M.L. 2010 Project Abstract
For the Period Ending June 30, 2014

PROJECT TITLE: Biological Control of European Buckthorn and Garlic Mustard
PROJECT MANAGER: Laura Van Riper
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2010, Chp. 362, Sec. 362, Subd. 6a and M.L. 2013, Chapter 52, Section 2, Subdivision 17

APPROPRIATION AMOUNT: $300,000

Overall Project Outcome and Results

European/common buckthorn (*Rhamnus cathartica*) and garlic mustard (*Alliaria petiolata*) are non-native invasive plants that severely threaten native plant communities and degrade wildlife habitat. They are widely distributed in the state and current control options, such as mechanical and chemical control, are labor and cost-intensive. They are of the highest priority for development of long-term management solutions, such as biological control. The purpose of this research was to determine 1) if there are suitable insects that can be used to reduce impacts caused by buckthorn and 2) implement introduction of insects to control garlic mustard and assess their establishment and success.

Over 30 specialized insects were identified as potential common buckthorn biocontrol. Most of these species were discarded because they lacked host-specificity. Two psyllids were host-specific, but did not cause significant damage to buckthorn and the insects were infected with the plant disease '*Candidatus* Phytoplasma rhamni' (buckthorn witches' broom). A seed-feeding midge proved too difficult to work with in a research setting. After 11 years of searching for a biological control insect that is host-specific and damaging to buckthorn, we conclude that there are not promising agents at this time.

Four *Ceutorhynchus* weevil species are being studied as biological control agents for garlic mustard. Petitions for release were submitted to the USDA-APHIS Technical Advisory Group starting in 2008, but they have requested additional host-specificity testing over time. No biological control insects have been approved for release as of 2014. Studies conducted in the University of Minnesota Containment Facility allowed the development of efficient and consistently reliable methods to rear *C. scrobicollis* from garlic mustard plants. Long-term monitoring at twelve sites in Minnesota shows that garlic mustard populations can fluctuate widely from year to year. There is little garlic mustard herbivory in Minnesota. Garlic mustard cover is negatively correlated with cover of other species.

Project Results Use and Dissemination

Buckthorn biological control research has been disseminated in the following ways:
Peer reviewed journal publication (pdf attached):

- Gassmann, A. and I. Tosevski. 2014. Biological control of Rhamnus cathartica: is it feasible? A review of work done in 2002–2012. Journal of Applied Entomology 138: 1-13.

CABI Report Summary (pdf attached):

- Gassmann, A., A. Leroux, M. Bennett, M. Penic, N. Haefliger, R. Eschen, J. Jović and I. Toševski. 2012. Report 2010–12: Biological control of common buckthorn, *Rhamnus cathartica*. CABI Europe-Switzerland. CABI Ref: VM01730.

Poster presentations at conferences:

- Gassman, Andre, Laura C. Van Riper*, and Luke C. Skinner. Conclusions from 11 Years of Buckthorn Biocontrol Research. Ecological Society of America Conference. 4-9 August 2013. La Crosse, WI.
- Gassman, Andre, Laura C. Van Riper*, and Luke C. Skinner. Conclusions from 11 Years of Buckthorn Biocontrol Research. Upper Midwest Invasive Species Conference. 29-31 Oct 2012. La Crosse, WI.
- Gassman, Andre, Laura Van Riper*, and Luke C. Skinner. Developing Biological Control for Common and Glossy Buckthorn. Invasive Plants Symposium, Dec. 2011. Milwaukee, WI.
- Gassman, Andre, Laura Van Riper*, and Luke C. Skinner. Developing Biological Control for Common and Glossy Buckthorn. Minnesota-Wisconsin Invasive Species Conference, 2-10 Nov 2010. St. Paul, MN.

Webpage created on MN DNR website:

- http://www.dnr.state.mn.us/invasives/terrestrialplants/woody/buckthorn/biocontrol.html

Garlic mustard biological control research has been disseminated in the following ways:
Peer reviewed journal publication (pdf attached):

- Becker, R.L., E.J.S. Katovich, H.L. Hinz, E. Gerber, D.W. Ragsdale, R.C. Venette, D.N. McDougall, R. Reardon, L.C. Van Riper, L.C. Skinner, and D.A. Landis. 2013. The Garlic Mustard (*Alliaria petiolata*) Case, What Makes a Good Biological Control Target. The Intersection of Science, Perspectives, Policy and Regulation. pp. 332-339 *In* Proc. XIII International Symposium on Biological Control of Weeds (ISBCW). Sept. 11-16, 2011. Waikoloa, Hawaii. Wu, Yun; Johnson, Tracy; Sing, Sharlene; Raghu, S.; Wheeler, Greg; Pratt, Paul; Warner, Keith; Center, Ted; Goolsby, John; and Reardon, Richard, Editors. USDA Forest Service, FHTET-2012-07. January 2013. 536 p. http://www.invasive.org/publications/xiiisymposium/

U.S. Forest Service Technology Transfer document:

- Becker, Roger, Esther Gerber, Hariet L. Hinz, Elizabeth Katovich, Brendon Panke, Richard Reardon, Mark Renz, and Laura Van Riper. 2013. Biology and Biological Control of garlic Mustard. US Forest Service Forest Technology Enterprise Team publication FHTET-2012-05. http://www.fs.fed.us/foresthealth/technology/pdfs/GarlicMustardBiocontrol_FHTET-2012-05.pdf [Accessed May 2014].

Reports to the Environment and Natural Resources Trust Fund (pdfs attached):

- Katovich, E.J. and Becker, R.L. 2014. Garlic mustard biological control: Developing biological control insects, working towards field release.
- Van Riper, L.C. and Becker, R.L. 2014. Garlic mustard (*Alliaria petiolata*) monitoring in Minnesota: 2005-2013.

Presentations:

- E. J. S. Katovich. Upper Midwest Invasive Species Conference. November, 2010. St. Paul, MN. Biocontrol of Garlic Mustard and Buckthorn, an Update.

- E. J. S. Katovich. XIII International Symposium on Biological Control of Weeds. September, 2011. Waikoloa, Hawaii. Biological Control of Garlic Mustard, *Alliaria petiolata*, with the Root and Crown- Boring Weevil, *Ceutorhynchus scrobicollis*.
- E. J. S. Katovich. Ontario Invasive Plant Council, Annual General Meeting and Conference. October, 2011. Picton, Ontario. Potential for the Biological Control of Garlic Mustard.
- E. J. S. Katovich. Upper Midwest Invasive Species Conference. October, 2012. La Crosse, WI. Biological Control of Garlic Mustard with a Seed-Feeding Weevil.
- E. J. S. Katovich. Biological Control of Northeastern Weeds-2013 Cooperators Meeting. February, 2013. Trenton, New Jersey. Garlic Mustard Biocontrol: Current Status and Future Directions.
- E. J. S. Katovich. Technical Advisory Group For the Biological Control of Weeds, Annual Meeting. June, 2013. Washington, D.C. *Ceutorhynchus scrobicollis* as a Potential Biocontrol Agent of Garlic Mustard, *Alliaria Petiolata.*
- E. J. S. Katovich. University of Minnesota, guest lecturer for AGRO 4505: Biology, Ecology and Management of Invasive Plants. Biological Control of Invasive Plants. 2010-2014.

2010 Environmental and Natural Resources Trust Fund ENRTF
Work Program Amendment Final Report

Date of Report: August 15, 2014
Date of Next Status Report: Final Report
Date of Work program Approval: June 3, 2010
Project Completion Date: June 30, 2014

I. PROJECT TITLE: Biological Control of European Buckthorn and Garlic Mustard

Project Manager: Laura Van Riper
Affiliation: Minnesota Department of Natural Resources
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St. Paul, MN 55155-4025
Telephone number: 651-259-5090
Email: laura.vanriper@state.mn.us

Location: State, county and federal parks, forests, nature preserves and wildlife management areas; roadsides private woodlots and agricultural lands statewide.

Total ENRTF Project Budget: **ENRTF Appropriation: $ 300,000**
Minus Amount Spent: $ 300,000
Equal Balance: $ 0

Legal Citation: M.L. 2010, Chp. 362, Sec. 362, Subd. 6a and **M.L. 2013, Chapter 52, Section 2, Subdivision 17**

Appropriation Language:
The availability of the appropriations for the following projects are extended to June 30, 2014: (6) Laws 2010, chapter 362, section 2, subdivision 6, paragraph (a), Biological Control of European Buckthorn and Garlic Mustard

$300,000 is from the trust fund to the commissioner of natural resources in cooperation with the commissioner of agriculture to continue the development and implementation of biological control for European buckthorn and garlic mustard. This appropriation 14.2 is available until June 30, 2014, by which time the project must be completed and final products delivered.

An extension is requested to complete activity 2: Introduction and evaluation of Garlic Mustard biological control agents in MN. While a petition for release of the biocontrol weevil *Ceutorhynchus scrobicollis* was submitted to the USDA in 2011, no decision has been made by the agency. Without the insect being approved for release, we were not able to complete the tasks in the work plan. We are requesting a one year extension to continue to work on the actions in Activity 2. This one-year extension is contingent on legislative approval.

Amendment Approved: May 9, 2013

II. FINAL PROJECT SUMMARY AND RESULTS:

European/common buckthorn (*Rhamnus cathartica*) and garlic mustard (*Alliaria petiolata*) are non-native invasive plants that severely threaten native plant communities and degrade wildlife habitat. They are widely distributed in the state and current control options, such as mechanical and chemical control, are labor and cost-intensive. They are of the highest priority for development of long-term management solutions, such as biological control. The purpose of this research was to determine 1) if there are suitable insects that can be used to reduce impacts caused by buckthorn and 2) implement introduction of insects to control garlic mustard and assess their establishment and success.

Over 30 specialized insects were identified as potential common buckthorn biocontrol. Most of these species were discarded because they lacked host-specificity. Two psyllids were host-specific, but did not cause significant damage to buckthorn and the insects were infected with the plant disease '*Candidatus* Phytoplasma rhamni' (buckthorn witches' broom). A seed-feeding midge proved too difficult to work with in a research setting. After 11 years of searching for a biological control insect that is host-specific and damaging to buckthorn, we conclude that there are not promising agents at this time.

Four *Ceutorhynchus* weevil species are being studied as biological control agents for garlic mustard. Petitions for release were submitted to the USDA-APHIS Technical Advisory Group starting in 2008, but they have requested additional host-specificity testing over time. No biological control insects have been approved for release as of 2014. Studies conducted in the University of Minnesota Containment Facility allowed the development of efficient and consistently reliable methods to rear *C. scrobicollis* from garlic mustard plants. Long-term monitoring at twelve sites in Minnesota shows that garlic mustard populations can fluctuate widely from year to year. There is little garlic mustard herbivory in Minnesota. Garlic mustard cover is negatively correlated with cover of other species.

III. PROGRESS SUMMARY AS OF *(12/30/13)*:

Update (12/30/13):

The buckthorn project (Activity 1) had been completed as of 6/30/2013. Since that time a journal article resulting from that work has been accepted for publication by the Journal of Applied Entomology.

A contract with the University of Minnesota for the garlic mustard work in Activity 2 was written and signed. Garlic mustard monitoring data was collected in October 2013. Also in October 2013 a letter was submitted to the USDA APHIS Technical Advisory Group (TAG) with the plant species that we propose for additional host-specificity testing.

Update (06/30/13):

Buckthorn biocontrol research was completed and a final report submitted by CABI. CABI has also developed a draft journal article that will be submitted for publication in the journal "Biological Control". The final report is attached to this summary. Numerous potential biocontrol insects for common and glossy buckthorn were screened for host-specificity and impacts. After 11 years of searching for a biocontrol insect that is both host-specific and damaging to common buckthorn, we conclude that we do not have any promising agents at this time. The journal article will be summarize the results of the buckthorn biocontrol research so the results will be available if a new buckthorn biocontrol project is initiated in the future.

Garlic mustard monitoring of field sites was conducted in October 2012 and June 2013. Lab studies continued to develop mustard propagation methods and *C. scrobicollis* rearing protocols in the High Containment facility the University of Minnesota in anticipation of permission to release *C .scrobicollis* into the field for the biocontrol of garlic mustard. In May and June 2013 we received communication from the USDA APHIS Technical Advisory Group regarding the petition for release of *C. scrobicollis*. The petition was rejected based on the desire to see additional Threatened and Endangered mustard species undergo host-specificity testing. Dr. Katovich and Dr. Becker will work with the US Fish and Wildlife Service on developing a list of species to be tested and obtaining the seeds. At this time, we do not know the final number of additional species that need to be tested, but it appears that it will be fairly small as many of the species have already been tested.

Update (09/30/12):
Buckthorn biocontrol research was completed and a final report submitted by CABI. Due to the difficulties surrounding currently studied agents and the low probability of finding additional potential agents, it has been decided that buckthorn biocontrol research will not be pursued into the future. The remaining psyllid potential biocontrol agents had issues with a lack of impact on buckthorn and potentially carrying a phytoplasma (plant disease) that is not known to be in the United States. The researchers were not able to work with the remaining seed-feeding midge in a research setting as they could not obtain fruiting trees of buckthorn species. The research that has been done on buckthorn biocontrol will be written into a journal article so that others may learn from this research and to provide a starting point if someone were to reinitiate buckthorn biocontrol research in the future.

There has been no notification from the USDA Technical Advisory Group (TAG) as to the status of the petition for the release of *Ceutorhynchus scrobicollis* as a biological control insect for garlic mustard. The petition was submitted to TAG in September of 2011. Garlic mustard monitoring data was collected from the 12 permanent monitoring sites in June 2012. Studies to maximize the reliability and production of *C. scrobicollis* rearing have been carried out. A 3 month aestivation time allows for the greatest production of insects. A study looking at the effect of the soil mixtures has found that the addition of 3 to 4 cm of greenhouse soil can aid in *C. scrobicollis* pupal survival and result in an increase in the number of insects reared. Work continues on updating and revising a manual for propagating garlic mustard and rearing *C. scrobicollis*.

Update (02/28/12):
Garlic mustard monitoring was conducted in October 2011. Data has been entered and data analysis has begun. Garlic mustard continues to be widespread. Of the 12 monitoring sites, the average percent cover of garlic mustard in June 2011 ranged from 6% garlic mustard cover at the lowest cover site to 65% garlic mustard cover at the highest cover site. There has been no notification from the USDA Technical Advisory Group (TAG) as to the status of the petition for the release of *Ceutorhynchus scrobicollis* as a biological control insect for garlic mustard.

Buckthorn biological control research at CABI Europe-Switzerland found that the potential biocontrol insect *Trichochermes walkeri* proved to be infected with '*Ca.* Phytoplasma rhamni' at a very high rate in almost all sampled localities. In Europe, *R. cathartica* trees were found to be infected with '*Candidatus* Phytoplasma rhamni' at almost all surveyed localities. Researchers did not find evidence of negative plant-soil feedback by mature *R. cathartica* on conspecifics that could explain low seedling numbers of *R. cathartica* in the native range.

Sixty buckthorn plants throughout Minnesota and the Midwest were sampled and tested for the presence of the buckthorn phytoplasma disease '*Candidatus* Phytoplasma rhamni'. None of the samples were found to be positive for the phytoplasma.

Update (09/30/11):
The garlic mustard biocontrol host-specificity was completed by researchers at CABI Europe-Switzerland and the University of Minnesota. The results were written up and the petition was submitted to the USDA Technical Advisory Group (TAG) on September 8, 2011. The petition was a supplement to the original petition number 08-05, submitted April 2008. The petition title was: 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 petiolota*) in North America . The petitioner was Dr. Luke Skinner. The Technical Advisory Group will review the petition and recommend to USDA-APHIS whether or not the garlic mustard biocontrol insect *C. scrobicollis* should be approved for release in the United States.

Research continues at CABI Europe-Switzerland on buckthorn biological control. Work focuses on the insects *T. walkeri, W. krumbholzi* and the phytoplasma disease '*Candidatus* Phytoplasma rhamni'. Using LCCMR funds, a contract was written with Dr. Roger Becker at the University of Minnesota to test buckthorn plants from the United States for presence of the phytoplasma.

Using LCCMR funds, a contract was written with Dr. Roger Becker at the University of Minnesota for garlic mustard monitoring in June and October 2011.

Update (02/28/11): A two year contract was written with CABI Europe-Switzerland for continued research on buckthorn biological control. Goals for July 1, 2010 to June 30, 2011 include continuing to assess the feasibility of using insects *Trichochermes walkeri*, *Cacopsylla rhamnicolla*, and *Wachtiella krumbholzi* as biological control agents for *Rhamnus cathartica*. Additional study of the plant disease phytoplasma '*Candidatus* Phytoplasma rhamni' is necessary to determine if *T. walkeri* could be used as a biological control agent. Additionally, researchers will work to determine the causes of the high levels of seed and seedling mortality of *R. cathartica* observed in Europe as a step toward identifying additional potential biological control agents. CABI researchers have collected samples of *Rhamnus* species and *T. walkeri* for detection of the phytoplasma. Samples of the *Rhamnus* have been analyzed and the phytoplasma has been detected in four of the countries they sampled, but trees did not show visible symptoms of the disease. Additional work has been completed in preparation for additional host specificity testing of the target insects.

A contract was written with the University of Minnesota for garlic mustard monitoring in October 2010. The results of that research are currently being analyzed. Final host specificity testing of the garlic mustard biocontrol agent *Ceutorhynchus scrobicollis* is expected to be completed by May 2011. Then a proposal for approval for release will be submitted to the USDA-APHIS Technical Advisory Group. TAG will give a recommendation as to whether the insects may be released in the United States.

IV. OUTLINE OF PROJECT RESULTS*:*

Result/Activity 1: Investigate potential insects as biological control of European Buckthorn

Description: Researchers from the CABI Europe-Switzerland will continue to locate, identify and collect potential natural enemies of *Rhamnus cathartica* and *Frangula alnus* of *Rhamnus* spp in Europe. Host specificity studies (make sure the insects will not eat plants native to MN and the U.S.) will continue on the high priority insect species. Insects will be prioritized based on their perceived potential to cause damage to buckthorn by impairing growth and/or reproduction, reduce vigor, or cause structural damage. These factors can potentially lead to buckthorn mortality. Expected results include a priority list of potential control agents with information on their host specificity to native buckthorn species and other plants as determined. This information will guide future research and eliminate candidate insects that are not good potential agents. Testing is done in Europe due to availability if insects and reduce risk of importing any species prior to release. Most species are collected from the wild as cuttings or as seed. Precautions are taken to ensure no soil or other plant parts are shipped with the test plants. The plants are then grown by the researcher in Switzerland and used in testing the insects. Testing procedures are determined once the insects have been identified.

Summary Budget Information for Result/Activity 1:	**ENRTF Budget**	**$150,000**
	Amount Spent	**$150,000**
	Balance	**$0**

Deliverable/Outcome	Completion Date	Budget
Field collection and host specificity testing of agents in 2010 and annual report summarizing results for 2010	2/28/11	$30,000
Field collection and host specificity testing of agents in 2011	9/30/11	$30,000
Annual report summarizing results for 2011	2/28/12	$30,000
Field collection and host specificity testing of agents in 2012	9/30/12	$30,000
Final report with findings and recommendations	6/30/13	$30,000

Completion Date: 6/30/14

Results Status as of (12/30/13):
This project was completed as of 6/30/2013. The research paper that resulted from this work was accepted for publication by Journal of Applied Entomology. The article is currently in press.

Results Status as of (6/30/13):
Buckthorn biocontrol research was completed and a final report submitted by CABI (Attached). CABI has also developed a draft journal article that will be submitted for publication in the journal "Biological Control". Numerous potential biocontrol insects for common and glossy buckthorn were screened for host-specificity and impacts. Early on, glossy buckthorn biocontrol was eliminated from consideration due to lack of promising agents. Research continued on common buckthorn. After 11 years of searching for a biocontrol insect that is both host-specific and damaging to common buckthorn, we conclude that we do not have any promising agents at this time. The journal article will summarize the results of the buckthorn biocontrol research so the results will be available if a new buckthorn biocontrol project is initiated in the future.

Result Status as of (9/30/12)
Research in Europe: An impact study of the effect of leaf galling by *T. walkeri* on eight-month-old *R. cathartica* seedlings was set up in August 2011. A total of 714 eggs were laid on infected trees. However, in 2012, no galls were recorded and the test was terminated without having obtained conclusive results.

Buckthorn biocontrol research projects were completed; work now focuses on writing the results and conclusions. CABI submitted their final report to MN DNR in September 2012. There is low potential for the remaining potential biocontrol insects to provide control of buckthorn. The psyllid species may be implicated in the spread of the phytoplasma (a type of plant disease) witches'-broom of buckthorn. Many buckthorn plants in Europe have the phytoplasma, but show no symptoms. There is no evidence that the buckthorn phytoplasma is present in the US. This possibility of a biocontrol agent spreading a phytoplasma that is not already present in the US makes it unlikely that the insects would gain approval for release. The remaining biocontrol insects, the seed feeding midges, proved too difficult to work with in a lab setting. The researchers could not obtain reproductive trees of buckthorn species, so therefore could not pollinate female buckthorn flowers or synchronize fruit development with midge oviposition and larval development. Without fruits of buckthorn species, the researchers could not screen the midges as to their host specificity.

Due to the difficulties surrounding currently studied agents and the low probability of finding additional potential agents, it has been decided that buckthorn biocontrol research will not be pursued into the future. The lead researcher will write up the final results of the buckthorn biocontrol research project for publication.

