

1. PROJECT TITLE: Biological control of garlic mustard: Completion of host-specificity testing of *Ceutorhynchus constrictus* and *C. scrobicollis* following TAG petition review.

2. PRINCIPAL INVESTIGATOR(S)

Dr. Roger Becker

Dept. of Agronomy and Plant Genetics

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3. COOPERATORS AND OTHER PARTICIPATING INSTITUTIONS:

Cooperators:

Dr. Elizabeth J. Katovich

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Biological Control of Invasive Plant Technical Working Group Contact:

John Kyhl

Entomologist

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4. Budget Amount: \$84,000

Project Leveraging: \$46,900

Start Date: January 1, 2014

End Date: Sept. 30, 2016

5. Project Goals and Supporting Objectives: The goal of this project is to complete what is anticipated to be the last round of host-specificity testing to gain approval to release the crown-boring weevil, *Ceutorhynchus scrobicollis* and to complete host-specificity testing of the seed-feeding weevil, *Ceutorhynchus constrictus* as biological control agents to control garlic mustard (*Alliaria petiolata*).

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

Objective 2: Submit complementary data on host-range testing for *C. constrictus* and *C. scrobicollis* to the USDA, APHIS, Technical Advisory Group for Biocontrol of Weeds.

6. Project Justification/Urgency:

Justification: Compared to when this effort was started in 1998, the need for biological control of garlic mustard is well known. This entrenched, invasive plant, native to Europe, poses a threat to native herbaceous and woody plants in the forest understory in North America (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 has been rated as the top invasive plant species in 20 Northeastern area states in Forest Service surveys (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.

Through abundant seed production garlic mustard is able to rapidly colonize mesic forests and 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). The need for biological control is generally understood and of interest to land managers.

Urgency: Host-specificity testing of agents for biological control of garlic mustard has previously been supported with funding from the Technology Development for the Biological Control of Invasive Native and Non-Native Plants program for both *C. constrictus* and *C. scrobicollis*. We are requesting additional supplemental funding to complete what we anticipate will be the final round of testing to gain approval for release of *C. scrobicollis*. This additional host specificity work was requested by the USDA APHIS Technical Advisory Group for Biocontrol of Weeds (TAG) this past summer (June, 2013) after reviewing, supplemental documents submitted in response to Petition 08-05, *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 garlic mustard (Alliaria petiolata) in North America*. If the additional testing described in this proposal continues to show specificity, we see no impediments to approval of *C. scrobicollis* for release based on host specificity of the weevil. Efforts to gain approval of *C. constrictus* for release will follow. The seed-feeder, *C. constrictus*, is the second priority agent after *C. scrobicollis*. We are expanding the list of species to test for *C. constrictus* to match the species to test for *C. scrobicollis*.

As the phylogeny of the Brassicaceae has continued to evolve, it has necessitated adding plant species to our host-range testing program. Since the initiation of the garlic mustard biocontrol program in 1998, we have had to add additional plant species to our host range testing several times as the result of changes in Brassicaceae phylogeny. Currently, garlic mustard is placed in Lineage II of the Brassicaceae phylogeny (Franzke et al. 2011, Al-Shehbaz 2012). This information was not available when we submitted our original petition in 2008, nor prior to submitting the supplemental work to TAG in 2011. Based on reviewers' assessments and discussions with TAG at their meeting in June of 2013, we will focus additional host range testing efforts on Brassicaceae species from Lineage II and on Threatened and Endangered (T and E) species. Accordingly, we produced a list of 16 additional species which was submitted to TAG for comment/approval in October 2013.

In our Proposed Test Plant List submitted to TAG for comment, eight species are on the USFWS Federal List of T and E and Candidate Species. Six species are surrogates for T and E species that cannot be tested directly since seed are not available. Taxonomically related species were chosen as surrogates that have 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. The two remaining of the 16 proposed species are not T and E species, but are from tribes not yet tested and were recommended for testing by a TAG reviewer. With the testing of these 16 proposed species, the majority of Brassicaceae tribes encompassing species native to North America will have been included in our *C. scrobicollis* host-range testing. The proposed additional testing will include no-choice oviposition and larval development tests as well as field single-choice tests. Host-range tests will be conducted at both the University of Minnesota quarantine facility and at CABI in Delémont, Switzerland. The species tested at each site are based on who has access to seed or has been able to successfully germinate and grow the species, not always achievable with T and E species.

