able to conduct field level *C. constrictus* screening which is not legally possible in the U.S. prior to approval for release in the U.S. They also have some of the test plants in Europe that are required for host-range testing. Their work plan and budget are shown in Appendix I and Appendix II, respectively.

Cost Share: Matching funding of 25% is required and is in the form a portion of the of unrecoverable indirect costs rate of 28.2% (\$15,297) and 1% of salary and fringe benefit costs for Roger Becker, Principal Investigator (\$3453) for a total of \$18750 for total in-kind services, 25% of the funding requested.

Literature Cited:

- Blossey B., V. Nuzzo, H. Hinz and E. Gerber. 2001. Developing biological control of *Alliaria petiolata* (M. Bieb.) Cavara and Grande (garlic mustard). *Natural Areas J.* 21:357-367.
- Cavers, P. B., M. I. Heagy and R. F. Kokron. 1979. The biology of Canadian weeds. 35. *Alliaria petiolata* (M. Bieb.) Cavara and Grande. *Can. J. Plant Science.* 59:217-229.
- Davis, A. S., D.A. Landis, V. Nuzzo, B. Blossey, E. Gerber and H. Hinz. 2006. Demographic models inform selection of bicontrol agents for garlic mustard (*Alliaria petiolata*). *Ecol. Applicatons.* 16(6):2399-2410.
- Gerber, E., H. L. Hinz, N. Guazzone, J. McKenny, S. Michler and M. Zuefle. 2001. Biological control of garlic mustard, *Alliaria petiolata*. CABI Bioscience. Switzerland Centre. Annual Report.
- Gerber, E., D. Fife and H. L. Hinz. 2011. Biological control of garlic mustard (*Alliaria petiolata*). Annual Report 2010. CABI-Europe Switzerland. 18 pages.
- Meekins, J. F. and B. C. McCarthy. 2002. Effect of population density on the demography of an invasive plant, *Alliaria petiolata*, (*Brassicaceae*) population in a Southeastern Ohio forest. *American Midland Naturalist.* 147:256-278.
- Meekins, J. F. and B. C. McCarthey. 1999. Competitive ability of *Alliaria petiolata* (garlic mustard, *Brassicaceae*), an invasive, nonindigenous forest herb. *International J. Plant Science.* 160:743-752.
- Plants.usda.gov. Plants database. Accessed 5-17/2010.
- Roberts, K. J. and R. C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Bieb. Cavara Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *American Midland Naturalist.* 146:146-152.
- Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallett, D. Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms.
- Vaughn, S. F. and M. A. Berhow. 1999. Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *J. Chemical Ecology.* 25:2495-2504.
- Welk, E. K. Schubert and M. H. Hoffmann. 2002. Present and potential distribution of invasive garlic mustard (*Alliaria petiolata*) in North American. *Diversity and Distributions.* 8:219-233
- Wolfe, B. E., V. L. Rogers, K. A. Stinson, and A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *J. Ecol.* 96:777-783.

Appendix I. Information on Sub-Contractor: CABI Europe-Switzerland

CABI Europe-Switzerland (CABI E-CH) is one of seven overseas Centres of CAB International, an intergovernmental not-for-profit organization. The Centre in Switzerland was established nearly 60 years ago and has since been conducting foreign exploration for the classical biological control of invasive plant and insect pests, mainly for North America. For instance, the biological control agents currently being used to good effect against leafy spurge, knapweeds, purple loosestrife, Dalmatian toadflax and houndstongue were mostly researched and supplied by our Centre. At present, our Weed Biological Control Group is working on 17 invasive weed species on behalf of the USA and Canada with the aim to find and supply biological control agents for introduction (<http://www.cabi.org/default.aspx?site=170&page=1309>). Apart from the search for natural enemies and host-specificity testing, our work includes behavioral studies, studies on the impact of agents, their potential interactions, and plant population dynamics.

The Centre is equipped with an insect quarantine facility, laboratories for pathogen and insect work and chemical analyses, five greenhouses, and extensive garden space to grow plants and conduct experiments. Besides, scientists at CABI E-CH have established an extensive network of collaborators and contacts across Europe and Asia to facilitate field surveys and conduct open-field tests.

The Centre currently employs 21 research scientists, 15 in Switzerland, one each in Serbia and Hungary, and four at our newly established joint lab in Beijing, China, six PhD students, three MSc students, about 20 temporary technical assistants, one gardener and two garden helpers and three administrative staff. In addition to classical weed biological control, the Centre has expertise in arthropod biological control, integrated crop management, ecosystem management, risk analyses and invasion ecology.

Appendix II. Sub-Contractor: CABI Europe-Switzerland Work Program for 2012/13/14

Investigations on *Ceutorhynchus constrictus*, a potential biological control agents of garlic mustard, *Alliaria petiolata*.

Objectives

1. Collect adults of *C. constrictus* in spring 2012, 2013 and 2014 for shipments to Minnesota and additional host-specificity tests at CABI;
2. Conduct no-choice and single-choice tests with plant species available at CABI that are not available in Minnesota;
3. Conduct single-choice cage tests and open-field tests with critical test plant species;
4. Prepare progress reports on work conducted at CABI and contribute to writing up the release petition for *C. constrictus*.

Workplan

1. Adults of *C. constrictus* will be collected at field sites of garlic mustard in the vicinity of Delémont, Switzerland. This will be done during April, when females start to lay eggs. Some of the collected adults will be shipped to the quarantine facility in Minnesota and the rest will be used for additional screening tests at the Centre (see objectives 2 + 3).
2. No-choice oviposition and development tests still need to be conducted with seven plant species and single-choice tests with two species.
3. Plants with complete *C. constrictus* development under no-choice conditions will be tested in single-choice cage and/or open-field tests to further evaluate the natural host-choice behavior of *C. constrictus*. These tests will include *Brassica juncea*, brown or Indian mustard, a species from which in no-choice tests in 2010 two mature larvae were recovered, however, from which subsequently no adults emerged in spring 2011. In 2011, a single-choice cage test with *Brassica juncea* was conducted and although no attack was found on *B. juncea* under these conditions, we plan to carry out additional cage tests and/or an open-field test in 2012.
4. In late 2012 and 2013, data collected will be summarized and an Annual project report will be prepared. In 2013, information on *C. constrictus* relevant for the field release petition will be compiled. This will include data on host specificity but also on taxonomy, morphology, distribution, parasitoid attack and impact of the species on garlic mustard.