Result Status as of (2/28/12)
Research in Europe:

- In Europe, *R. cathartica* trees were found to be infected with '*Candidatus* Phytoplasma rhamni' at almost all surveyed localities, although the presence of witches' broom symptoms were not observed. Phytoplasma was not detected in any of the other *Rhamnus* species analyzed, which suggests a very specific host association of this phytoplasma with its plant host.
- *Trichochermes walkeri* proved to be infected with '*Ca.* Phytoplasma rhamni' at a very high rate in almost all sampled localities. However, *T. walkeri* infection with phytoplasma only shows that this psyllid is acquiring the phytoplasma during feeding on infected plants, but not a capability to re-inject the phytoplasma during feeding. The latter will be tested in transmission trials that were started in 2011 and will be completed in 2012.
- Researchers did not find evidence of negative plant-soil feedback by mature *R. cathartica* on conspecifics that could explain low seedling numbers of *R. cathartica* in the native range.

Research in the US:
Sixty buckthorn plants throughout Minnesota and the Midwest were sampled and tested for the presence of the buckthorn phytoplasma disease '*Candidatus* Phytoplasma rhamni'. None of the samples were found to be positive for the phytoplasma.

Result Status as of (9/30/11)
Research in Europe:

- 100 *T.walkeri* adults were collected in Serbia from *Rhamnus cathartica* trees which had known to be infected with '*Ca.* Phytoplasma rhamni'. A phytoplasma transmission experiment was set up to see if those insects can infect non-infected trees.
- A study was initiated on the potential impact of *T. walkeri* on young buckthorn plants.

- About 30 adults of *W. krumbholzi* were collected, but none of the lab's potted *R. cathartica* flowered or fruited, thus no oviposition tests could be carried out. Researchers suggest discarding *W. krumbholzi* from the list of potential agents.
- An experiment was established to test the hypothesis that seed and seedling mortality of *R. cathartica* in Europe is affected by negative plant–soil feedbacks.

Research in the US: The presence of the phytoplasma in the potential biocontrol agent *T. walkeri* raises questions about the possibility of using this agent for biocontrol. It is not known if the phytoplasma is present in North American populations of buckthorn, but this is necessary information to assess the potential for *T. walkeri* as a biocontrol agent. Researchers at the University of Minnesota conducted a preliminary survey for this phytoplasma in Minnesota and coordinated with partners in other states to have them send in buckthorn samples for laboratory analysis. The results of the lab testing are not available at this time.

Result Status as of (02/28/11): The 2010 annual report will be submitted March 2011. '*Candidatus* Phytoplasma *rhamni*' is a witches'-broom disease of European buckthorn (*Rhamnus cathartica*). Phytoplasmas are non-culturable, insect-transmitted, wall-less bacteria. In 2009, the presence of '*Candidatus* Phytoplasma *rhamni*' was detected in the adults of the potential biocontrol insect *Trichochermes walkeri* (a sap-sucking psyllid) in two locations in Switzerland. Psyllids may be vector for transmitting the virus. The biology and transmittal of the phytoplasma is not well understood. Additional research on the phytoplasma and *T. walkeri* is necessary to assess whether *T. walkeri* is still viable as a potential biocontrol insect for buckthorn in the United States.

In 2010, CABI researchers sampled trees (*R. cathartica*, other *Rhamnus* species, *Frangula alnus*) and insects (*T. walkeri*) in a number of sites within five countries in Western Europe for the detection of the phytoplasma '*Candidatus* Phytoplasma *rhamni*'. The phytoplasma was detected in *R. cathartica* samples at several sites in all countries surveyed, except for Montenegro, but not in any of the other *Rhamnus* species sampled or in *F. alnus*. The researchers did not observe symptoms of the witches'-broom disease and cannot associate the presence of the phytoplasma with any particular symptoms in the trees. A high rate of phytoplasma has been found at the two sites where the positive *T. walkeri* samples had been collected in 2009. The psyllid *T. walkeri* samples collected in 2010 are being analyzed and results will be available by the end of March 2011.

In preparation for continued host specificity testing of the gall midge *Wachtiella krumbholzi*, CABI researchers collected buckthorn fruits attacked by the gall midge. They can use these insects for host-specificity testing. Additionally, CABI researchers collected mature fruits and seeds of *R. cathartica* in Europe to be used in a plant-soil feedback study. This study may identify other potential biocontrol agents and help explain why buckthorn is held in check in Europe. They have also done germination experiments in order to prepare for this soil feedback study.

Final Report Summary:

Biological control of common buckthorn (*Rhamnus cathartica*) research focused on assessing the feasibility of using the psyllids *Trichochermes walker*, *Cacopsylla rhamnicola*, and *Trioza rhamni* and the seed-feeding midge *Wachtiella krumbholzi* as biological control agents, determining the biology and transmittal of the witches broom disease '*Candidatus* Phytoplasma rhamni' which

was found to be present in the psyllids, and determining the causes of the high levels of seedling mortality and post-dispersal seed mortality of common buckthorn observed in Europe as compared to North America.

While research indicated the three psyllid species were host-specific to common buckthorn, there were two issues that complicated their use as biocontrol insects. There was the potential that the psyllids could bring the buckthorn witches broom disease ('*Ca.* Phytoplasma rhamni') to the United States. It was also not clear that the psyllids could cause enough damage to common buckthorn to be an effective control agent.

Little was known about 'Ca. Phytoplasma rhamni' so additional research was necessary. In Europe, common buckthorn trees were found to be infected with '*Ca.* Phytoplasma rhamni' at almost all surveyed localities, confirming previous reports of host association of this phytoplasma with common buckthorn, although the presence of witches' broom symptoms were not observed. The phytoplasma was not detected in any of the other *Rhamnus* species analyzed, which suggests a very specific host association of this phytoplasma with its plant host, and also a very specific relationship between the insect vector of the pathogen and its host plant. Work on '*Ca.* Phytoplasma rhamni' in North America was carried out by Dr. Roger Becker and Dr. Dimitre Mollov, University of Minnesota, St Paul, USA. '*Ca.* Phytoplasma rhamni' was not detected in 75 *R. cathartica* populations from North America suggesting either that the phytoplasma has not been introduced in the exotic range of its host plant, or that the absence of a suitable vector for phytoplasma propagation constrained its establishment in North America. *Trichochermes walkeri* proved to be infected with '*Ca.* Phytoplasma rhamni' at a very high rate in almost all sampled localities. Transmission trials strongly suggest that *T. walkeri* is not a vector of '*Ca.* Phytoplasma rhamni'. *Trichochermes walkeri* acquires the phytoplasma during feeding on infected plants, but it is not capable of re-injecting the phytoplasma during feeding. The phytoplasma was also found in *Cacopsylla rhamnicola* and *Trioza rhamni* although the role they play in spreading the phytoplasma is not clear.

An impact study of the effect of leaf galling by *T. walkeri* on eight-month-old *R. cathartica* seedlings was set up in August 2011. A total of 714 eggs were laid on infected trees. However, in 2012, no galls were recorded and the test was terminated without having obtained conclusive results.

The seed-feeding midge *W. krumbholzi* was found at most common buckthorn (*R. cathartica*) sites where searched. Midge larvae have also been discovered in the fruits of rock buckthorn (*R. saxatilis* ssp. *tinctorius*) at one site in Serbia, where common buckthorn also occurs. Based on the mitochondrial COI (cytochrome c oxidase) gene, midges from common buckthorn (*R. cathartica*) and *R. saxatilis* ssp. *tinctorius* are clearly two closely related but distinct species. This further confirms the likely high degree of host specificity of *W. krumbholzi.* CABI was unable to do host-specificity testing since they did not succeed in obtaining reproducing trees of the host, *R. cathartica,* when grown in pots or fruiting trees of other test species. CABI finds it will not be feasible to successfully screen *W. krumbholzi* in the near future. Without host-specificity testing, *W. krumbholzi* could not be approved for release.

A study found no evidence of negative plant–soil feedback by mature *R. cathartica* on conspecifics that could explain low seedling numbers of *R. cathartica* in the native range. There was however a positive plant–soil interaction in the rate of seedling emergence. A small difference in the number of days to seedling emergence probably explains most of the variation in seedling growth.

Due to the difficulties surrounding currently studied agents and the low probability of finding additional potential agents, it has been decided that the project will be stopped and we conclude that there are not suitable biological control insects for *R. cathartica* at this time.

Result/Activity 2: Introduction and evaluation of Garlic Mustard biological control agents in MN

Description: Activities will include selection of potential release sites, collection of pre-release plant community data, development of rearing methods for control agents, introduction of control agents and initial evaluation of establishment of agents. In anticipation of biological control agents becoming available for garlic mustard, 12 field sites have been selected in different habitat types to implement a biological control program in Minnesota. At these chosen sites, we will continue to collect data on the abundance of both garlic mustard and native plants prior to release, to establish a baseline for assessing the long-term impact of introduced biological control insects. Work will also take place to develop rearing methods for control agents. Once biological control insects are introduced, we will evaluate insect establishment and plant community response to the biological control.

Summary Budget Information for Result/Activity 2:	**ENRTF Budget**	**$150,000**
	Amount Spent	**$150,000**
	Balance	**$0**

Deliverable/Outcome	Completion Date	Budget
Introduction of first biological control agent	2/28/11	$20,000
Monitor release sites; implement rearing	9/30/11	$40,000
Insect rearing protocol completed	2/28/12	$30,000
Monitor release sites; implement rearing	9/30/12	$40,000
Final report with findings and recommendations	6/30/13	$20,000

Completion Date: 6/30/14

Results Status as of (12/30/13):
A contract was written with the University of Minnesota for the balance of the funds remaining. Monitoring of garlic mustard plots was conducted in October 2013. Data was entered and submitted.

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 bicontrol 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.

Results Status as of (6/30/13):

Garlic mustard field monitoring was conducted in October 2012 and June 2013. Lab experiments were conducted to develop the most efficient and consistently reliable methods to rear *C. scrobicollis* from garlic mustard plants. *Ceutorhynchus scrobicollis* has been successfully reared on caged garlic mustard plants in a growth chamber by alternating growth chamber temperatures and photoperiods to mimic natural conditions in its native range. In Germany, *C. scrobicollis* produces one generation per year and F-1 adults emerge in late May. Simulating a three-month summer aestivation period, followed by a week of fall, and three weeks of winter resulted in optimum levels of oviposition. After receiving shipments of *C. scrobicollis* from Europe, it will be necessary to rear a minimum of one generation in a containment facility to ensure that the endoparasitoid, *Perilitus conseutor*, is not introduced along with adult *C. scrobcollis*. A method was developed to rear parasitoid-free *C. scrobicollis.* A bill is expected from U of M after June 30, 2013. At that point, we will write a contract with the University of MN for the remaining fund amount.

In May and June 2013 we received communication from the USDA APHIS Technical Advisory Group regarding the petition for release of *C. scrobicollis*. The petition was rejected based on the desire to see additional Threatened and Endangered mustard species undergo host-specificity testing. Dr. Katovich and Dr. Becker will work with the US Fish and Wildlife Service on developing a list of species to be tested and obtaining the seeds. At this time, we do not know the final number of additional species that need to be tested, but it appears that it will be fairly small as many of the species have already been tested. The TAG chair indicated that there was strong support for *C. scrobicollis* as garlic mustard biocontrol, but that the additional host-specificity testing was necessary. We will work with TAG and USFWS to obtain an agreed upon list of species to be tested and work with them to obtain seeds.

Result Status as of (9/30/12)

There has been no notification from the USDA Technical Advisory Group (TAG) as to the status of the petition for the release of *Ceutorhynchus scrobicollis* as a biological control insect for garlic mustard.

Garlic mustard monitoring data was collected from all 12 sites in June 2012. Data has been entered and research analysis is beginning. Among all 240 plots, the mean garlic mustard percent cover was 19%. Garlic mustard seedling density averaged 74 seedlings/m^2 and garlic mustard adult density averaged 12 adults/m^2.

Work continues on updating and revising a manual for propagating garlic mustard and rearing *C. scrobicollis*. Progress has been made in establishing rearing methods for the biocontrol insects. In Europe, *C. scrobicollis* adults emerging in the spring require a summer aestivation period before adult females are able to lay eggs. Researchers conducted a study to determine the minimum length of summer aestivation required for adult females to reach maturity and lay eggs when reared in growth chambers in the containment facility. By using the shortest length of aestivation required, insects can be reared more quickly and then more can be produced over time. Results showed that total numbers of eggs per leaf were highest with the standard 3 month aestivation period as opposed to the 1 or 2 month aestivation periods.

Rearing *C. scrobicollis* in the containment facility has not been reliable and researchers have not been able to consistently rear adults on garlic mustard plants. Since *C. scrobicollis* pupate in the soil, it is possible that pupae had low rates of survival in the soil mixes used to propagate garlic

mustard. For this reason, researchers tested whether the addition of 3 to 4 cm of a standard greenhouse soil mix added to the top of a soil-less greenhouse mix would affect the number of *C. scrobicollis* adults emerging from garlic mustard soils. The results of the study showed a significantly greater number of F1 adults emerged from plants with 3 to 4 cm of greenhouse soil mix placed over the soil surface. The conclusion was that the addition of greenhouse soil can aid in *C. scrobicollis* pupal survival. For future *C. scrobicollis* rearing efforts, soil will be added to the top of the peat-based mix prior to placing adults on garlic mustard plants.

Result Status as of (2/28/12)
There has been no notification from the USDA Technical Advisory Group (TAG) as to the status of the petition for the release of *Ceutorhynchus scrobicollis* as a biological control insect for garlic mustard.

Garlic mustard monitoring data was collected in October 2011 at all 12 sites. Data was entered and data analysis of the 2011 data has begun. For the 12 sites, the average percent cover of garlic mustard in June 2011 ranged from 6% garlic mustard cover at the lowest cover site to 65% garlic mustard cover at the highest cover site. Average garlic mustard cover at the sites in October 2011 ranged from 0% to 13% cover of garlic mustard. For the 12 sites, the average density of adult stems of garlic mustard ranged from 1-40 stems/m^2 in June 2011, the average density of garlic mustard seedlings ranged from 46-655 seedlings/m^2 in June 2011, and the average density of garlic mustard rosettes ranged from 0-58 rosettes/m^2 in October 2011.

Result Status as of (9/30/11)
The petition for release of the garlic mustard biocontrol insect *Ceutorhynchus scrobicollis* was submitted to the USDA Technical Advisory Group (TAG) on Sept. 8, 2011. At this time no insects are approved for garlic mustard biocontrol release. We await the recommendation from TAG. From March-September 2011, work continued to focus on monitoring garlic mustard plots. Data was collected on the 12 permanent garlic mustard monitoring plots in June 2011. 2011 data has been collected and entered, but has not been analyzed.

Results of garlic mustard monitoring in 2010 showed that garlic mustard population density in 2010 was similar to previous years in showing high variability among sites. Garlic mustard is decreasing at two sites which have received management (Luce Line and Pine Bend Bluffs). At Luce Line, herbicide applications have resulted in a decrease in garlic mustard. At Pine Bend Bluffs, cutting trees and converting the site from a forest to savannah has resulted in a decrease in garlic mustard. A common pattern for other garlic mustard sites is for cycling where one life stage (seedling or adult) to dominate in any given year, then the next year, the other life stage dominates. In 2010, three sites showed strong population cycling with the sites alternating between being dominated by the seedling/rosette 1st year life stage in one year and then dominated by the adult 2nd year life stage the next. Three sites showed some cycling, but not consistently. These sites had declines in adult plants in 2009, followed by an increase in 2010. Three sites had increasing garlic mustard from 2005-2008, but now the populations are beginning to cycle and hold steady. One site is showing a decline in garlic mustard.

Result Status as of (02/28/11):

A contract was written with the University of Minnesota to carry out the LCCMR funded research on garlic mustard in fall 2010. The main goal was to continue monitoring established permanent plots to monitor garlic mustard populations in anticipation of biological control insect release. From 2005-present, monitoring sites have been surveyed twice yearly with data collected on garlic mustard population density, percent cover, insect damage, and heights and numbers of siliques of the second year plants. In October 2010 data was collected on the garlic mustard monitoring plots. Monitoring data from June and October 2010 is being analyzed and summarized. No biological control agents have been approved for release in the US at this time.

Final Report Summary:
Four *Ceutorhynchus* weevil species are being studied to determine their suitability as biological control agents for garlic mustard. Petitions for release have been submitted to the USDA-APHIS Technical Advisory Group (TAG) starting in 2008, but TAG has requested additional host-specificity testing over time. No biological control insects for garlic mustard have been approved for release as of 2014.

In order to develop *C. scrobicollis* as a biocontrol agent for garlic mustard, it was necessary to design reliable and consistent methods to rear the weevils. Studies were conducted to develop mustard propagation methods and *C. scrobicollis* rearing protocols in our High Containment facility the University of Minnesota in anticipation of permission to release *C .scrobicollis* into the field for the biocontrol of garlic mustard. The experiments that were conducted allowed the development of efficient and consistently reliable methods to rear *C. scrobicollis* from garlic mustard plants.

A second focus of research for this report has been monitoring garlic mustard populations in Minnesota to collect pre-release data so efficacy of biocontrol can be measured once insects are released. Long-term monitoring shows that garlic mustard 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. Twenty permanent $0.5m^2$ monitoring plots were established at 12 sites throughout Minnesota. Data was collected each June and October from 2005 to 2013. Nine years of monitoring data show that garlic mustard is currently experiencing very little herbivory in Minnesota and that garlic mustard populations can vary considerably from year to year. As of 2013, garlic mustard is still present in almost all of the plots. Garlic mustard cover is negatively correlated with cover of other species.

V. TOTAL TRUST FUND PROJECT BUDGET:

Contract Services: $300,000 (CABI for buckthorn research; and Univ. of MN for garlic mustard implementation)

TOTAL ENRTF PROJECT BUDGET: $300,000

VI. PROJECT STRATEGY:

A. Project Partners:

Dr. Andre Gassmann, CABI Europe-Switzerland, Delemont, Switzerland will be under contract to continue the ongoing buckthorn research ($150,000). CABI has been working on buckthorn biological control since 2001. CABI is responsible for research on purple loosestrife bio-control agents and many leafy spurge bio-control agents that are currently used in the U. S. and Canada.

Drs. David Ragsdale, Roger Becker and Elizabeth Stamm Katovich, University of Minnesota, will carry out garlic mustard biological control research under contract ($150,000). This amount may change based on future role of Minnesota Department of Agriculture; see below). Drs. Becker and Ragsdale will spend 5% and of their time on this project. Dr. Katovich will spend 60% of her time on garlic mustard.

Monika Chandler, MN Department of Agriculture, will work closely with DNR staff to rear biological control agents and implement evaluations of garlic mustard biological control in the field. Ms. Chandler will spend 5% of her time (in-kind) on this project.

B. Project Impact and Long-term Strategy:

Development and implementation of biological control for buckthorn could take up to ten years. This research will determine whether there are suitable bio-control agents, whether further research into these potential agents is warranted, and make recommendations for future work. If potential control agents are found, further research would be needed to continue screening the insects to ensure they are host specific and won't feed on other plants. Several insects for garlic mustard control are near completion of host specificity testing and one or more species are expected to be approved for introduction in the United States in 2010. Our time will be spent over the next 5-7 years evaluating the success of the insects introduced. Both European buckthorn and garlic mustard biological control efforts will follow research processes similar to those used for highly successful purple loosestrife and leafy spurge programs that have been funded through the LCCMR process.

C. Other Funds Proposed to be Spent during the Project Period:

An estimated $3,500 in-kind directly related to this project (e.g. general fund-supported project manager staff time) is expected to be contributed to this project (but not tracked for reporting purposes). Approximately $42,000 in Department Operations and Division Support charges accruing to this project will be covered by Division general funds or other eligible Division funds (see Attachment B.)

Buckthorn related spending: The Department of Natural resources will contribute approximately $30,000 in additional funding towards this project.

D. Past Spending:

Buckthorn related spending: The DNR spent $20,000 in 2001 to initiate research on buckthorn bio-control. The DNR received $125,000 from the U.S. EPA (2001-2005) to continue the buckthorn research. LCMR funding $109,000 (2003) and $110,000 (2005) recommended funding along with an additional $30,000 from the United States Fish and Wildlife Service (through Minnesota Department of Natural resources) is being used to

continue this research. The Department of Natural Resources contributed an additional $30,000 in 2007.

Garlic mustard related spending: The DNR spent $25,000 in 1999 supporting garlic mustard biological control research. Between 2002 and 2008, the DNR received $265,000 from the U.S.D.A.-Forest Service to continue host specificity testing of garlic mustard agents. LCCMR funded $90,000 (2005) and 135,000 (2007) for garlic mustard research.

VII. DISSEMINATION: 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.

Buckthorn biological control research has been disseminated in the following ways:
Peer reviewed journal publication (pdf attached):

- Gassmann, A. and I. Tosevski. 2014. Biological control of Rhamnus cathartica: is it feasible? A review of work done in 2002–2012. Journal of Applied Entomology 138: 1-13.

CABI Report Summary (pdf attached):

- Gassmann, A., A. Leroux, M. Bennett, M. Penic, N. Haefliger, R. Eschen, J. Jović and I. Toševski. 2012. Report 2010–12: Biological control of common buckthorn, *Rhamnus cathartica*. CABI Europe-Switzerland. CABI Ref: VM01730.

Poster presentations at conferences:

- Gassman, Andre, Laura C. Van Riper*, and Luke C. Skinner. Conclusions from 11 Years of Buckthorn Biocontrol Research. Ecological Society of America Conference. 4-9 August 2013. La Crosse, WI.
- Gassman, Andre, Laura C. Van Riper*, and Luke C. Skinner. Conclusions from 11 Years of Buckthorn Biocontrol Research. Upper Midwest Invasive Species Conference. 29-31 Oct 2012. La Crosse, WI.
- Gassman, Andre, Laura Van Riper*, and Luke C. Skinner. Developing Biological Control for Common and Glossy Buckthorn. Invasive Plants Symposium, Dec. 2011. Milwaukee, WI.
- Gassman, Andre, Laura Van Riper*, and Luke C. Skinner. Developing Biological Control for Common and Glossy Buckthorn. Minnesota-Wisconsin Invasive Species Conference, 2-10 Nov 2010. St. Paul, MN.