This additional funding will enable us to leverage the resources of the University of Minnesota with its Level 2 High Security Containment Facility and the resources of CABI Switzerland to complete the required host range testing for *C. constrictus* and *C. scrobicollis*. The University of Minnesota Level 2 High Security Containment Facility is currently the only location in the country where work on these *Ceutorhynchus* spp. is being conducted. We have been rearing and conducting host-specificity testing with *Ceutorhynchus* species at the quarantine facility in Minnesota for the past ten years. Scientist at CABI Switzerland have been working on these biological control agents since 1998. Our interdisciplinary team of entomologists, weed scientists and plant ecologists have over twenty years of experience in weed biological control development and implementation. The experience and expertise at CABI Switzerland and at the University of Minnesota containment facility renders our team uniquely qualified to implement and successfully complete this project.

7. Approach: Host-specificity testing for *C. constrictus* and *C. scrobicollis* will be conducted in our quarantine facility at the University of Minnesota, St. Paul, MN and sub-contracted work will be conducted at CABI in Delémont, 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* and *C. scrobicollis* 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* or *C. scrobicollis* will be placed on a caged inflorescence with developing seed pods (*C. constrictus*) or a leaf (*C. scrobicollis*) of a test species for 2-3 days. Seeds or leaves will be 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. After each exposure period on a test plant, weevils will be provided garlic mustard to ensure that they continue to lay eggs.

Single-choice oviposition test: One pair of *C. constrictus* or *C. scrobicollis* will be offered an inflorescence with developing seed pods (*C. constrictus*) or a leaf (*C. scrobicollis*) from a test species and garlic mustard simultaneously. After 2-3 days, seeds/leaves will be inspected 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 of *C. constrictus* or *C. scrobicollis* will be placed in gauze bags tied around seed-bearing inflorescences (*C. constrictus*) or a whole plant (*C. scrobicollis*) of potted test or garlic mustard (control) plants for 5-7 days. After infestation, individual pods (*C. constrictus*) or leaves (*C. scrobicollis*) will be removed from each plant and inspected for eggs. If eggs are found, the inflorescence/plant will be re-covered so larval development could be monitored in the remaining pods/plant. To retrieve mature *C. constrictus* larvae, 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. For *C. scrobicollis*, potted plants will be regularly checked for emerged adults and these counted and removed.

Field screening single-choice or open-field tests: Test plant species that supported larval development of *C. constrictus* or *C. scrobicollis* will be exposed under more natural conditions at CABI 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). Mated, egg laying pairs of *C. constrictus* or *C. scrobicollis* will be placed into each screen cage. After one- to-two weeks, plants will be removed and inspected for eggs. For open-field tests, potted test and garlic mustard plants will be placed in the field and mating pairs of *C. constrictus* or *C. scrobicollis* will be released in the vicinity of plants. After one- to two-weeks, plants will be removed and inspected for eggs. Since contamination from oligophagous weevil species (e.g. *C. obstrictus*) occurring naturally cannot be excluded in these tests, all eggs or larvae found during dissections will need to be stored in 95% ethanol and sent for molecular analyses to confirm species identity.

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* and *C. scrobicollis* 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 Switzerland and submitted to TAG.

8. Expected Products and Outcomes: The expected product of the proposed research is to complete host range testing for the seed-feeding weevil, *C. constrictus* and *C. scrobicollis* 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* and *C. scrobicollis* for use on garlic mustard. This action can reduce the time before widespread control of garlic mustard can be achieved and reduce the impact of this invasive plant on forest habitat.

Additional Items Requested (not in 5 page limit):

1. Curriculum Vitae Vita for Roger Becker

Title: Extension Agronomist - Weed Scientist

Department: Agronomy and Plant Genetics

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Telephone: (612) 625-5753 **E mail:** becke003@umn.edu

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:

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.

- 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 calmariensis* 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/>
- 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.

Vita for Elizabeth J. Katovich

Elizabeth J. Stamm Katovich

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Telephone: (612) 625-5230

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.

Relevant Publications:

- 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.
- 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.
- Strefeler, M. S., E. Darms, 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.* 83:265-273.
- Strefeler, M. S., E. Darms, 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.