Webpage created on MN DNR website:

- http://www.dnr.state.mn.us/invasives/terrestrialplants/woody/buckthorn/biocontrol.html

Garlic mustard biological control research has been disseminated in the following ways:
Peer reviewed journal publication (pdf attached):

- Becker, R.L., E.J.S. Katovich, H.L. Hinz, E. Gerber, D.W. Ragsdale, R.C. Venette, D.N. McDougall, R. Reardon, L.C. Van Riper, L.C. Skinner, and D.A. Landis. 2013. The Garlic Mustard (*Alliaria petiolata*) Case, What Makes a Good Biological Control Target. The Intersection of Science, Perspectives, Policy and Regulation. pp. 332-339 *In* Proc. XIII International Symposium on Biological Control of Weeds (ISBCW). Sept. 11-16, 2011. Waikoloa, Hawaii. Wu, Yun; Johnson, Tracy; Sing, Sharlene; Raghu, S.; Wheeler, Greg;

Pratt, Paul; Warner, Keith; Center, Ted; Goolsby, John; and Reardon, Richard, Editors. USDA Forest Service, FHTET-2012-07. January 2013. 536 p. http://www.invasive.org/publications/xiiisymposium/

U.S. Forest Service Technology Transfer document:

- Becker, Roger, Esther Gerber, Hariet L. Hinz, Elizabeth Katovich, Brendon Panke, Richard Reardon, Mark Renz, and Laura Van Riper. 2013. Biology and Biological Control of garlic Mustard. US Forest Service Forest Technology Enterprise Team publication FHTET-2012-05. http://www.fs.fed.us/foresthealth/technology/pdfs/GarlicMustardBiocontrol_FHTET-2012-05.pdf [Accessed May 2014].

Reports to the Environment and Natural Resources Trust Fund (pdfs attached):

- Katovich, E.J. and Becker, R.L. 2014. Garlic mustard biological control: Developing biological control insects, working towards field release.
- Van Riper, L.C. and Becker, R.L. 2014. Garlic mustard (*Alliaria petiolata*) monitoring in Minnesota: 2005-2013.

Presentations:

- E. J. S. Katovich. Upper Midwest Invasive Species Conference. November, 2010. St. Paul, MN. Biocontrol of Garlic Mustard and Buckthorn, an Update.
- E. J. S. Katovich. XIII International Symposium on Biological Control of Weeds. September, 2011. Waikoloa, Hawaii. Biological Control of Garlic Mustard, *Alliaria petiolata*, with the Root and Crown- Boring Weevil, *Ceutorhynchus scrobicollis*.
- E. J. S. Katovich. Ontario Invasive Plant Council, Annual General Meeting and Conference. October, 2011. Picton, Ontario. Potential for the Biological Control of Garlic Mustard.
- E. J. S. Katovich. Upper Midwest Invasive Species Conference. October, 2012. La Crosse, WI. Biological Control of Garlic Mustard with a Seed-Feeding Weevil.
- E. J. S. Katovich. Biological Control of Northeastern Weeds-2013 Cooperators Meeting. February, 2013. Trenton, New Jersey. Garlic Mustard Biocontrol: Current Status and Future Directions.
- E. J. S. Katovich. Technical Advisory Group For the Biological Control of Weeds, Annual Meeting. June, 2013. Washington, D.C. *Ceutorhynchus scrobicollis* as a Potential Biocontrol Agent of Garlic Mustard, *Alliaria Petiolata*.
- E. J. S. Katovich. University of Minnesota, guest lecturer for AGRO 4505: Biology, Ecology and Management of Invasive Plants. Biological Control of Invasive Plants. 2010-2014.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than February 2011, September 2011, February 2012, September 2012, June 2013, and December 2013. A final work program report and associated products will be submitted by June 30, 2014.

Final Attachment A: Budget Detail for 2010 Projects

Date: August 15, 2014

Project Title: Biological Control of European Buckthorn and Garlic Mustard (111-D)

Project Manager Name: Laura Van Riper

Trust Fund Appropriation: $ 300,000

1) See list of non-eligible expenses, do not include any of these items in your budget sheet

2) Remove any budget item lines not applicable

2010 Trust Fund Budget	**Result 1 Budget:**	**Amount Spent** *(08/15/14)*	**Balance** *(08/15/14)*	**Result 2 Budget:**	**Amount Spent** *(08/15/14)*	**Balance** *(08/15/14)*	**TOTAL BUDGET**	**TOTAL BALANCE**
	Buckthorn biological control - Europe			Garlic Mustard biological control				
BUDGET ITEM								
Contracts	$150,000.00	$150,000.00	$0.00	$150,000.00	150,000.00	$0.00	$300,000.00	$0.00
Professional/technical *(with whom?, for what?)*	CABI Europe-Switzerland: Research in Europe	$130,338.00		University of Minnesota: Research in Minnesota	150,000.00			
	University of Minnesota: Research in Minnesota	$19,662.00						
COLUMN TOTAL	**$150,000.00**	**$150,000.00**	**$0.00**	**$150,000.00**	**$150,000.00**	**$0.00**	**$300,000.00**	**$0.00**

Garlic Mustard (*Alliaria petiolata*) Monitoring in Minnesota: 2005-2013

August 15, 2014

Authors

Laura C. Van Riper, Minnesota Department of Natural Resources
Roger L. Becker, University of Minnesota

Report to the Legislative-Citizen Commission on Minnesota Resources

Environmental and Natural Resources Trust Fund Project Title:
Biological Control of European Buckthorn and Garlic Mustard
Project Manager: Laura Van Riper
Legal Citation: M.L. 2010, Chp. 362, Sec. 362, Subd. 6a

ABSTRACT

Garlic mustard (*Alliaria petiolata*) is an invasive forb that is native to Europe and has become abundant in forested regions in the US. Garlic mustard can form dense populations in the forest understory and crowd out native species. Garlic mustard also exudes allelopathic chemicals which can impede seed germination and reduce populations of native mycorrhizal soil fungi. Four *Ceutorhynchus* weevil species are being studied to determine their suitability as biological control agents for garlic mustard. Garlic mustard is a biennial and its population can vary widely from year to year. Long-term monitoring shows that garlic mustard populations can fluctuate from year to year. The populations can then be followed post-release to determine if the biological control agent had its intended effect of reducing garlic mustard. 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. Twenty permanent $0.5m^2$ monitoring plots were established at 12 sites throughout Minnesota. Data was collected each June and October from 2005 to 2013. Nine years of monitoring data show that garlic mustard is currently experiencing very little herbivory in Minnesota and that garlic mustard populations 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^{st} or 2^{nd} year plants in any given year. The other sites had more variable garlic mustard populations. Garlic mustard is still present in almost all of the plots. Garlic mustard cover is negatively correlated with cover of other species. We also observed variation in garlic mustard adult plant height and silique production from year to year. It is expected that after biological control release, garlic mustard populations as a whole will decrease and shoot heights and silique production of individual plants will decrease as well.

INTRODUCTION

Garlic mustard (*Alliaria petiolata*) is a non-native, biennial, herbaceous plant that has become abundant in wooded areas in Minnesota and the eastern United States (Meekins et al. 2001; Rodgers et al. 2008). Garlic mustard can form dense cover on the forest floor and negatively impact native species (Nuzzo 1999; Blossey et al. 2001; Stinson et al. 2006). In order to better understand garlic mustard populations in Minnesota and to collect baseline data in the event of biological control insect release (Blossey et al. 2001), a garlic mustard monitoring was initiated in Minnesota in 2005. The results of the monitoring data collected from 2005 to 2008 are presented in Van Riper et al. 2010. This report summarizes the results as of data collected up to 2013.

Garlic mustard and associated plant communities were monitored at 12 deciduous forests sites in Minnesota. Garlic mustard populations can fluctuate dramatically from year to year (Meekins and McCarthy 2002; Winterer et al. 2005; Pardini et al. 2009). Multiple years of monitoring are necessary to produce baseline data on garlic mustard populations and to determine the impacts of biological control agents, should they be released (Blossey 1999). It is expected that releasing biological control agents would decrease the population density and cover of garlic mustard and reduce garlic mustard plant height and silique production (Blossey et al. 2001; Davis et al. 2006; Gerber et al. 2007a, b). Data were collected on garlic mustard population density, cover, height, and silique production so the current population could be characterized and so comparisons could be made should biocontrol agents be released in the future. Additionally, data were collected on the current levels of insect herbivory garlic mustard experiences in Minnesota.

METHODS

Methods follow the standard protocol of the Ecology and Management of Invasive Plants Program developed in 2003 (available at http://www.invasive plants.net) and described in Van Riper et al. 2010. Data were collected from the 12 Minnesota permanent garlic mustard monitoring sites in June and October of each year (Table 1 lists monitoring sites and locations). Each site consisted of 20 permanent 1-m by 0.5-m monitoring plots. The term 1^{st}-year plants refers to seedlings and rosettes; 2^{nd}-year plants refers to overwintered, flowering adults. From 2010-2012, the data collection protocol was modified to collect a smaller subset of data than that reported in Van Riper et al. 2010. Data was collected on garlic mustard density (1^{st} and 2^{nd} year plants), percent cover of garlic mustard (1^{st} and 2^{nd} year plants), percent leaf damage to garlic mustard plants, type of leaf damage observed, and percent cover of all other species besides garlic mustard. Other species in the plots besides garlic mustard were not identified to species or their individual percent cover recorded Data was not collected on litter depth and ground cover (percent bare soil, leaf, rock, and wood) in 2010, but was collected in 2011 and 2012.

Garlic mustard is a biennial plant and can have complicated population dynamics (Pardini et al. 2009). Data were collected on the various life-stages of garlic mustard. A garlic mustard seed germinates early in the spring. By the fall monitoring period (October) the seedlings had grown into basal rosettes of leaves. The rosettes over-winter and in the following spring, they bolt to form flowering, adult plants. Flowering occurs April through May in Minnesota. By June they have formed siliques which are counted in the monitoring protocol. Adult plants fully mature and drop seeds and senesce by late July to August. Therefore, in the June monitoring period both seedling and adult stages of garlic mustard are present, but in October only the rosette stage is present.

Observers changed over the course of the study. Laura Van Riper collected data 2005-2009. From 2010 to 2013, a variety of students and staff from the University of Minnesota collected data.

Since a number of the measures are visual estimates of percent cover, differences in percent cover in 2010 from previous years may also have a component of variation due to the change in observers in addition to actual changes in percent cover over time. Efforts were made to standardize estimates by marking the plot frames help delineate the size of various percent covers. Even with these efforts, observer differences still exist. Data such as stem, seedling, and rosette counts have insignificant observer variability.

A few unexpected events occurred during the course of the study. On May 25, 2008 Warner Nature Center was hit by a tornado. A number of trees were knocked down in the area of the garlic mustard monitoring plots. This opened up the canopy to more light than the site had experienced in previous years. At the Luce Line Trail, garlic mustard plants in plots 1 to 10 and 16 to 20 were treated with 2% Roundup (glyphosate) herbicide on May 29-30, 2008. Plants were still alive for June 2008 data collection at Luce Line, but died soon after. In May 2010, Luce Line garlic mustard plants were again treated with herbicide. Plants had died by the time of June 2010 data collection. At Pine Bend Bluffs SNA, in an effort to reduce heavy infestations of woody invasive trees present, *Rhamnus cathartica* L. (common buckthorn) and *Lonicera spp*. (nonnative honeysuckles) were cut down in April 2009 in the area with garlic mustard monitoring plots 1-10 and 16-20. The tree clearing resulted in a dramatic increase in light to the plots and a loss of some plots as they were covered in brush piles. In 2013 it was determined that too many plots were irrevocably damaged and the Pine Bend Bluffs plot stakes were removed. Unforeseen events are to be expected in any long-term monitoring project. Having 12 monitoring sites provided broad sampling of environments and habitats of garlic mustard infestations in Minnesota. Data from the Luce Line and Pine Bend sites were assessed separately to determine if those management events impacted garlic mustard and other plant species present.

RESULTS

Fluctuations in garlic mustard population density over time – Figures 1, 2, and 3

Garlic mustard populations in Minnesota are highly variable from year to year (Figs. 1, 2, and 3). Two sites received unanticipated management over the years decreasing garlic mustard population densities (Fig. 3a). Several of the sites show consistent cycling between dominance of the first- and second-year life-stages (Fig. 3b). The remaining sites show few clear patterns (Figs. 3c and 3d).

Two sites [Luce Line (LL) and Pine Bend (PB)] received management during the course of the study, even though plots were established at the sites with the expectation that there would not be management. Luce Line (LL) had showed population cycling in the first several years of monitoring, but with repeated herbicide treatments in 2008 and 2010, there were very few plants in 2010 (Fig. 1, Fig. 2, Fig. 3a). However, by 2011 recruitment from the seedbank resulted in a rebound in rosettes population densities (Fig 1c) which in turn resulted in a rebound in adults population densities in 2012 (Fig. 1c, Fig. 3a). At Pine Bend, half of the study plots occurred in an area that had tree removal in April 2009. The site was extensively cut to remove invasive buckthorn and honeysuckle and to move to a savannah restoration in that area. After the tree removal in 2009 adult garlic mustard decreased, so that by 2011 the mean adult garlic mustard population density was 0.8 plants/m^2 and by 2012 there were no adult garlic mustard plants in the plots (Fig. 3a). Seedling and rosette population densities were also low at Pine Bend in 2012 (34 seedlings/m^2, 3 rosettes/m^2) (Fig.1). This is likely the result of the fact that half of the study plots occurred in the area that had trees removed in April 2009 for the purpose of savannah restoration. Garlic mustard is not known to persist in savannahs. In our studies, the largest declines in garlic mustard populations occurred at these two sites which had management, with the caveat that garlic mustard populations appear to be rebounding after herbicide application stopped at Luce Line.

A common pattern for garlic mustard infestations at various sites is for one life-stage (seedling or adult) to dominate in any given year and then in the next year, the other life-stage dominates. For example, at Warner Nature (WN), garlic mustard seedlings were abundant in 2005 and there were few adults (Fig. 1, Fig. 2). By the following spring of 2006, those seedlings had matured to adults and adults were abundant but not seedlings. Dense adult plant population densities competed with seedlings killing a large cohort of 1st year plants that year. This high mortality in 2006 resulted in few adult plants by 2007. Warner Nature Center (WN) and Westwood Hills (WH) have shown consistent life-stage cycling for most years of study (Fig. 3b). Plainview (PL) also showed clear life-stage cycling in adult population densities (Fig. 3b). Plainview also had higher population densities of seedlings and rosettes during the years of high adult population density than did Warner Nature Center and Westwood Hills, such that the cycling of seedlings and rosettes were not as clear (Fig. 1). Looking at adult population density only, Plainview, Warner Nature Center, and Westwood Hills all showed consistent cycling with the total population density of adults remaining fairly consistent in their relative highs and lows (Fig. 3b).

Baker Park (BP), Coon Rapids (CR), Cottage Grove (CG) show some cycling (e.g. 2009 to 2011 Fig. 3c), but do not have consistent patterns. These sites all show a strong decline in adults 2009, followed by an increase in 2010 (Fig. 3c). 2008 was a very dry year with high mortality for seedlings (Van Riper et al. 2010) which progressed to low adult population density numbers in 2009. Lower seedling mortality in 2009 than in 2008 at Coon Rapids and Cottage Grove (Fig. 1) may have contributed to the increase in adults by 2010. These sites may continue to cycle in the future. Interestingly, Baker Park had been quite consistent its adult garlic mustard population density until 2009 (Fig. 3c). Cottage Grove had seen 3 years of consistent decline before then increasing in 2010. Coon Rapids had 2 years of adult population density decline before increasing in 2010 (Fig. 3c). Baker Park, Coon Rapids, and Cottage Grove may be starting to show population cycling in the years 2008 through 2010 (Fig. 3c).

Garlic mustard population density has been variable at Fort Snelling, Hilloway Park, Nerstrand, and Willmar. Garlic mustard population density has been holding fairly steady at Fort Snelling (FS) and Hillloway Park (HP) (Fig. 3d). Nerstrand (NE) has shown life-stage cycling in some years, but it hasn't been consistent. There was cycling in the first three years of monitoring at Nerstrand, but low rosette population density in 2008 due to drought resulted in low adult population density in 2009, breaking the pattern of life-stage cycling (Fig. 1, Fig. 3d). Initially, rosette population density at Willmar (WI) increased each year until 2009 when the rosette population density decreased (Fig. 1c). At Willmar, adult garlic mustard population density increased from 2006-2009, then has been at low levels from 2010-2012 (Fig. 3d). From discussions with the landowner and observing the lack of garlic mustard in surrounding areas, monitoring at the Willmar site likely started at the early stages of the invasion. Over time, the population may have built up to a point where the cover of adult garlic mustard was dense enough to increase seedling mortality, initiating the pattern of life-stage cycling.

Garlic mustard mortality over time – Table 2

Garlic mustard mortality shows strong year to year variation (Table 2). The mean mortality for garlic mustard plants from the seedling stage in June to the rosette stage in October ranged from 47% mortality in 2005 to 86% mortality in 2013. The winter mortality for plants advancing from the rosette stage in October to adults in June of the next year ranged from a low of only 7% mortality in 2005 to 2006 to a high of 57% mortality in 2011-2012. The likelihood that a seedling that emerged in June of the first year to make it to an adult in June of the second year ranged from 38% in 2007-2008 to only 8% in 2011-2012 (62 and 92% mortality, respectively). There appears to be a trend of increased mortality over the years.

Mortality could vary from year to year for a number of reasons, such as weather differences from year to year e.g. very dry years or extreme winters could increase mortality. As noted in Van Riper et al. 2010, the summer of 2008 was much drier than average. Additionally, Minnesota experienced

near-record to record setting low rainfall amounts for the period of July through September for three consecutive years from 2011 to 2013 (Appendix A). This likely is the dominant factor in increased seedling mortality and poor wither survival of rosettes in recent years. This high summer and early fall mortality was exacerbated by near-record to record setting excessive rainfall from March through June in the same years, conditions favoring high levels of recruitment from the seedbank that in turn are unlikely to survive the ensuing drought, failing to produce seed further depleting the seedbank. Alternatively, sites may become less favorable for garlic mustard over time due to reasons such as negative soil feedback, but significant mortality due to diseases was not observed at any of the sites.

Percent cover of adult garlic mustard over time – Figures 4, 5

Over time, the percent cover of adult garlic mustard in June has ranged from a high of 61% cover at Warner Nature Center in 2006 to a low of 0% cover at Pine Bend 2012 (Fig.4, Fig. 5a,b). Looking at the percent cover of adult garlic mustard in the spring over time, garlic mustard cover appears to be getting less variable. For example, in 2006 adult percent cover ranged from 3% to 61% while in 2012, percent cover ranged from 0% to 21% (Fig. 4).

The trends of adult percent cover mimic the trends in adult stem population density. Cover decreased at Luce Line due to accidental spraying with herbicide but is rebounding (Fig. 5a). Cover also decreased at the Pine Bend site due disturbance from renovations to create an oak savannah confounding our monitoring efforts, and was abandoned in the spring of 2013. Plainview, Warner Nature, and Westwood Hills show cycling in adult percent cover (Fig. 5b). Adult percent cover appears to be decreasing at Coon Rapids and Cottage Grove, while Coon Rapids cover has been cycling the past four years (Fig. 5c). Percent cover of adults at Fort Snelling has remained fairly steady (Fig. 5d). Hilloway Park and Willmar show decreasing adult cover and Nerstrand shows some cycling (Fig. 5d).

Fluctuations in garlic mustard plant height and reproductive output – Figures 6, 7

We had no reason to expect large changes in mean garlic mustard plant height or mean number of siliques per garlic mustard stem over this monitoring period. If biocontrol agents were introduced, we would expect a decrease in the mean height and in the mean number of siliques per stem. As plant heights were getting shorter, we'd expect that the number of siliques per stem would also be reduced. Mean adult garlic mustard stem height per site generally varied over time and may be getting more variable among sites over time (Fig. 6). In 2011, mean adult stem height ranged from 23 cm at Baker Park to at 93 cm Coon Rapids (Fig. 6). Since plant heights were staying fairly consistent (Fig. 6), it makes sense that the mean number of siliques per garlic mustard stem also stayed fairly consistent at most sites (Fig. 7). Lower numbers of adult plants, thus lower intra-specific competition can have a strong influence increasing the mean height and numbers of siliques per stem. Overall, the highest mean number of siliques per stem was 34.3 siliques/stem at Luce Line in 2011; the lowest was 1.5 siliques/stem, also at Luce Line in 2009, corresponding with the tallest and the shortest mean plant height in 2011 and 2009, respectively.

Garlic mustard presence – Table 3

When establishing plots at a monitoring site, the protocol called for garlic mustard to be present in all of the plots. The percent of plots with garlic mustard present are summarized in the first column of Table 3. In spring 2005, when five monitoring sites were established, 100% of the plots contained garlic mustard. In spring 2006, when all 12 sites had been established, 98% of the plots had garlic mustard in them. In spring of 2012 and 2013, the percent was almost the same with 97% of the plots having garlic mustard present. This tells us that although garlic mustard cover and population density are changing, garlic mustard is remaining present on the landscape. It is an artifact of the life-cycle of garlic mustard that the percent of plots that contain garlic mustard decreases in the fall because adult

plants die after flowering before the October sampling period. Additionally, some plots may have lost garlic mustard due to the high mortality of seedlings that emerged that spring before reaching the rosette stage that fall.

Garlic mustard herbivory levels – Table 3

Data was taken on herbivory on garlic mustard to characterize the amount of insect herbivory on garlic mustard currently to compare with the level of herbivory post-release of garlic mustard biocontrol (data summarized in Table 3). Garlic mustard herbivory levels are generally low, ranging from 1.4 to 8.8% of leaf area. Edge feeding and hole feeding are the most common types of leaf feeding (over time present within 74 to 100% of the plots with garlic mustard). Leaf mining and window-pane feeding were less common types of leaf damage. Mean leaf area removed was low with a range from 1.4% of leaf area in spring 2009 to 8.5% of leaf area in fall 2011.

Over the course of the study, an aphid species has been observed occasionally forming large colonies on the tips of garlic mustard plants. Aphids collected at Cottage Grove and Warner Nature by Laura Phillips-Mao were identified by Doris Lagos of the University of Illinois as *Lipaphis brassicae*. In 2009, aphids were observed at five of the sites (CG, FS, NE, WH, WN) although were not always present within observation plots. The aphids were present in plots at BP, CR, and HP In 2010, at BP and WN in 2011, and at LL and WI in 2012. Aphids have now been observed at 9 of the 12 sites. The only sites without aphid observations are Pine Bend Bluffs, Plainview, and Willmar.

Litter depth and bare ground – Table 4

All sites had low amounts of leaf litter on the soil surface (Table 4). Low depths of leaf litter are associated with the presence of non-native earthworms. Their invasion can decrease litter layer depth from 10 cm to 0 cm and cause negative impacts to the plant community (Hale et al. 2005). In June of 2011 and 2012, five sites had mean litter depth of 0 to 1 cm (BP, CR, LL, PB, WH), six sites had mean litter depths between 1 to 2 cm (FS, HP, NE, PL, WN, WI), and 1 site had mean litter depths between 2 to 3 cm (CG). Overall, litter depth was low at all sites. Thus, litter depth likely is not a strong variable to explain differences in garlic mustard population density, height, cover, or number of siliques, nor differences in life-cycle dynamics among sites.

Cover of other species besides garlic mustard – Figure 8, Table 5

The visual percent cover was estimated for all species other than garlic mustard in each plot (Fig. 8). In spring 2012, the mean cover of other species ranged from 3% cover at Luce Line to 69% cover at Pine Bend. In fall 2012, the mean cover of other species ranged from 2.6% cover at Luce Line to 29% cover at Baker Park. Luce Line has been extensively treated with herbicide for buckthorn and garlic mustard which may be a cause of the very low cover of other species at the Luce Line site. Pine Bend had extensive tree removal in April 2009 which opened up the canopy and may account for the increase in cover of other species. Coon Rapids plots can have dense cover of the ground cover species creeping Charlie (*Gleochoma hederacea*) which could account for the high cover of other species at that site.

The correlation between the percent cover of garlic mustard and the percent cover of other species is shown in Table 5. The cover of garlic mustard in the spring (adult garlic mustard cover plus seedling garlic mustard cover) was negatively correlated with the cover of other species in the spring or fall in all years except 2009 or 2010, respectively. The fall cover of garlic mustard rosettes was also negatively correlated with the cover of other species in the fall in all years. Where cover of garlic mustard is high, there tends to be low cover of other species.

DISCUSSION

Garlic mustard population densities were highly variable over time and the tendency for life-stages to cycle can make discerning patterns difficult. Garlic mustard is still present in almost all of the plots after nine years of monitoring. We see some trends towards decreasing garlic mustard populations, particularly following successive years of excessive spring rainfall followed by late-summer droughts that last into the fall sampling period. This type of weather pattern would be expected to cause high mortality of garlic mustard seedlings. The wet early season weather would promote high levels of germination depleting the seedbank with seedlings attempting to establish in saturated soils, developing shallow root systems leaving plants ill-equipped to survive the seasonal-drought that ensued. This is compounded by the fact that vulnerable garlic mustard seedlings are attempting to establish in the understory of established, deeply rooted perennial trees and shrubs that have a distinct advantage competing for limited soil moisture. Garlic mustard cover is negatively correlated with cover of other species. Our monitoring data shows that garlic mustard continues to be widespread on the landscape. Where garlic mustard populations have declined or is absent in monitoring plots, garlic mustard is still present in the surrounding landscape and though not quantified, observations show spatial variability of this seed-bank dependent species across the landscape over time, similar in some respects to that observed with seed-bank dependent annual weeds in agricultural systems (Cardina et al. 1997).

In the future, if we are able to release biocontrol insects at some sites, this long-term pre-release monitoring data will enable us to determine the impacts of the biocontrol agent on areas impacted by garlic mustard, which was the intent of the study design at the onset. We forced transects into areas of intense pressure of garlic mustard to attempt to have garlic mustard present in all monitoring plots. This non-random placement is not well suited to denote increasing or decreasing abundance of garlic mustard across the landscape. Since we selected intense populations of garlic mustard at the onset, we would anticipate that population densities would moderate with time as we arguably placed monitoring transects in areas where garlic mustard was present at or nears its maximum population carrying- capacity for that site and environment.

Research and discussions on the impacts of garlic mustard and the suitability of garlic mustard for biocontrol continue (Becker et al. 2013*a*). Research continues on garlic mustard biocontrol insects and an informational manual has been published (Becker et al. 2013*b*). During the delay in gaining approval to release biocontrol agents for garlic mustard in North America, this long-term monitoring dataset is providing novel information on the biology and population dynamics of garlic mustard in Minnesota.

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TABLES

Table 1. Garlic mustard monitoring sites in Minnesota, USA. The ID column lists the abbreviation for that site as found in the figures (from Van Riper et al. 2010).

Site no.	ID	Site Name	City	County	Habitat type	Latitude Longitude
1	BP	Baker Park Preserve*	Maple Plain	Hennepin	Upland	45° 02.427’ 93° 37.195’
2	CR	Coon Rapids Dam Regional Park	Coon Rapids	Anoka	Floodplain	45° 07.975’ 93° 17.841’
3	CG	Cottage Grove Ravine Regional Park	Cottage Grove	Washington	Upland	44° 48.480’ 92° 53.960’
4	FS	Fort Snelling State Park*	Saint Paul	Ramsey	Floodplain	44° 52.373’ 93° 11.634’
5	HP	Hilloway Park	Minnetonka	Hennepin	Upland	44° 57.552’ 93° 26.098’
6	LL	Luce Line	Long Lake	Hennepin	Upland	44° 58.441’ 93° 35.137’
7	NE	Nerstrand State Park, Prairie Creek SNA*	Nerstrand	Rice	Upland	44° 21.527’ 93° 05.809’
8	PB	Pine Bend Bluffs SNA*+	Inver Grove Heights	Dakota	Upland	44° 47.076’ 93° 01.732’
9	PL	Plainview – private land	Plainview	Winona	Upland	44° 06.600’ 92° 03.821’
10	WN	Warner Nature Center*	Marine on St. Croix	Washington	Upland	45° 10.853’ 92° 49.641’
11	WH	Westwood Hills Nature Center	St. Louis Park	Hennepin	Upland	44° 58.301’ 93° 23.692’
12	WI	Willmar - private land	Willmar	Kandiyohi	Upland	45° 19.356’ 94° 59.667’

*= one of five sites established in time for spring 2005 data collection

+=plots removed in 2013 as the site had been converted to savannah and brush had been piled on many plots

Table 2. Mean garlic mustard mortality (mean for all the sites).

Year	June to October mortality for seedling to rosette (%)	Year	Winter mortality from October rosette to June adult (%)	Mortality from seedling in June year 1 to adult in June year 2 (%)
2005	NA	**2005-2006**	7%	NA
2006	47%	**2006-2007**	45%	70%
2007	52%	**2007-2008**	18%	62%
2008	77%	**2008-2009**	34%	89%
2009	80%	**2009-2010**	43%	88%
2010	56%	**2010-2011**	43%	85%
2011	82%	**2011-2012**	57%	92%
2012	80%	**2012-2013**	47%	90%
2013	86%			

Table 3. Garlic mustard presence and types of insect feeding at 12 sites in Minnesota, USA, 2005 to 2013 (modified from Van Riper et al. 2010). The percentage of plots with garlic mustard present out of the 20 plots at each of 12 study sites in Minnesota over 9 years are presented (5 study sites established spring 2005, 11 study sites left by 2013, 12 study sites for all other dates). Of the plots with garlic mustard present, the percentages of those plots with various types of visual leaf damage estimates are listed by the type of feeding damage.

	Plots with garlic mustard present	Plots with feeding by this insect type (of plots with garlic mustard present)				
Time		Edge feeding	Holes	Leaf miner	Windowpane feeding	Mean leaf removal
	----------	----------	----------	% ----------	----------	----------
Spring 2005	100	96	98	31	4	1.6
Fall 2005	87	99	98	1	1	1.5
Spring 2006	98	96	97	31	9	1.5
Fall 2006	84	97	98	<1	<1	2.0
Spring 2007	99	100	100	33	0	1.8
Fall 2007	88	97	96	1	0	2.4
Spring 2008	99	100	98	12	4	2.3
Fall 2008	63	97	91	0	<1	3.0
Spring 2009	99	97	98	8	<1	1.4
Fall 2009	78	95	89	0	0	2.4
Spring 2010	91	74	89	0	3	8.8
Fall 2010	64	86	78	<1	1	3.4
Spring 2011	97	92	95	6	64*	2.7
Fall 2011	55	89	89	7	61	8.5
Spring 2012	97	95	96	36	33	3.7
Fall 2012	64	97	86	8	18	6.6
Spring 2013	97	100	92	8	31	2.8
Fall 2013	50	42	37	1	10	5.0

*Suspect that field staff at this point began counting something different from what were counted as windowpanes in previous years.

Table 4. Mean litter depth and percent bare ground. Measurements from June 2011 - 2013.

ID	2011 Litter depth (cm)	2012 Litter depth (cm)	2013 Litter depth (cm)	2011 bare ground (%)	2012 bare ground (%)	2013 bare ground (%)
BP	0.2	0.2	0.3	72	83	81
CR	1.0	0.7	0.7	65	51	37
CG	2.3	2.6	2.4	10	5	2
FS	1.2	1.1	1.2	18	10	19
HP	1.1	1.1	1.7	35	25	13
LL	0.1	0.3	0.7	69	58	60
NE	1.0	1.4	1.6	56	43	21
PB*	0.9	0.8	NA	65	51	NA
PL	2.0	1.4	0.9	27	34	51
WN	2.6	1.1	1.4	26	30	29
WH	0.6	1.0	0.7	58	32	34
WI	1.7	1.6	2.7	32	9	2

BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

*Pine Bend plots were removed in 2013.

Table 5. Correlations of garlic mustard percent cover with the percent cover of all other species besides garlic mustard for the 12 sites by year (11 sites in 2013).

Correlation	2005	2006	2007	2008	2009	2010	2011	2012	2013
Spring adult + seedling garlic mustard vs. other species in the spring	-0.06	-0.34	-0.45	-0.48	0.01	-0.06	-0.36	-0.47	-0.24
Spring adult + seedling garlic mustard vs. other species in the fall	-0.40	-0.19	-0.49	-0.30	-0.13	0.17	-0.52	-0.43	-0.30
Fall garlic mustard rosettes vs. other species in the fall	-0.42	-0.04	-0.25	-0.32	-0.32	-0.16	-0.50	-0.25	-0.47

FIGURES

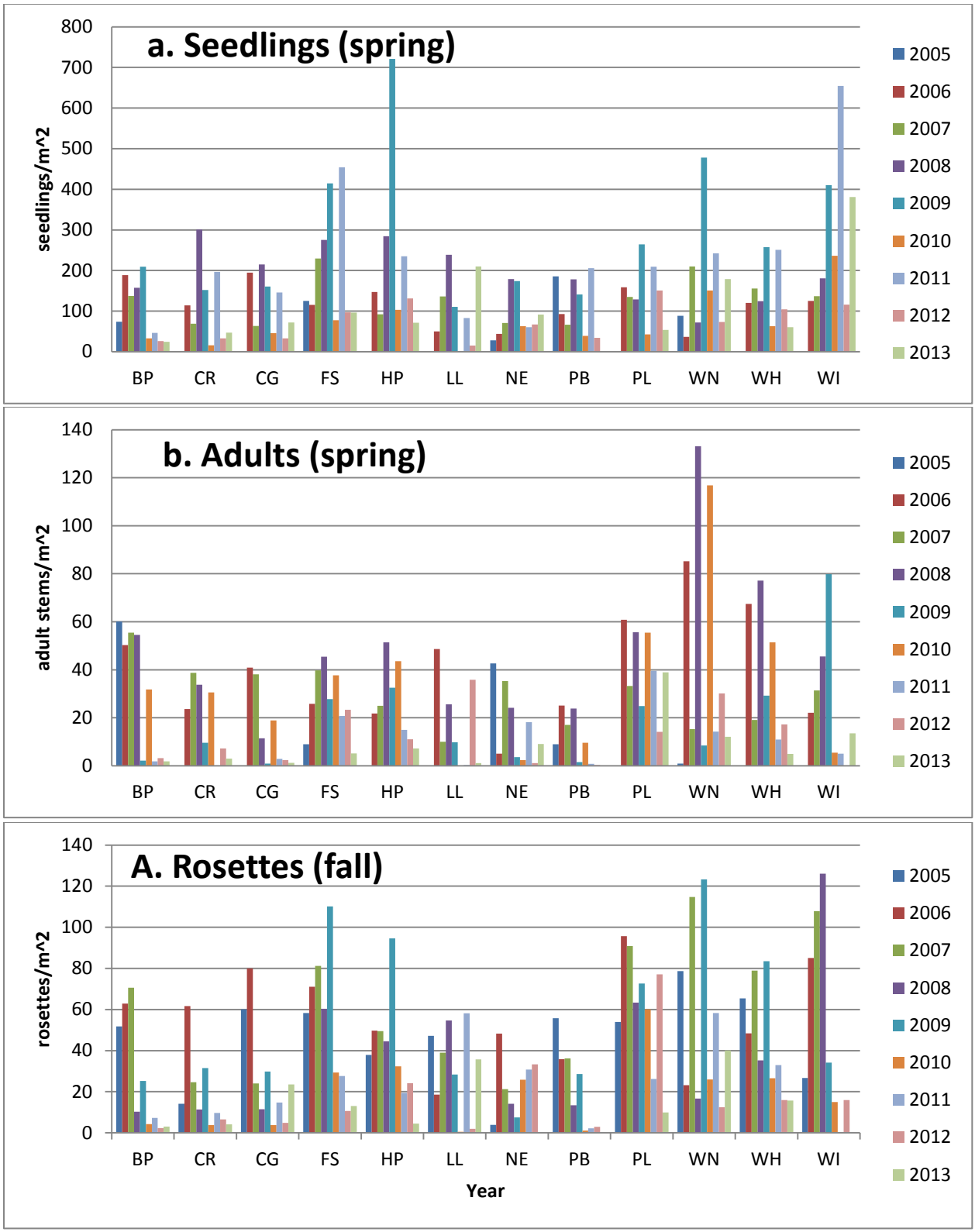


Figure 1. Mean garlic mustard population density of seedlings (a), adults (b), and rosettes (c) from 2005-2013 at 12 garlic mustard monitoring sites in Minnesota. Note that the y-axes vary. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

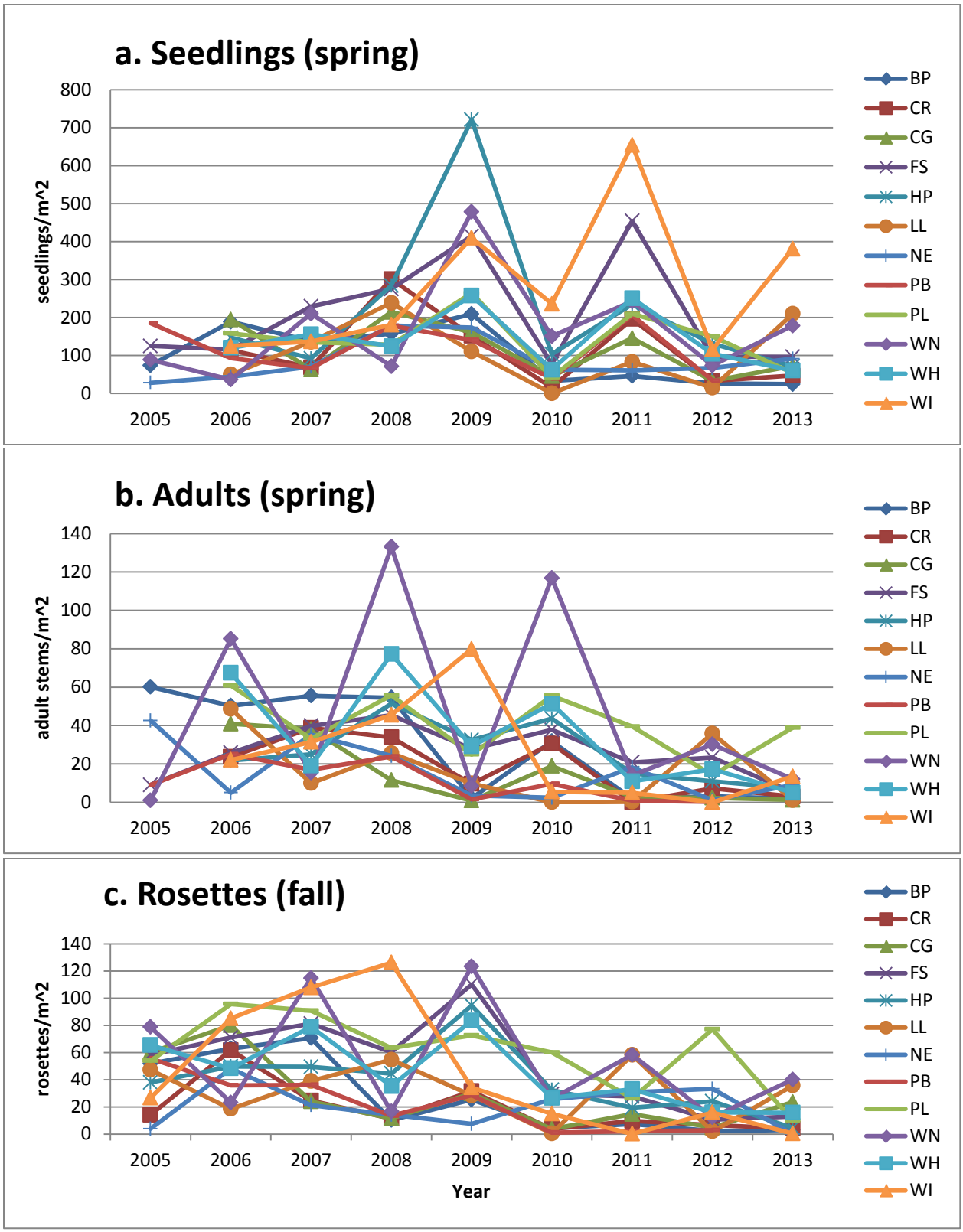


Figure 2. Mean garlic mustard population density of rosettes (a), seedlings (b), and adults (c) from 2005-2013 at 12 garlic mustard monitoring sites in Minnesota. Note that the y-axes vary. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

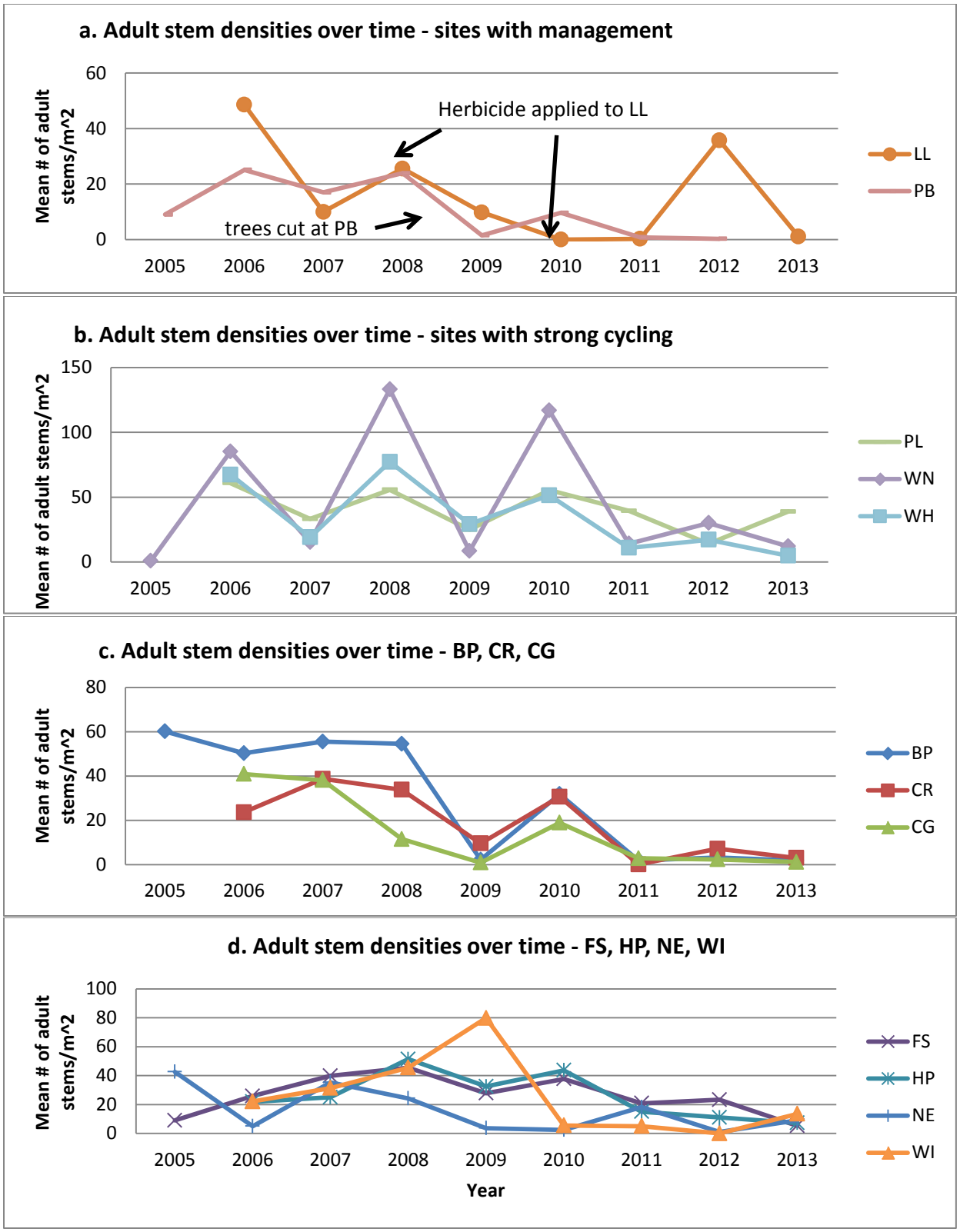


Figure 3. Mean garlic mustard population density of rosettes (a), seedlings (b), and adults (c) from 2005-2013 at 12 garlic mustard monitoring sites in Minnesota. Note that the y-axes vary. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