2. Budget

Biological control of garlic mustard: Completion of host-specificity testing of <i>Ceutorhynchus constrictus</i> and <i>C. scrobicollis</i> following TAG petition review.				
USDA Forest Service, Technology Development for the Biological Control of Invasive Native and Non-Native Plants. FY 2014 – 2016 RFP: John Kyl				
			25% Match Required	
	Item	Requested Funding	25% min. In-Kind Other-Source Funding	Source 25% Min. of USFS Contribution + In Kind Cost Sharing Total = \$25386
FY2014				
Administration	Salary	\$17,000	\$15,421	Portion of the USDA 30% Indirect Costs Not Collected by U of M
	Fringe benefits	\$6,109	\$3,365	Salary for PI (3%)
	Travel	\$10	\$268	Benefits for PI
Procurement	Services	\$12,564		
	Space rental	\$0		
	Supplies	\$300		
Year Totals		\$35,983	\$19,055	
FY2015				
Administration	Salary	\$16,900	\$15,438	Portion of the USDA 30% Indirect Costs Not Collected by U of M
	Fringe benefits	\$6,102	\$3,365	Salary for PI (3%)
	Travel	\$10	\$268	Benefits for PI
Procurements	Services	\$12,564		
	Space rental	\$0		
	Supplies	\$237		
Year Totals		\$35,813	\$18,982	
FY2016				
Administration	Salary	\$5,600	\$5,230	Portion of the USDA 30% Indirect Costs Not Collected by U of M
	Fringe benefits	\$2,031	\$3,365	Salary for PI (3%)
	Travel	\$0	\$268	Benefits for PI
Procurements	Services	\$4,573		
	Space rental	\$0		
	Supplies	\$0		
Year Totals		\$12,204	\$8,863	
Total		\$84,000	\$46,900	

BUDGET REQUEST EXPLANATION: This grant will be administered as a Cooperative Agreement with the US Forest Service with a 75/25 matching requirement. In this request, \$55,300 is budgeted for the work at the University of Minnesota and a sub-contract of \$ 28,700 is budgeted for work at CABI Switzerland. Annual budgets for work at the University of Minnesota include salary costs for a 0.31 FTE scientist for Year 1 and Year 2 and a 0.10 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. Fringe benefits are included in overhead costs (36.8% of salary) with total salary and fringe = \$22,572 in Year 1 and 2 and \$7524 Year 3 for the scientist position. A part time worker with fringe benefits at 7.4% of salary with total salary and fringe = \$537 for Year 1, \$430 for Year 2, and \$107 for Year 3. Travel requested will cover the costs for the PI's to travel to a professional meeting, get supplies, or conduct business relevant to this grant. Supplies requested will cover supplies required to implement host range studies. General operating services include weekend watering services, fees for insect shipment, etc. CABI Switzerland is budgeted \$12,063.50 per year for Year 1 and 2, \$4573 for Year 3, a total of \$28,700 is budgeted including non-salary items. CABI Switzerland is able to conduct field level *C. constrictus* and *C. scrobicollis* screening which we can not do in Minnesota since the agents have not been approved to move out of quarantine. CABI's budget includes lab service fees for molecular analysis of samples to detect contamination from oligophagous weevil species e.g. *C. obstrictus*. CABI is also in possession of some of the test plants required for host-range testing.

Cost Share: Matching funding is in the form of unrecoverable indirect costs at a rate of 30% in the form of indirect costs (\$36,000) and 3% of salary and fringe benefit costs for Roger Becker, Principal Investigator for a total of \$46,900 for total in-kind services.

3. Timetable: We expect to complete the host-range testing by April, 2016 and complete and submit a petition to TAG by September 30, 2016. Progress reports will be submitted as required. Work may proceed faster depending on how rapidly we are able to procure seed and develop methods to successfully germinate and propagate test plant species and match developmental staging with that of the weevils. Effort charged in FY periods and timetables will be adjusted accordingly.

4. Literature Cited:

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- 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.
- Franzke, A., M. A. Lysak, I. A. Al-Shehbaz, M. A. Koch and K. Mummenhoff. 2011. Cabbage family affairs: the evolutionary history of Brassicaceae. *Trends in Plant Sciences.* 16(2):108-116.
- 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.
- 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. McCarthy. 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 below-ground 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 t expertise in arthropod biological control, integrated crop management, ecosystem management, risk analyses and invasion ecology.

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.