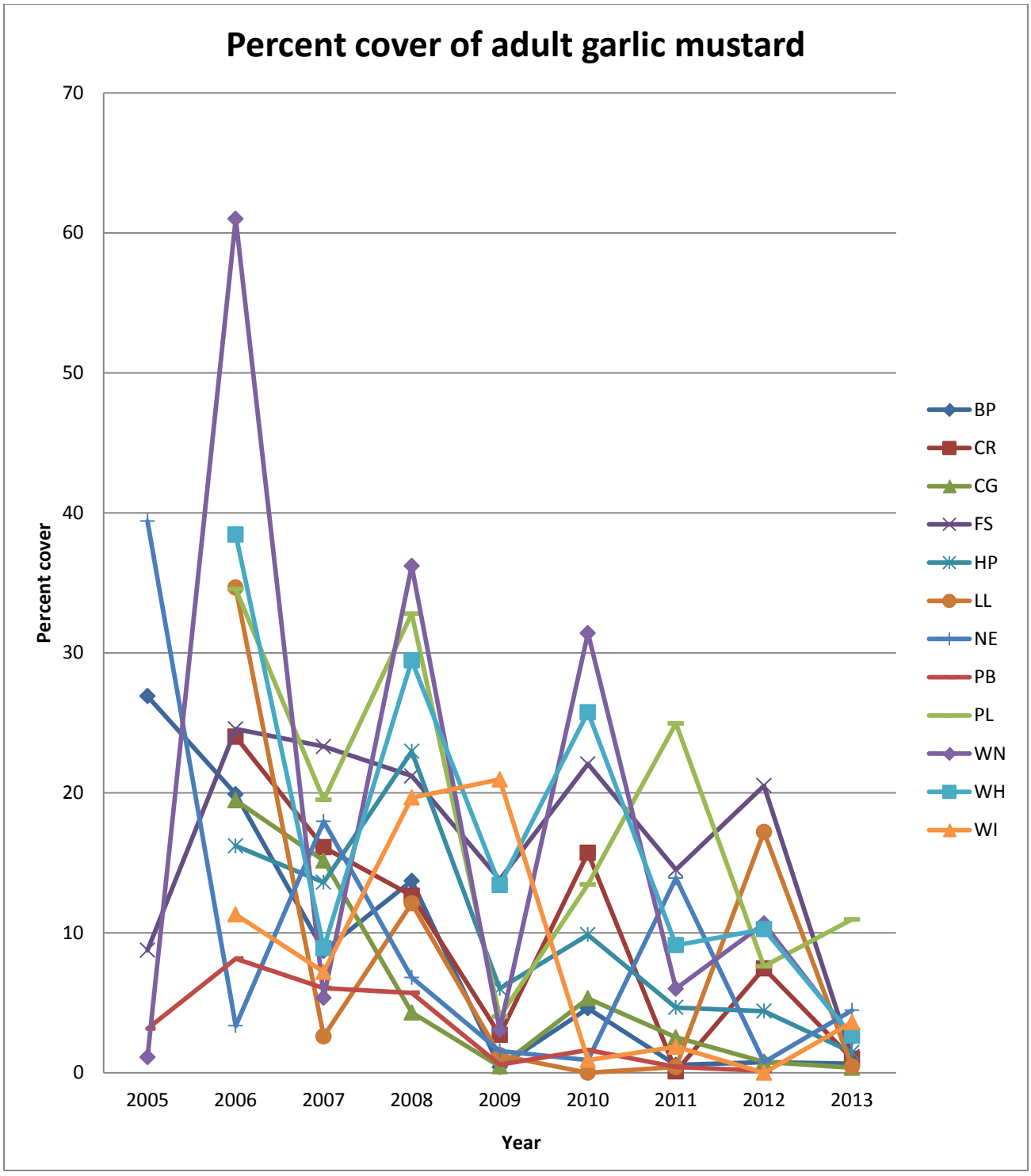


Figure 4. Mean visual percent cover of adult garlic mustard at each garlic mustard monitoring site in June from 2005-2013. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

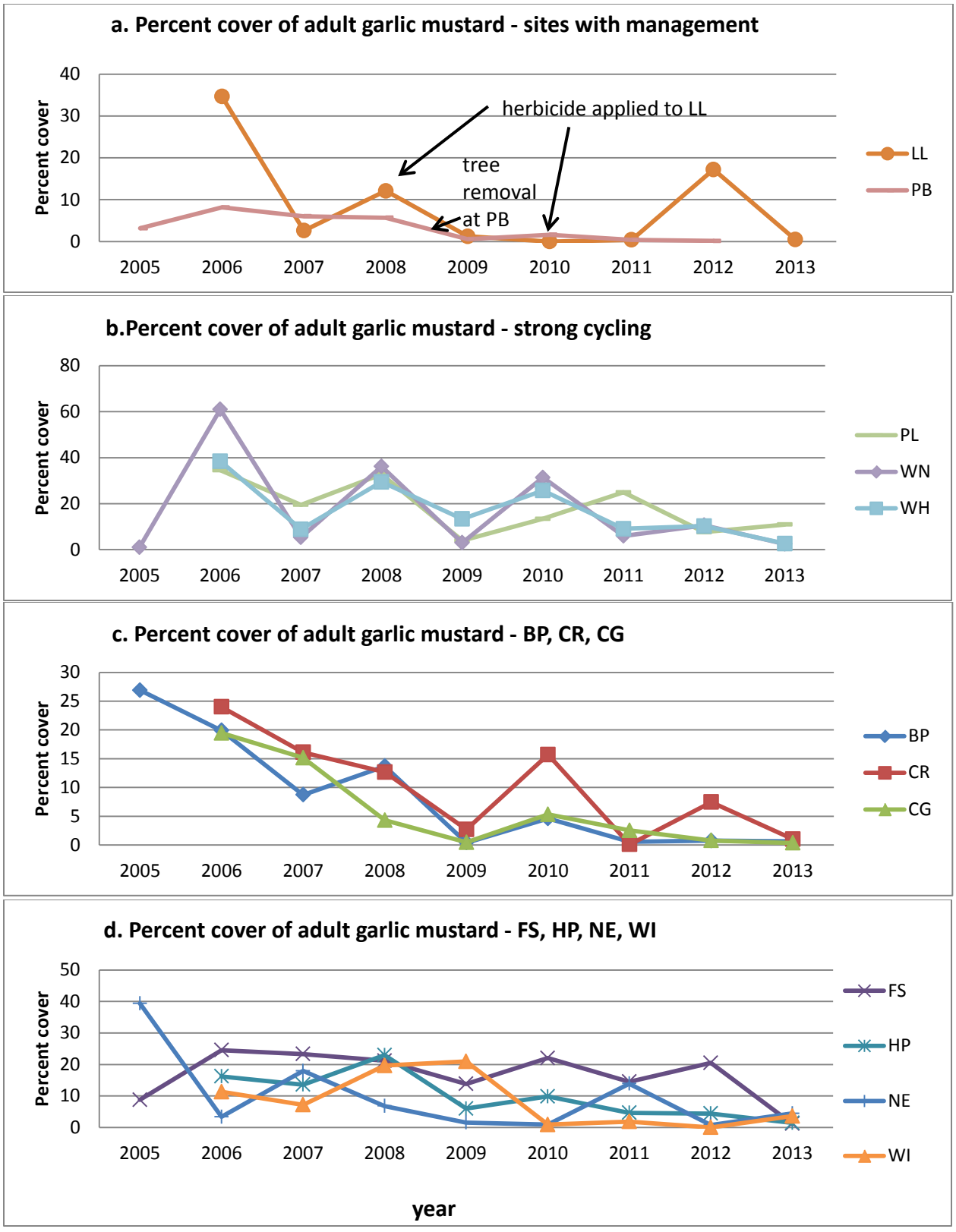


Figure 5. Mean visual percent cover of adult garlic mustard at each garlic mustard monitoring site in June from 2005-2013. Note that the y-axes vary. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

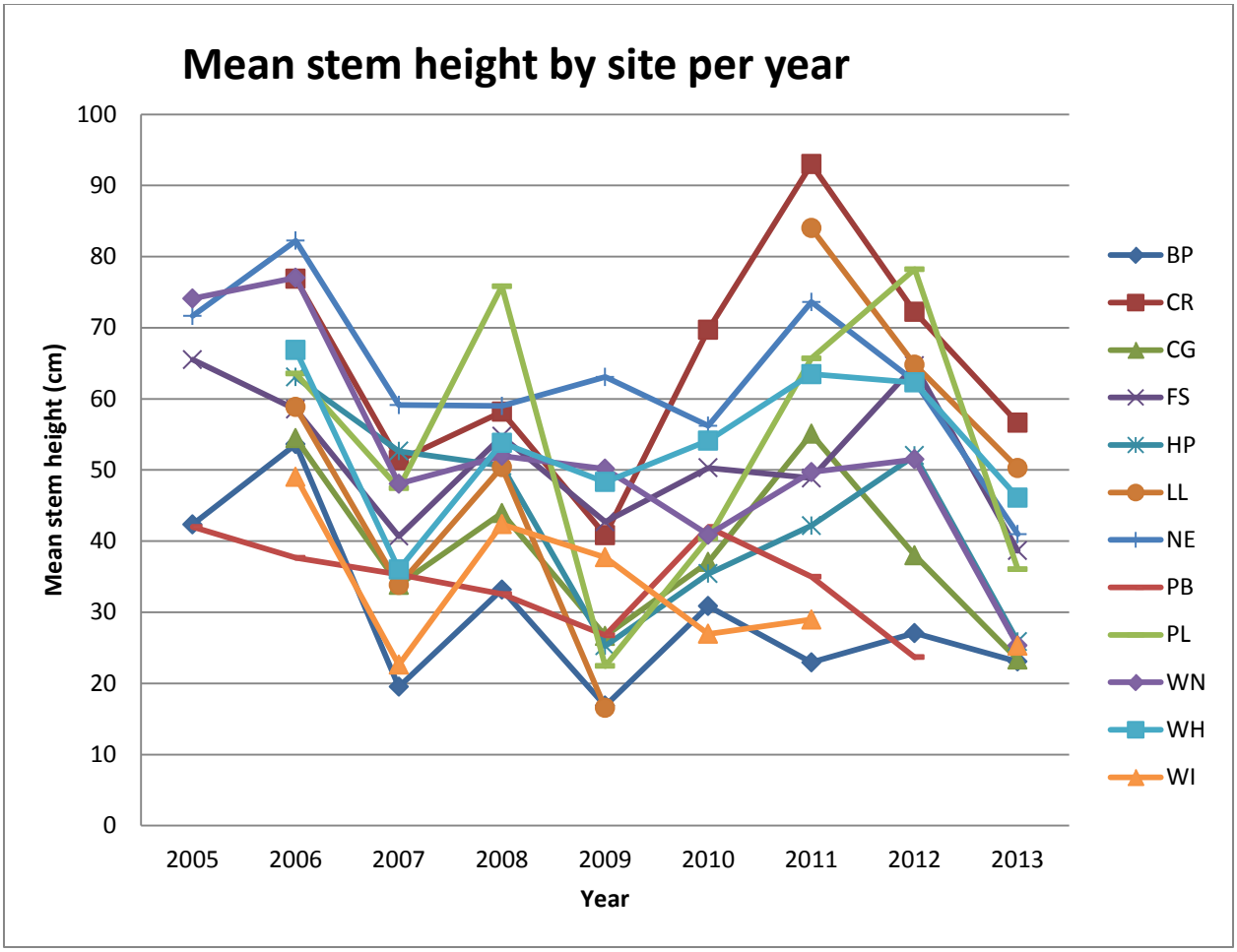


Figure 6. Mean adult garlic mustard stem heights as measured in June of 2005-2013. Luce Line (LL) had no adult plants in 2010 and Willmar (WI) had no adult plants in 2012. Pine Bend (PB) plots were removed in 2013, so no data was collected in 2013. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

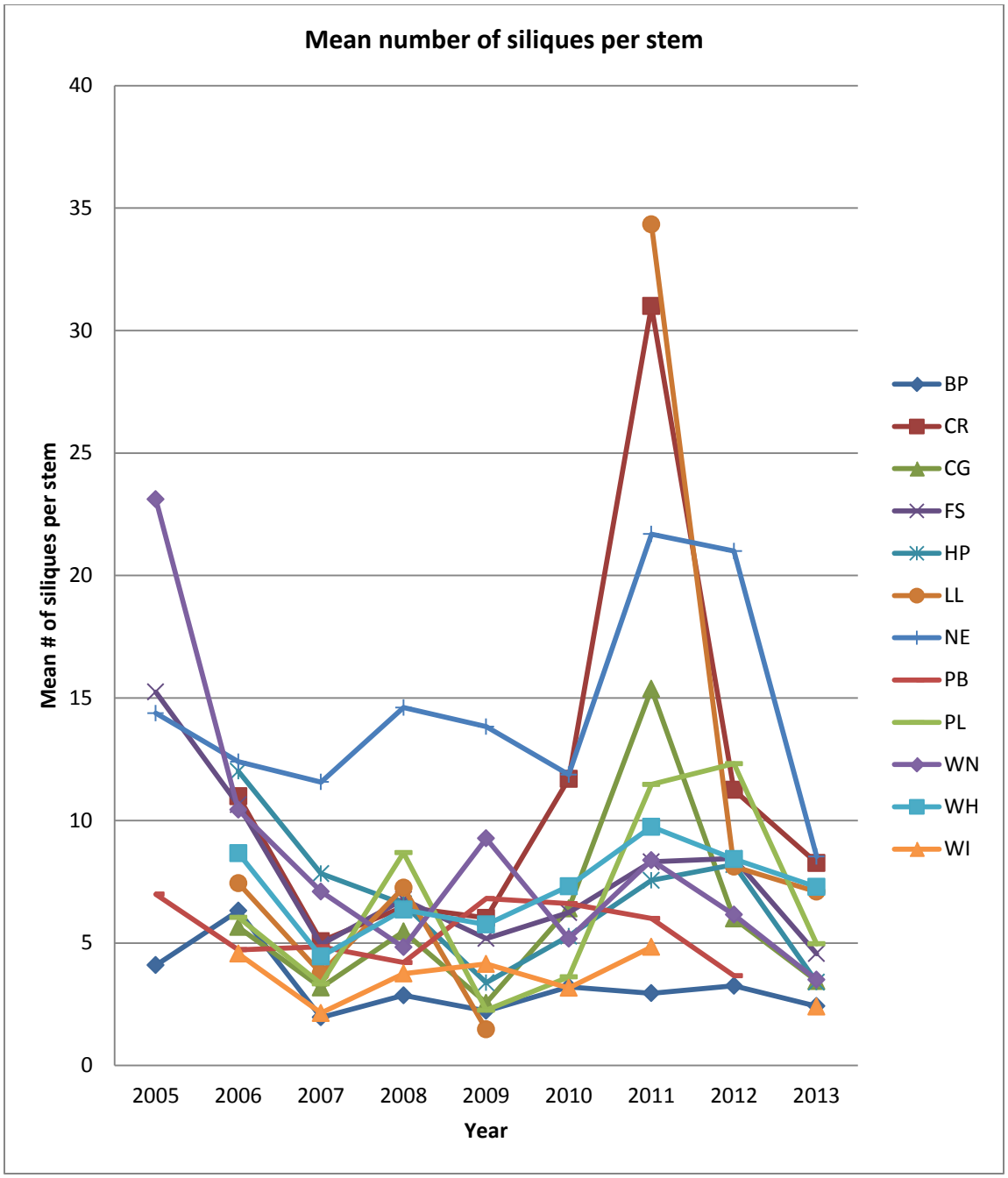


Figure 7. Mean number of siliques per adult garlic mustard stem as measured in June of 2005-2012. Luce Line (LL) had no adult plants in 2010 and Willmar (WI) had no adult plants in 2012. Pine Bend (PB) plots were removed in 2013, so no data was collected in 2013. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

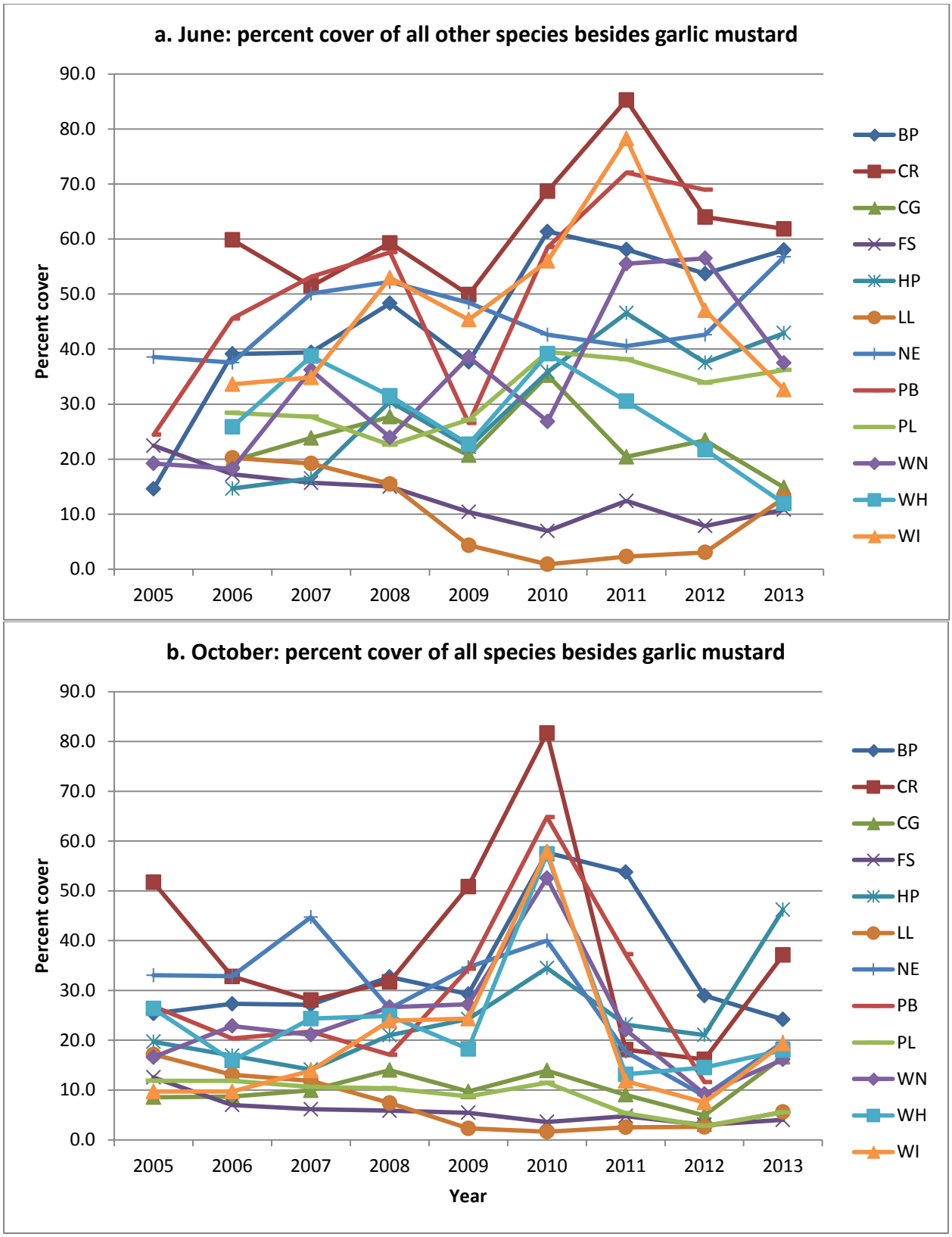
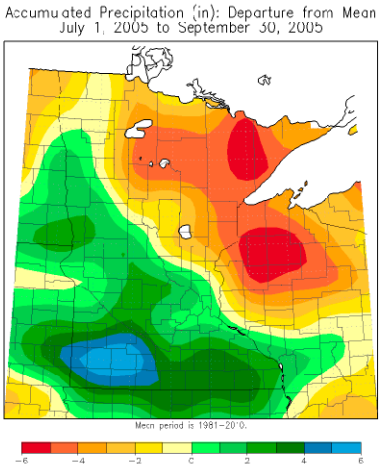
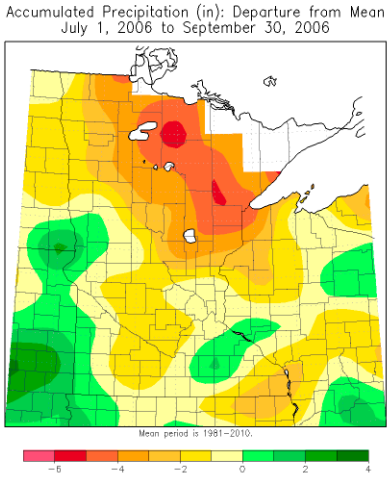


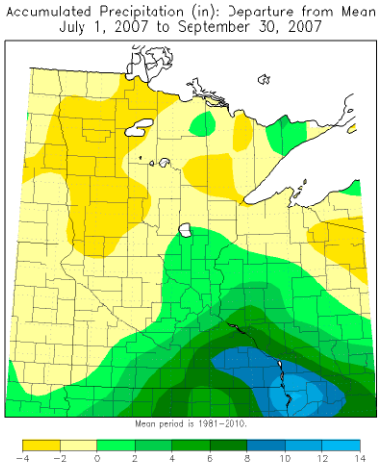
Figure 8. Mean percent cover of all other species besides garlic mustard in June (a) and October (b) from 2005-2013. (BP=Baker Park, CR=Coon Rapids, CG=Cottage Grove, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar)

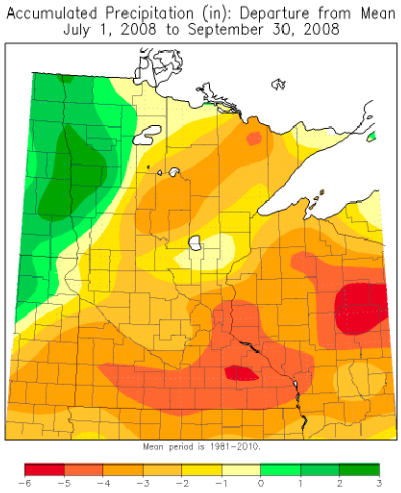
APPENDIX A

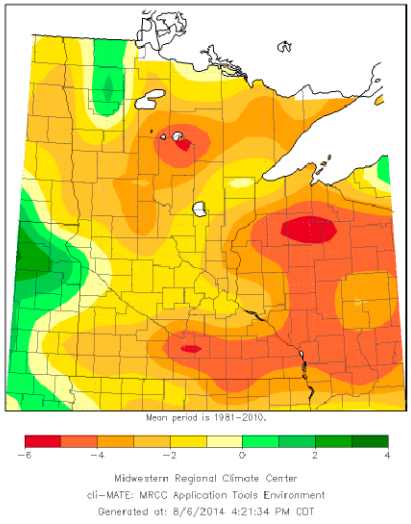
Accumulated precipitation and departure from mean for July through September In Minnesota, 2005 to 2013. http://mrcc.isws.illinois.edu/CLIMATE/Maps/map_btd.jsp (Accessed August 6, 2014)

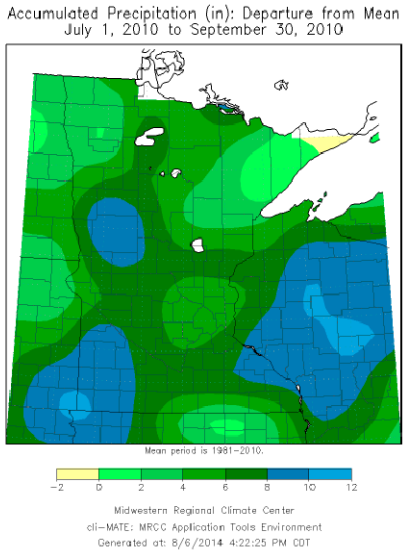




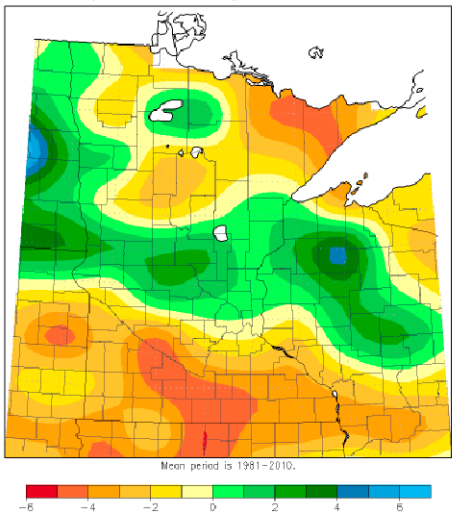








Accumulated Precipitation (in): Departure from Mean
July 1, 2011 to September 30, 2011



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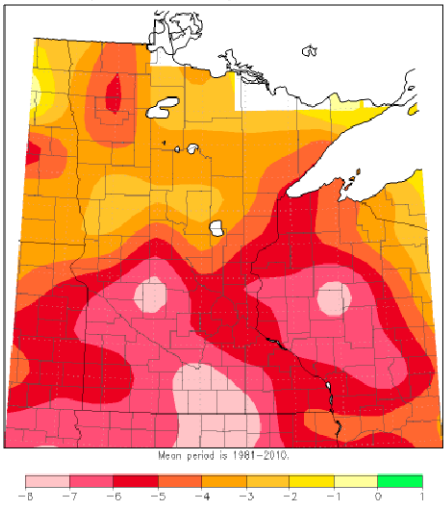

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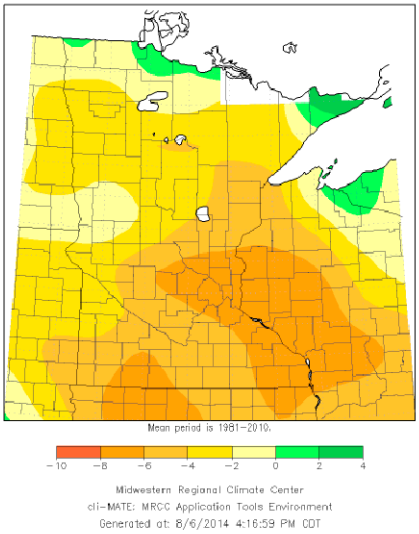
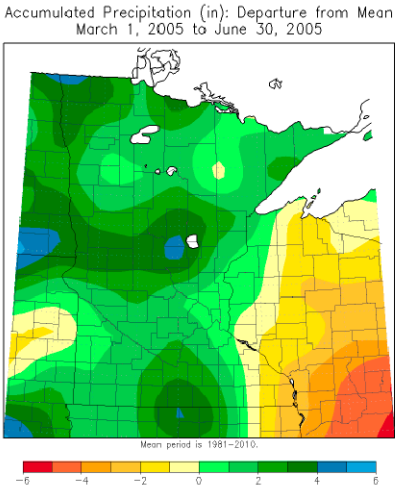
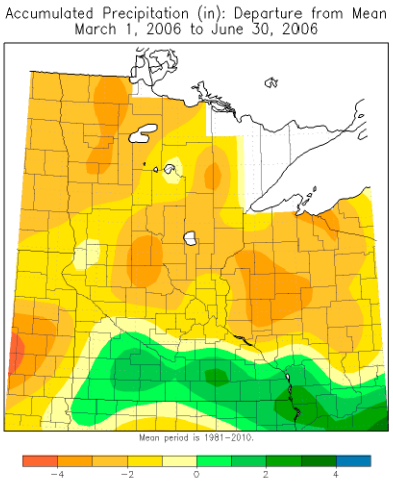
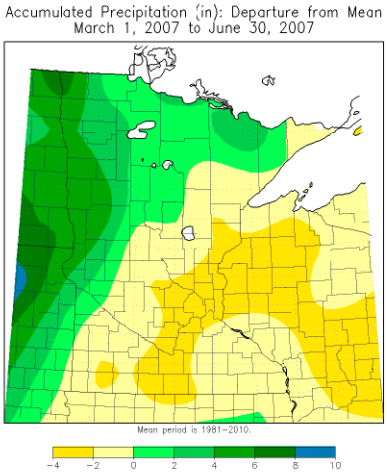
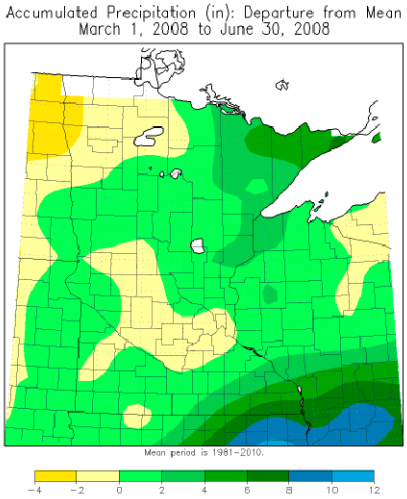


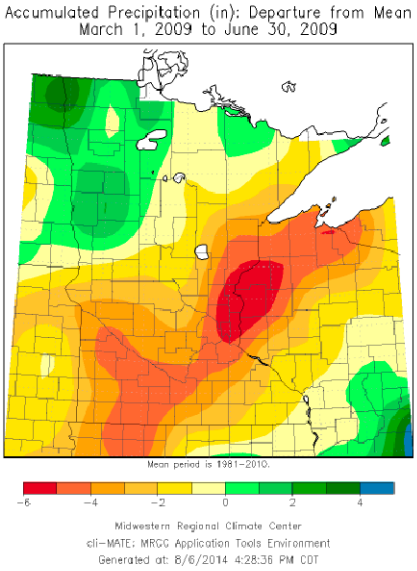
Appendix B. Accumulated precipitation and departure from mean for March through June In Minnesota, 2005 to 2013. http://mrcc.isws.illinois.edu/CLIMATE/Maps/map_btd.jsp (Accessed August 6, 2014)

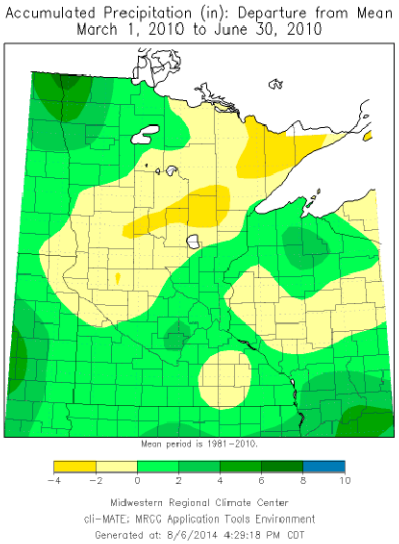












Accumulated Precipitation (in): Departure from Mean
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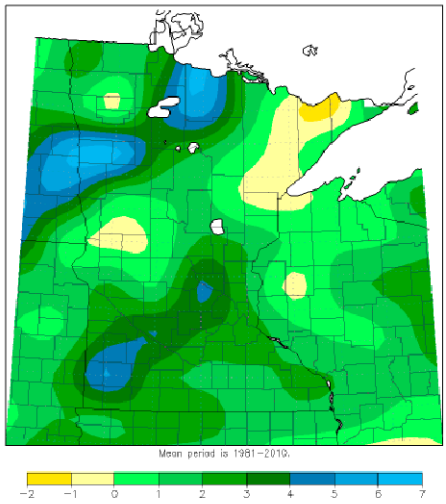

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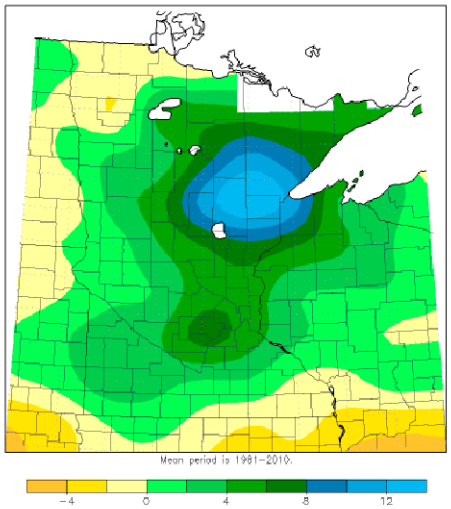

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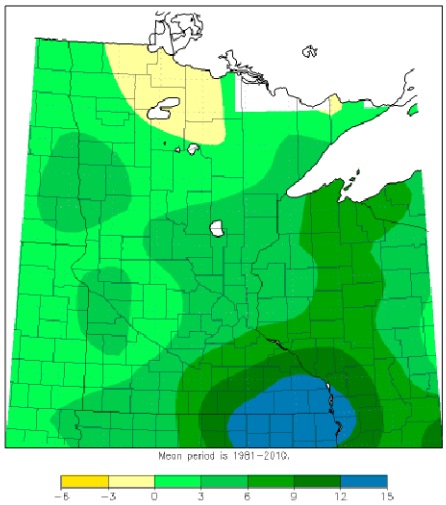

Forest Health Technology
Enterprise Team

TECHNOLOGY
TRANSFER

Biological Control

BIOLOGY AND BIOLOGICAL CONTROL OF GARLIC MUSTARD

Roger Becker, Esther Gerber, Hariet L. Hinz, Elizabeth Katovich, Brendon Panke, Richard Reardon, Mark Renz, and Laura Van Riper

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November 2013

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Cover photos
Background photo: Field of flowering garlic mustard; photo by Steven Katovich
Left inset: Adult *Ceutorhynchus alliariae*; photo by Albert de Wilde
Middle inset: Garlic mustard silique; photo by Elizabeth Katovich
Right inset: Flowering garlic mustard plant; photo by Elizabeth Katovich

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Biology and Biological Control of Garlic Mustard

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CHAPTER 1: INTRODUCTION

Invasive Plants

Most invasive plants in North America are not native to this continent. They arrived here from other regions of the world through accidental or deliberate introduction. Some invasive plants, such as garlic mustard (Figures 1-1 and 1-2), were brought to the new world by immigrants because of their valued medicinal or herbal properties. Others, such as purple loosestrife, reached North America via ship ballast or were introduced as ornamentals.

When invasive plants are introduced into a new region, their natural enemies are often not brought along with them. These natural enemies comprise the complex of insects and pathogens that regulate plant populations in their native range. Without these natural enemies, an invasive plant may become a strong competitor in its new range and crowd out native plant species.

Classical Biological Control of Weeds

The goal of classical weed biological control is to re-unite an invasive plant with its insect or pathogen enemies from its native range into the introduced range. The reunion of the natural enemy and invasive plant can reduce the abundance or competitiveness of the invasive against native plant communities. An insect natural enemy complex is comprised of several insect species

Figure 1-1. Garlic mustard invasion of a forested site. (Steven Katovich, USDA Forest Service)

Figure 1-2. Garlic mustard invasion of a forested site. (Steven Katovich, USDA Forest Service)

that each attack different plant parts. Some insect species defoliate leaves, others destroy shoots, attack developing flowers and seeds, or tunnel through stems, roots and crowns. Introducing a series of insect natural enemies, with different attack strategies, can increase the effectiveness of a weed biological control program. In this manual we will focus solely on insect classical biological control.

An advantage of using biological control as a weed management option (Table 1-1) is that biocontrol insects are plant specific, only attack the target weed and rarely attack related species. For example, an application of a broadleaf herbicide may kill most broadleaved plants, but a biological control insect will only attack the target invasive plant. Also, once biocontrol insects are established at a site, they reproduce and naturally disperse into new areas, including those that are hard to access by land managers or equipment operators. For this reason, weed biocontrol programs are well suited to non-cropland areas, such as rangelands, wetlands and forested sites where it may not be economically feasible to control invasive plants with other management options. Weed biocontrol projects have initial upfront costs, but are cost effective over the long term.

There are also problems that may be encountered when implementing a weed biological control program (Table 1-1). First of all, it may require five to ten years for biocontrol insects to reach sufficient numbers to control the invasive plant. Secondly, not all biocontrol insects will successfully establish at all sites. This is why multiple biocontrol insects are often released against an invasive plant target. Lastly, once a biocontrol insect is released, it cannot be removed from the environment. This is why pre-release host specificity tests are critical to developing an insect for use as a biocontrol agent.

Host Specificity Testing

The host specificity of an insect is the range of plant species that the insect can complete its life cycle on. The ideal biocontrol insect can only complete its development on the target invasive plant. To determine the range of plants that the insect can use as a host, potential biocontrol

Table 1-1. Advantages and disadvantages to consider prior to implementing a weed biological control program.

Advantages
• Invasive plant is only species targeted by biocontrol insect • Release of biocontrol insects provides long-term control • Biological control insects can naturally disperse into sites difficult to access • Once established, biological control insects can self-perpetuate, so long term management costs are reduced. • Biocontrol is well suited for non-croplands, where it may not be economically feasible to control invasive plants through other management options.
Disadvantages
• Upfront initial costs are high • Not all biocontrol insects are effective in every habitat • Non-target effects on closely related plant species • Lengthy period before management of invasive plant occurs, often five to ten years • Some invasive plants species are not good targets for weed biocontrol programs

insects are rigorously tested to determine whether they can complete their life cycles on a series of plants. The plant species chosen for inclusion in host specificity testing range from those closely related to the invasive plant to plants of economic importance, such as crop plants, as well as plants growing in the same habitat as the invasive that may or may not be closely related or of economic importance (Figure 1-3).

An ideal biocontrol insect will have a life-cycle synchronized with the invasive plant. The insect will also effectively kill, damage, or prevent the development of seeds of the target plant. Often, successful weed biocontrol programs have released a series of insects that target different parts or life-cycles of the plant. For example, root and stem mining insects, leaf defoliators, and seed-feeders may be released to increase the effectiveness of the overall biological control program.

Code of Best Practices for Biological Control of Weeds

Biological control practitioners have adopted a Code of Best Practices for Biological Control of Weeds. By following the code, practitioners reduce the potential for causing environmental damage through the use of biological control by voluntarily restricting biological control activities to those most likely to result in success and that show little potential to impact non-target plants. The code of best practices was developed by delegates and participants to the

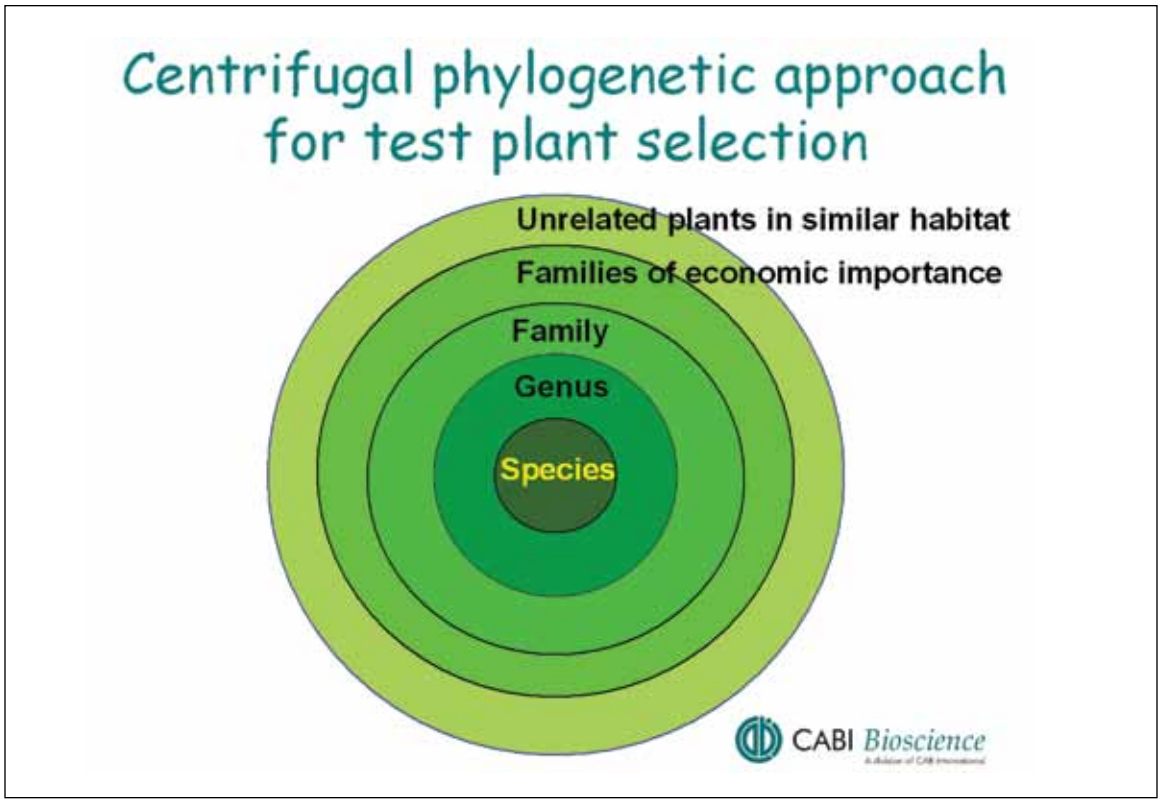

Figure 1-3. The Centrifugal Phylogenetic Approach for test plant selection provides a framework for host test plant selection. Plant species chosen for inclusion in host specificity testing start with those closely related to the invasive plant and expand to include plants less taxonomically related, such as plants of economic importance. (André Gassmann, CABI)

X International Symposium for Biological Control of Weeds. Although weed biological control is an effective and important weed management tool, it does not work in all cases and will not eradicate, or completely remove, the target weed. Often, biological control can be integrated with other chemical, mechanical, or cultural methods of weed control.

The United States Department of Agriculture – Animal and Plant Health Inspection Service – Plant Protection and Quarantine (USDA-APHIS-PPQ) is the federal agency responsible for authorizing the importation of biological control agents into the United States. The Canadian Food Inspection Agency (CFIA) serves the same role in Canada.

Federal laws and regulations are in place to minimize the risks to native plant and animal communities associated with introductions of exotic organisms to manage weeds. The Technical Advisory Group for Biological Control Agents of Weeds (TAG) is an expert committee with representatives from regulatory agencies, federal land management and environmental protection agencies from the United States, Canada, and Mexico. TAG reviews all petitions to import new biological control agents into the United States and makes recommendations to USDA-APHIS about the safety and potential impact of prospective biological control agents. Weed biological control researchers work closely with USDA-APHIS-PPQ and TAG to accurately assess the environmental safety of potential weed biological control agents and programs. The Canadian counterpart to TAG is the Biological Control Review Committee (BCRC). In addition, each state in the United States has its own approval process to permit field release of weed biological control agents.

About this Manual

This manual provides background information about garlic mustard and each of its potential biological weed control agents. It also provides guidelines for other garlic mustard management strategies, such as mechanical, cultural and chemical weed management options.

Chapter 2 provides a detailed description of garlic mustard's biology and lifecycle, including images of plant parts and life stages. The distribution of garlic mustard in North America is discussed, as well as the environmental impact of garlic mustard on forest ecosystems.

Chapter 3 describes the biology and lifecycle of each potential biological control agent and includes images of each insect, along with a description of plant parts attacked. The host range of each insect is discussed.

Chapter 4 provides detailed information on mechanical, cultural, and chemical management strategies for garlic mustard.

Appendix 1. Monitoring Garlic Mustard Biological Agents

References

Bourchier, R., R. Hansen, R. Lym, A. Norton, D. Olson, C.B. Randall, M. Schwarzlander, L. Skinner 2009. Biology and Biological Control of Leafy Spurge. Forest Health Technology Enterprise Team. USDA Forest Service. FHTET-2005-07.

CHAPTER 2: GETTING TO KNOW GARLIC MUSTARD

Description and Classification

Family: Brassicaceae (mustard family)
Tribe: Thlaspideae
Genus: *Alliaria*
Species: *petiolata*

Garlic mustard (Figures 2-1 and 2-2) is the only species of the genus *Alliaria* present in North America. Only two additional species in the tribe Thlaspideae are found in North America: roadside pennycress (*Thlaspi alliaceum*) and field pennycress (*Thlaspi arvense*) (Figures 2-3 and 2-4). Both of these species of pennycress, like garlic mustard, have been introduced into North America and are not native.

Figure 2-1. Flowering garlic mustard plant. (Reproduction of a painting by Carl Lindman, a Swedish botanist, 1856-1928)

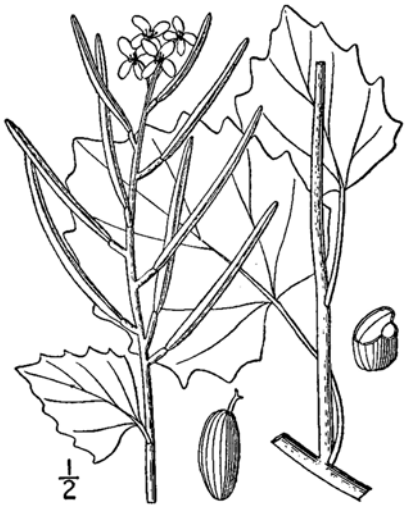

Figure 2-2. Second year flowering plant and siliques (seed capsules). (USDA-NRCS PLANTS Database; Britton, N.L., and A. Brown. 1913. *An illustrated flora of the northern United States, Canada and the British Possessions. 3 vols*. Charles Scribner's Sons, New York. Vol. 2: 170)

Figure 2-3. Field pennycress, one of two introduced plants in North America in the same tribe as garlic mustard. (USDA-NRCS PLANTS Database; Britton, N.L., and A. Brown. 1913. *An illustrated flora of the northern United States,Canada and the British Possessions. 3 vols*. Charles Scribner's Sons, New York. Vol. 2: 168)

Figure 2-4. Flowering field pennycress. (Mary Ellen Harte, Bugwood.org)

Garlic Mustard Biology

Life History Overview

Garlic mustard is an obligate biennial plant (it lives for two years). This plant is named "garlic" mustard because the leaves have a distinct garlic smell when crushed. Seeds germinate early in the spring, making the seedlings easy to identify. During the first summer, seedlings develop into rosettes with rounded leaves. The plant overwinters as a rosette and leaves remain green throughout the winter. In the spring of the second year, garlic mustard rosettes "bolt" to produce flowering stems, and plants flower from May to June. Each flower has four white petals with seed capsules, called siliques, forming soon after flowering. The siliques are initially green. By mid-summer (usually mid-July in the upper Midwest) siliques have matured and are long, brown, and curved, making them easy to identify. After the seeds have matured, the plants die and turn light brown. The main means of spread of garlic mustard is through seed dispersal.

Description

Seedlings. Cotyledons are elongated, paddle-shaped and average ¼ inches long (Figure 2-5). The first true leaves are heart-shaped with scalloped margins (Figure 2-6).

Figure 2-5. Garlic mustard seedling with cotyledon and first true leaves. (Roger Becker, University of Minnesota)

Figure 2-6. Garlic mustard seedling with heart-shaped leaf. (Roger Becker, University of Minnesota)

Rosettes. Garlic mustard seedlings develop into rosettes during the first summer of growth (Figure 2-7). Rosettes have round, glossy, scalloped-edged leaves, 2 to 5 inches in length. Leaves are dark green in color. Petioles are pubescent (hairy). Plants overwinter in the rosette stage and remain green throughout the winter.

Mature, second year bolting plant. After overwintering, rosettes bolt to produce several flowering stems early in the spring of the second year (Figure 2-8). Second year plants have basal heart-shaped leaves at the base and triangular, sharply-toothed leaves higher on the stem (Figure 2-9). Leaves are in an alternate arrangement on the bolting stem (Figure 2-10). Stems are pubescent (hairy). Leaves and stems smell like garlic when crushed and this can distinguish garlic mustard from other plants, such as violets (*Viola* sp.) or creeping Charlie/ground ivy (*Glechoma hederacea*).

Figure 2-7. First year garlic mustard rosette. (Steven Katovich, USDA Forest Service)

Figure 2-8. Second year bolting garlic mustard plant with flower buds. (Steven Katovich, USDA Forest Service)

Figure 2-9. Second year flowering garlic mustard plant with triangular toothed leaves. (Elizabeth Katovich, University of Minnesota)

Figure 2-10. Second year flowering garlic mustard plant showing alternate leaf arrangement. (Roger Becker, University of Minnesota)

Flowers. Flowers develop during May and June. Numerous flowers form in clusters at the end of stems and in leaf axils (Figure 2-11). Flowers are white, with four oblong petals in a cross shape. Petals are ¼ inch in length (Figure 2-12). Each flower has six stamens, four long and two short. Flowers can be cross-pollinated by bees, other small insects, or self-pollinated.

Figure 2-11. Flowering garlic mustard plant. (Elizabeth Katovich, University of Minnesota)

Figure 2-12. Garlic mustard flowers are white, with four petals in a cross shape. Petals are ¼ inch in length. (Roger Becker, University of Minnesota)

Seed capsules. After the flowers have been pollinated, green siliques form on plants (Figure 2-13). Seeds are mature by mid-July and the distinctive, mature siliques are long, light tan and curved (Figure 2-14). Each silique is 1 to 2.5 inches long with a single row of black, oblong seed (Figure 2-15). When mature, siliques split open to release the seed. After the seeds have matured, flowering plants die and turn light brown (Figure 2-16).

Figure 2-13. Green siliques develop after the flowers have been pollinated. (Elizabeth Katovich, University of Minnesota)

Figure 2-14. Mature siliques are long, brown and curved. (Elizabeth Katovich, University of Minnesota)

Figure 2-15. Each silique contains a single row of black, oblong seeds. (Elizabeth Katovich, University of Minnesota)

Figure 2-16. Garlic mustard plants turn a light brown color in late July and die after the seeds mature. (Elizabeth Katovich, University of Minnesota)

Seeds. Seeds are brownish black, oblong in shape, have longitudinal striations and are ¼ to ⅛ inch in length (Figures 2-17 and 2-18). Garlic mustard plants are prolific seed producers. It is estimated that one plant can produce up to 3500 seeds. Seeds are dormant at maturity and require a period of cold stratification to break dormancy. The majority of seed germinate after one winter but may be viable in the soil seed bank for up to five years. Garlic mustard is spread by seed, with most seed falling within the radius of the adult plant. Seeds can be water dispersed, especially during flooding.

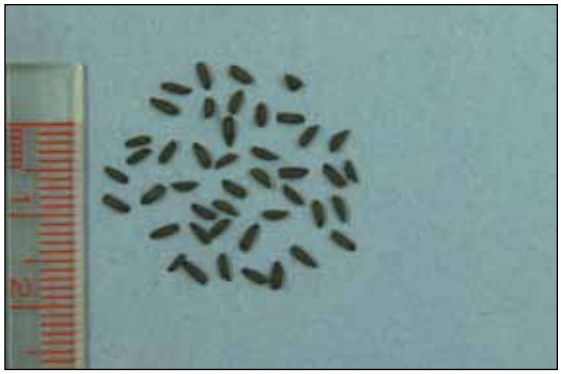

Figure 2-17. Garlic mustard seeds are black, oblong and approximately 3 mm (⅛ inch) in length. (Roger Becker, University of Minnesota)

Figure 2-18. Seeds are striated across the length of the seed. (Steve Hurst, USDA NRCS PLANTS Database, Bugwood.org)

Garlic Mustard Distribution in North America

Garlic mustard is native to Europe where it has historically been valued for its medicinal and herbal properties. This invasive plant was first recorded in North America at Long Island, NY in 1868. Since the initial introductions, genetic evidence suggests that garlic mustard has been introduced from Europe on multiple occasions.

From the first recorded sites in New York, garlic mustard has spread to the Northeast, Midwest, and West. Garlic mustard is now recorded in 37 states and 6 Canadian provinces (Figure 2-19) and has the potential for a wider distribution based on climate matching. Garlic mustard is listed as a noxious weed in eight states.

Figure 2-19. Garlic mustard is present in 37 states and 6 Canadian provinces. (USDA, NRCS. 2012. The PLANTS Database [http://plants.usda.gov], 6 November 2012. National Plant Data Team, Greensboro, NC 27401-4901 USA)

Garlic Mustard Biology and Ecology

As previously stated, garlic mustard is a biennial plant (plants live for two years). Seedlings germinate early in the spring and can form a dense carpet of seedlings before tree canopy closure (Figure 2-20). Overwintered, second year rosettes bolt and flower early in the growing season (Figure 2-21). Early germination and flowering allow garlic mustard plants to maximize soil nutrients and light while native species are still dormant and before tree canopy closure. These phenological attributes enable garlic mustard plants to displace spring ephemerals, tree seedlings and other native plants.

Figure 2-20. A carpet of garlic mustard seedlings form early in the spring in the forest understory before tree canopy closure. (Steven Katovich, USDA Forest Service)

Figure 2-21. Overwintered, second year rosettes bolt and flower early in the growing season. (Steven Katovich, USDA Forest Service)

Garlic mustard plants can adapt to available light levels. Garlic mustard is tolerant of shade and plants thrive in the forest understory and along forest edges in shaded and semi-shaded areas. It can also grow in full sun along the edges of forested areas as shown in Figure 2-22. Garlic mustard grows in a variety of soil types but plant growth may be limited in areas with peat, muck, or acidic soils. Plants also have a lower rate of survival at drier sites.

Due to the biennial life-cycle of garlic mustard, it is common to see one life-stage dominate at a location. For example, at some sites seedlings or first year rosettes may predominate (Figure 2-23). At other sites most plants may be flowering, second year plants (Figure 2-24). Thus, the most prominent life stage can alternate from year to year at a particular site. Conversely, some sites will have similar numbers of seedlings, rosettes and second year plants growing side by side (Figure 2-25).

Figure 2-22. Garlic mustard often grows along the edges of wooded areas. (Elizabeth Katovich, University of Minnesota)

Figure 2-23. A wooded site dominated by garlic mustard seedlings or first year rosettes. (Steven Katovich, USDA Forest Service)

Figure 2-24. Second year flowering plants dominate this garlic mustard site. (Steven Katovich, USDA Forest Service)

Figure 2-25. Seedlings and second year rosettes, prior to bolting and flowering, growing in the same site. (Steven Katovich, USDA Forest Service)

Garlic mustard seeds can remain viable in the soil for up to five years. With the presence of garlic mustard seed in the soil, seeds can germinate and produce a flush of seedlings that can re-invade a site, even if growing conditions were poor the previous season.

With their abundant seed production and early season germination and growth, garlic mustard plants are able to rapidly colonize forests and are more competitive than other woody understory species. Dense stands of garlic mustard in forested understory sites can reduce the abundance of sugar maple, white ash, oak, black cherry, and red maple seedlings as well as native grasses and herbs.

Garlic mustard plants produce phytotoxic chemicals that are exuded from root tissue. These phytotoxins can alter the properties of forest soils or directly inhibit growth of native hardwood seedlings, such as red maple, sugar maple, and white ash. Garlic mustard plant exudates can also disrupt the mutual associations between native tree seedlings and arbuscular mycorrhizal or ectomycorrhizal fungi that are critical for tree growth and survival.

Garlic mustard also lacks "natural controls", such as native insects and diseases, that could curtail its growth and survival. For example, in a Minnesota study, herbivores were found to damage less than 2 percent of the leaf area of garlic mustard plants. Even high levels of damage had little effect on seedling or rosette survival, as shown in a Michigan survey where 88 percent of study quadrats contained garlic mustard plants with insect damage, mammal browsing, or had symptoms of plant disease.

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CHAPTER 3: BIOLOGY OF GARLIC MUSTARD BIOCONTROL AGENTS

A project to investigate the potential for classical biological control of garlic mustard was initiated in 1998 by Prof. Bernd Blossey (Cornell University, Ithaca, NY). CABI's centre in Switzerland was mandated to explore potential biological control agents in the native range of garlic mustard in Europe and to carry out host range testing on prioritized potential agents. Since 2003, host specificity testing is also conducted in quarantine at the University of Minnesota.

Basic Insect Biology

Insects are a very large and diverse class of animals. An understanding of basic insect biology and anatomy will help land managers recognize and identify biological control agents of garlic mustard. Garlic mustard biological control insects have complete metamorphosis, a life cycle with four distinct stages: egg, larva, pupa and adult (Figure 3-1). Adult insects have an exoskeleton; a segmented body divided into three regions: head, thorax, and abdomen; three pairs or six segmented legs; and most have one or two pairs of wings. The head of the adult insect has one pair of compound eyes and antennae (Figure 3-2). Immature insects have an exoskeleton

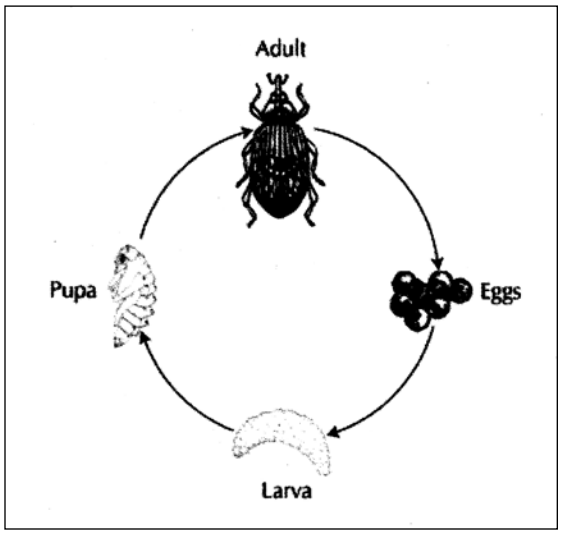

Figure 3-1. Garlic mustard biological control weevils have four life stages and complete metamorphosis. (bugwood.org)

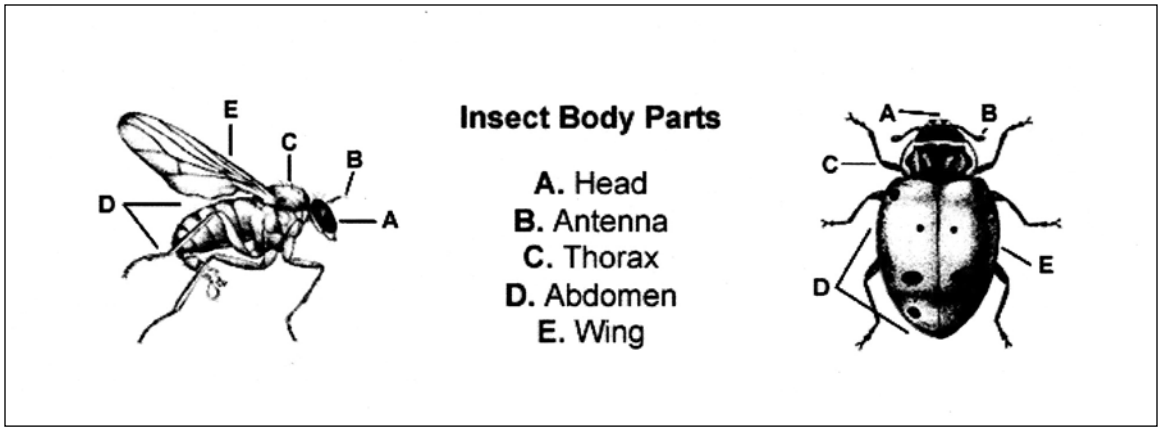

Figure 3-2. Generalized adult insect anatomy. (bugwood.org)

which must be shed, or molted, for immature insects to grow to the next stage. Larval stages between molts are called "instars." Larvae of garlic mustard biocontrol insects complete three instars before they molt into the pupal stage. During the pupal stage the insect changes from a larva to an adult. Insects do not feed during the pupal stage.

Insects and Garlic Mustard

In its native range, at least 70 insect species in 20 different families as well as seven fungi are recorded to be associated with garlic mustard. Insects include mainly beetles (48 percent of the species recorded) and butterflies (27 percent of species recorded). In addition, also flies (Diptera) and bugs (Hemiptera), as well as one sawfly (Hymenoptera) and one thrips (Thysanoptera) species are associated with garlic mustard in Europe. Most of these are however not specific enough to be considered as potential biological control agents; several species developing on garlic mustard are also known pests of cultivated crucifers. Five species in the genus *Ceutorhynchus* (Coleoptera; Curculionidae) and one fly species, *Ophiomyia alliariae* Hering (Diptera; Agromyzidae) are cited as monophagous on garlic mustard; i.e., garlic mustard is the only food plant known for these species.

Garlic Mustard Biocontrol Agents

Six species were found in the field at the start of the biological control project to test their potential as agents against garlic mustard. In addition to the five weevil species considered as monophagous on garlic mustard in the literature (*Ceutorhynchus alliariae*, *C. constrictus*, *C. roberti*, *C. scrobicollis*, *C. theonae*), a flea beetle, *Phyllotreta ochripes* (Curtis) (Coleoptera; Chrysomeldidae), was also investigated. Adults of *Ph. ochripes* were recorded in the literature to feed on a limited range of wild crucifers; larval development was only known to occur on great yellow-cress (*Rorippa amphibia* [L.] Bess.) and garlic mustard. Host specificity tests revealed however that the host range of this species is too broad for field release in North America. *Ceutorhynchus theonae* originates from the Caucasus region and collection of the species was logistically difficult. Since the species is a seed feeder and occupies the same niche on the plant as *C. constrictus*, a species common in Europe, work on *C. theonae* was discontinued. We never encountered the fly species, *O. alliariae*, described as monophagous on garlic mustard in the literature.

The remaining four species occupy different feeding niches on garlic mustard: *Ceutorhynchus alliariae* and *C. roberti* are stem-miners, *C. scrobicollis* is a root feeder, and *C. constrictus* develops in the seeds (Figure 3-3).

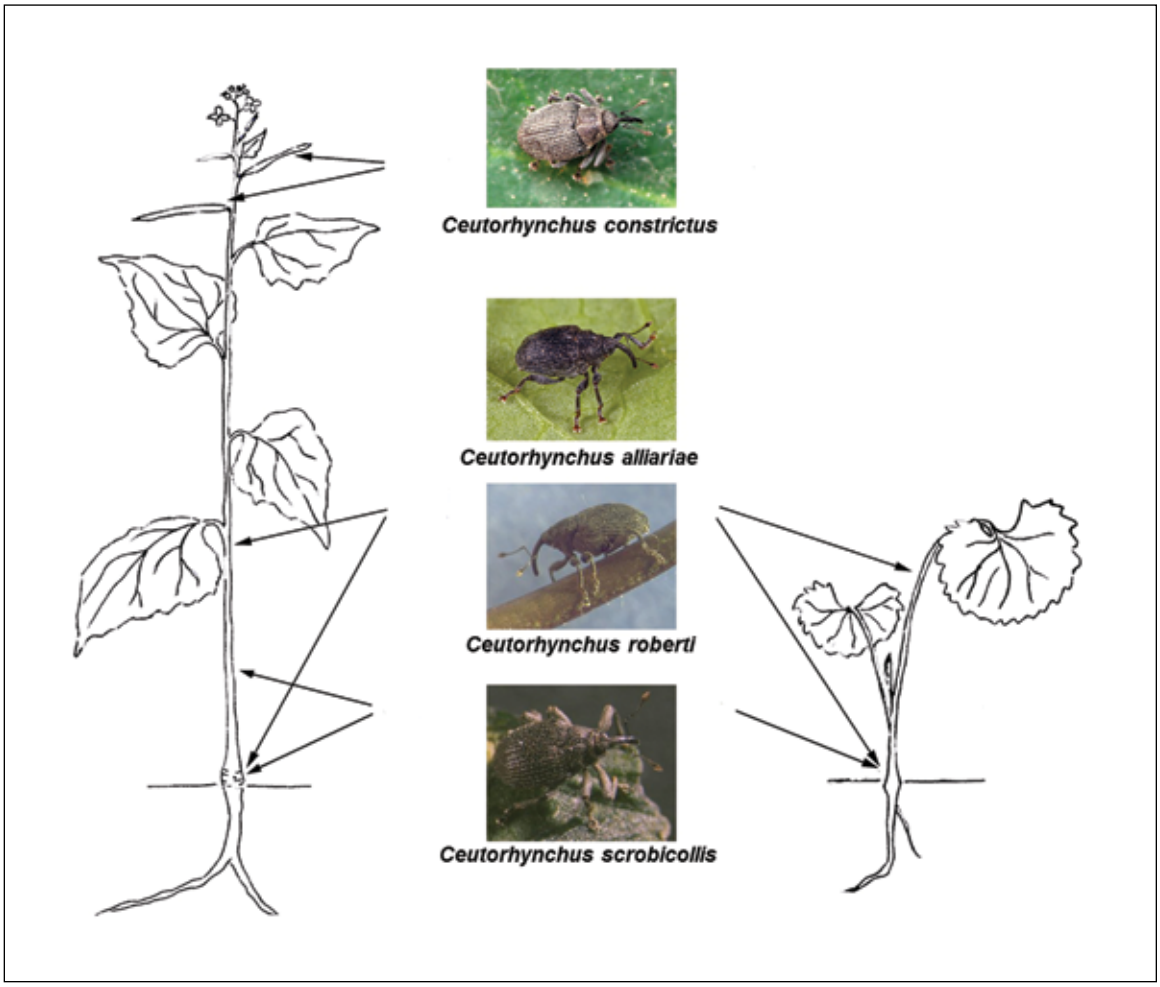

Figure 3-3. Four weevil species selected for biological control and their feeding niche on bolting plants (left) and rosettes (right) of garlic mustard. *Ceutorhynchus alliariae* and *C. roberti* have identical feeding niches.

Ceutorhynchus scrobicollis Nerensheimer & Wagner

Order: Coleoptera

Family: Curculionidae

Native Distribution

Central and eastern Europe, extending to Ukraine and eastern Caucasus region. *Ceutorhynchus scrobicollis* can be found in a wide range of habitats, such as road-sides, field edges, wastelands, and forests.

Original Source

Ceutorhynchus scrobicollis used in host-specificity tests originated from the Berlin region (Germany).

Description

Adult *C. scrobicollis* are 2.9 to 3.4 mm long (Figure 3-4). Their body is uniformly black; elytrae (hardened fore wings of beetles) are only sparsely covered with black hairs and appear glabrous at first sight. Eggs are 0.50 x 0.30 mm in size and pale yellow. The legless larvae have white bodies with clearly distinctive dark brown (1st instars) or reddish brown head capsules (2nd and 3rd instars).

Life History

Ceutorhynchus scrobicollis has one generation per year. Adults lay eggs into garlic mustard rosettes from mid-September until the beginning of April of the following year (Figure 3-5). Egg-laying stops if mean daily temperatures drop below –5 °C (23 °F). Based on laboratory observations, females lay around 230 eggs over this time period. Eggs are laid into petioles, leaves and the growing points of rosettes (Figure 3-6). Females use their long snout (rostrum) to bore holes into host plant tissue, deposit a single egg and subsequently cover the opening with secretion. Larvae pass through three instars and due to repeated oviposition on the same plants, all three larval instars and eggs can be found at the same time in the same plants. Mature larvae leave the plants to pupate in the soil and new adults emerge from early May to mid-June. After emergence, *C. scrobicollis* briefly feed on garlic mustard leaves, and then remain inactive in summer. From the beginning of September onwards, weevils become active again and their characteristic feeding marks reappear on leaves (Figure 3-7). In captivity, adults survived for more than one year and had a second, in some cases even a third oviposition period.

Figure 3-4. Adult *Ceutorhynchus scrobicollis*. (Tim Haye, CABI)

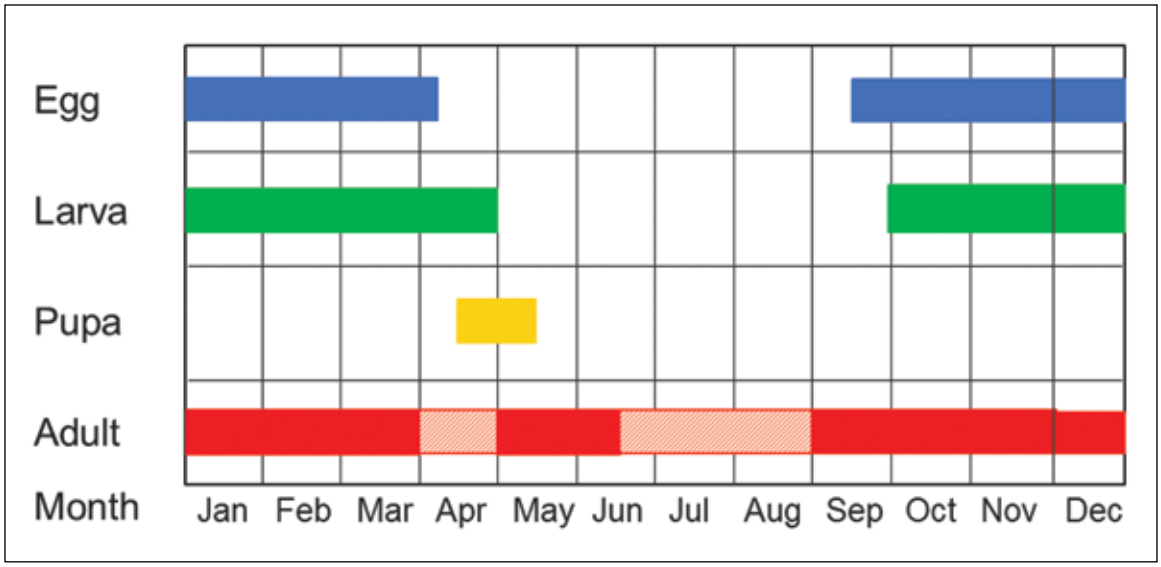

Figure 3-5. Life cycle of *Ceutorhynchus scrobicollis*. Bars indicate the approximate length for each life stage. Patterned bar for adults indicates periods without activity. (Esther Gerber, CABI)

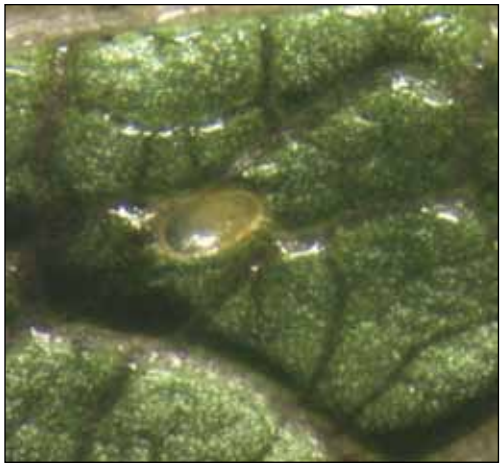

Figure 3-6. *Ceutorhynchus scrobicollis* egg laid into the leaf surface of garlic mustard. (Elizabeth Katovich, University of Minnesota)

Figure 3-7. *Ceutorhynchus scrobicollis* feeding marks on garlic mustard rosette leaves. (Hariet L. Hinz, CABI)

Feeding Stage and Host Impact

Adult weevils feed on garlic mustard foliage; at high densities, they can substantially reduce leaf area (Figure 3-7). The most damaging stage is however the larval stage. They mine petioles and root-crowns throughout the winter and can also be found in the base of shoots in early spring. At field sites, garlic mustard plants attacked by *C. scrobicollis* can easily be spotted: larval mining destroys the main shoot, leading to production of several weaker side shoots. Attack rates of up to 100 percent can be observed at field sites in its native range and up to 50 larvae were found in a single plant. In manipulative experiments, attack by these weevils significantly reduced rosette survival. Surviving plants produced more shoots, but these were of reduced height and their biomass and seed production was reduced.

Host Specificity

Tests were both conducted in the native range of the weevil and in quarantine in the United States and covered 86 species and subspecies, 55 in the family Brassicaceae, and the remaining in 23 different families.

Test results clearly show that plant species outside the family Brassicaceae are not at risk of being attacked by *C. scrobicollis*. Within the Brassicaceae, five test plant species allowed complete larval development.

- A single adult in a single replicate emerged from a variety of the commercially grown Savoy cabbage (*Brassica oleracea* var. *sabauda* L.), but subsequent extensive testing indicate that this unique attack must be considered as a laboratory artifact. No development in any other cabbage variety was found. A single native North American species, spreading yellowcress (*Rorippa sinuata* [Nutt.] Hitchc.) allowed *C. scrobicollis* to complete larval development under no-choice conditions. The species was, however, not attacked in single-choice tests, i.e., in the presence of garlic mustard, indicating that under field conditions, risk of attack of this species by *C. scrobicollis* is extremely low.
- The three remaining plant species that allowed development—field pennycress (*Thlaspi arvense* L.), garlic cress (*Peltaria alliacea* Jacq.) and watercress (*Nasturtium officinale* W.T. Aiton)—are of European origin. Additional tests with watercress showed that *C. scrobicollis* is not able to complete its development in water-saturated soils, the conditions present when the species is grown commercially.

Overall, *C. scrobicollis* can be considered a highly specialized herbivore and was proposed for introduction in North America in May 2008. Supplementary data were submitted upon requests by reviewers in September 2011. A decision by the United States government to introduce *C. scrobicollis* is pending.

Root-feeding insects have become popular weed biocontrol agents in the last 15-20 years because they have higher establishment rates than above-ground biocontrol agents (78 vs. 65 percent). They also contribute more to suppression of target weed populations (54 percent) compared to folivores (34 percent). Most of the successful root-feeding control agents are beetles, particularly in the families Curculionidae (weevils) and Chrysomelidae (leaf beetles). In addition, root feeders, by virtue of their feeding niche, are relatively safe from parasitism and predation, a factor often limiting establishment and population build-up of biocontrol agents. Finally, demographic modelling of garlic mustard combined with elasticity analysis predicted that *C. scrobicollis* will have the most significant impact on the plant's demography and that single agent releases of *C. scrobicollis* will control garlic mustard in many, though not all, situations.

Ceutorhynchus constrictus (Marsham)

Order: Coleoptera

Family: Curculionidae

Native Distribution

Western and central Europe, extending eastward to Bulgaria. *Ceutorhynchus constrictus* can be found in a wide range of habitats but prefers moist and nutrient rich sites.

Original Source

Ceutorhynchus constrictus used in host-specificity tests originated from the Delémont region (Switzerland).

Description

Adult *C. constrictus* are 2 to 2.5 mm long (Figure 3-8). Their body is uniformly black; elytrae (hardened fore wings of beetles) and pronotum (the dorsal plate of an insect's prothorax) are covered with white scales, giving the weevil an overall greyish appearance. Characteristic of the species are the yellowish scales that cover the apices of the mesepimera (lateral structure behind the episternum), which is also visible from above. Eggs are 0.40 x 0.28 mm in size and pale yellow. The legless larvae have white bodies with clearly distinctive dark reddish brown head capsules (Figure 3-9). Mature 3rd instar larvae are 2-3 mm long.

Life History

Ceutorhynchus constrictus has only one generation per year (Figure 3-10). Females lay eggs into pods containing developing seeds during May and June and subsequently cover the opening with secretion. Based on laboratory observations, females lay on average around 160 eggs over this time period. Females use their long snout (prolonged rostrum) to bore holes into host plant tissue, deposit a single egg and subsequently cover the opening

Figure 3-8. Adult *Ceutorhynchus constrictus*. (Gabi Krumm)

Figure 3-9. Third instar larva of *C. constrictus* next to a garlic mustard seed. (Esther Gerber, CABI)

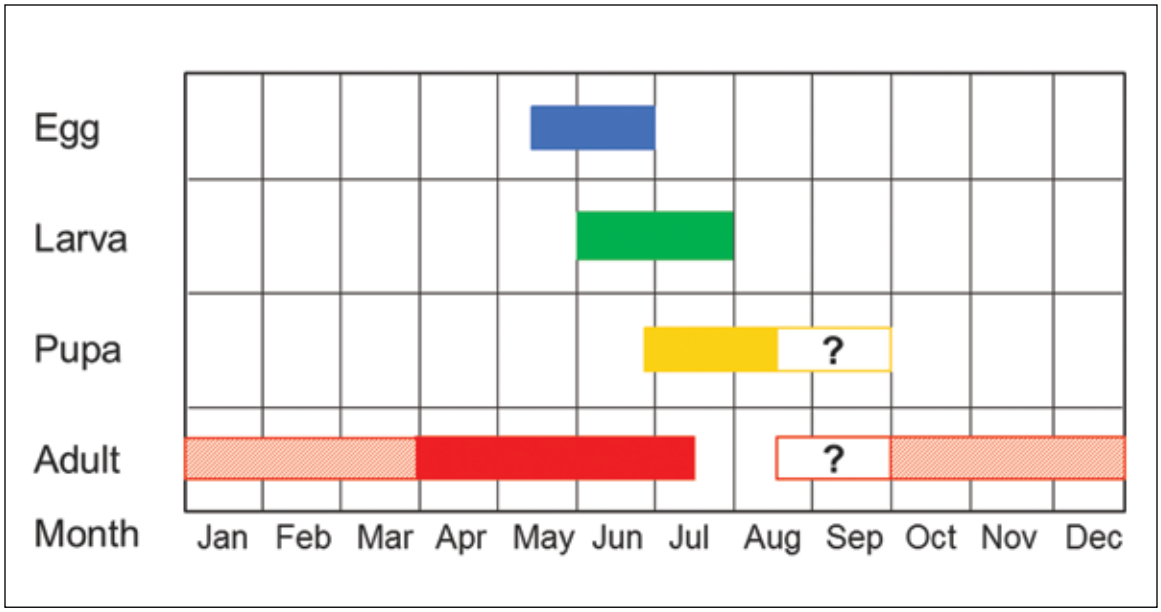

Figure 3-10. Life cycle of *Ceutorhynchus constrictus*. Bars indicate the approximate length for each life stage. Patterned bar for adults indicates period when fully developed adults remain inactive in the soil. (Esther Gerber, CABI)

with secretion. Several eggs can be laid into the same pod. Larvae feed on the ripening seeds and pass through three instars before leaving the pods to pupate in the soil by late June. Development from egg to mature larva takes about 6-7 weeks. Fully developed living adults were found in earthen cocoons in October but adults only emerge after overwintering in the following spring. After emergence, by the end of March or beginning of April, *C. constrictus* feed on garlic mustard leaves and flowers (Figure 3-11). Females may need to feed on pollen, flowers or developing pods of garlic mustard in order to develop their ovaries. All adults die after egg-laying.

Figure 3-11. *Ceutorhynchus constrictus* adults and their feeding damage on garlic mustard during a mass outbreak of the species in its native range. (Esther Gerber, CABI)

Feeding Stage and Host Impact

Adult weevils feed on garlic mustard foliage (Figure 3-11). The most damaging stage is however the larval stage. One larva consumes about two seeds during its development. At field sites in the native range of *C. constrictus*, up to 50 percent seed reduction has been found. In manipulative experiments, individual plants had up to 79 percent of seeds destroyed. A mass outbreak of *C. constrictus* was observed in the area of Delémont, Switzerland in 2007. During the outbreak, adult feeding had a considerable impact on leaf area and presumably also pod production, since weevils also heavily fed on developing flowers.

Host Specificity

Host-range evaluation for this potential biocontrol agent has not been completed yet. Tests are both conducted in the native range of the weevil and in quarantine in the United States and so far covered 77 species and subspecies, 57 in the family Brassicaceae and the remaining in 16 different families.

Test results clearly show that plant species outside the family Brassicaceae are not at risk of being attacked by *C. constrictus*. Within the Brassicaceae, so far two test plant species allowed complete larval development.

- In no-choice tests, *C. constrictus* emerged from the commercially grown black mustard (*Brassica nigra* [L.] W. D. J. Koch), a European species. Subsequent extensive testing indicated that under field conditions, risks to this species by *C. constrictus* are extremely low.
- Also the commercially grown Indian mustard (*Brassica juncea* [L.] Czern.) allowed development of *C. constrictus* in no-choice tests. Tests are currently being carried out to investigate attack of this species under more natural conditions. The results so far indicate a very low risk of Indian mustard being attacked by *C. constrictus* in the field. In addition, an extensive literature research revealed that no reports of *C. constrictus* attacking commercially grown mustards in Europe exist.

Comments

While seed predators can have large impacts on seed production, they are not necessarily successful in reducing populations of invasive weeds. Plants may either not be seed limited, and/or they compensate for seed loss through increased growth of the plants that did germinate at a site. Seed reduction has however been recognized as one of the factors affecting garlic mustard population growth rate in North America. Demographic modelling of garlic mustard combined with elasticity analysis predicted that seed reduction at levels inflicted by *C. constrictus* might be needed in combination with the root mining *C. scrobicollis* to control garlic mustard across the full range of its demographic variability.

Ceutorhynchus alliariae Brisout

Order: Coleoptera

Family: Curculionidae

Native Distribution

Southern parts of Northern Europe (Sweden), from Western Europe (France) to eastern Europe (Ukraine). *Ceutorhynchus alliariae* can be found in a wide range of habitats; some authors mention a higher preference of shaded habitats compared to the otherwise very similar *C. roberti*, but this was not confirmed in a recent study. The two species occur both geographically isolated (allopatric) and associated (sympatric) in Europe.

Original Source

Ceutorhynchus alliariae used in host-specificity tests originated from the Delémont region (Switzerland).

Description

Adult *C. alliariae* are 2.6 to 3.4 mm long (Figure 3-12). Their body is uniformly black; only the tarsi (final segments in the leg of insects) are reddish. Eggs are 0.58 x 0.37 mm in size and pale yellow. The legless larvae have white bodies with clearly distinctive reddish brown head capsules. Mature 3rd instar larvae are 6-7 mm long.

Life History

Ceutorhynchus alliariae has one generation per year (Figure 3-13). Adults lay eggs into the stem of bolting plants, occasionally also into large petioles of rosettes and subsequently cover the opening with secretion. Egg-laying occurs from mid-March to the beginning of June (Figure 3-13). Based on laboratory observations, females lay on average around 100 eggs over this time period. Females use their long snout (prolonged rostrum) to bore a hole into host plant tissue, deposit a single egg (Figure 3-14) and subsequently cover the opening with secretion. Several eggs can be laid into the same stem. Larvae mine within stems and pass through three instars before leaving the plants to pupate in the soil from early May onwards. Larval development requires approximately seven weeks from egg to mature larva. After emergence, *C. alliariae* feed on garlic mustard leaves, and then

Figure 3-12. Adult *Ceutorhynchus alliariae*. (Albert de Wilde)

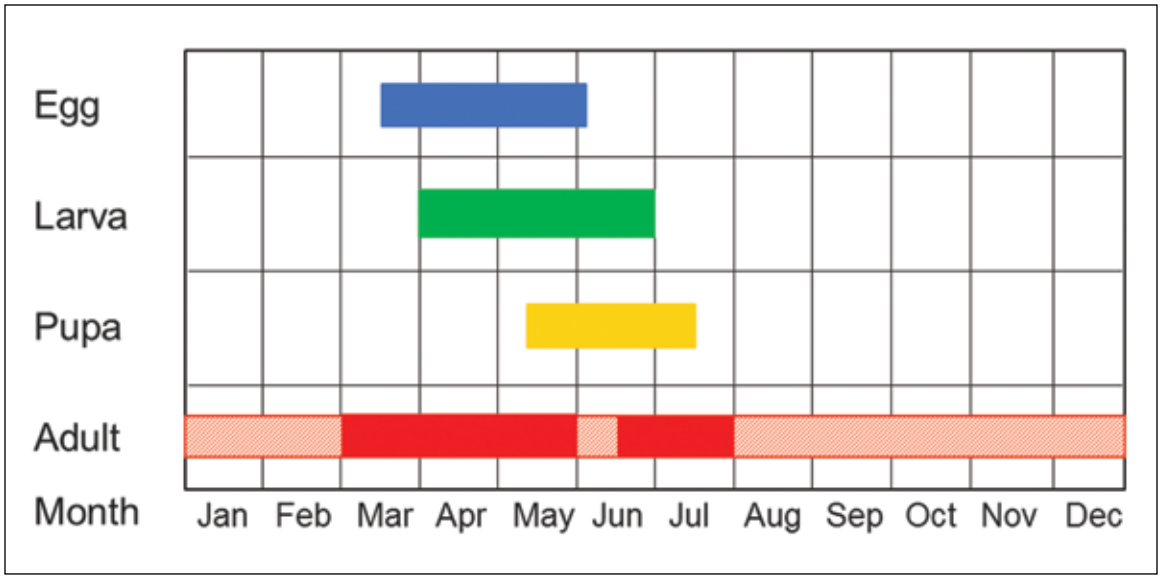

Figure 3-13. Life cycle of *Ceutorhynchus alliariae* and *C. roberti*. Bars indicate the approximate length for each life stage. Patterned bar for adults indicates periods without activity. (Esther Gerber, CABI)

Figure 3-14. *Ceutorhynchus alliariae* female boring a hole into a shoot (left; Albert de Wilde); cross section of a garlic mustard stem with an egg of *C. alliariae* (right; Tim Haye, CABI).

remain mainly inactive until the next spring. In western Europe, weevils become active again from the end of February onwards. In captivity, some adults survived for more than one year and had a second oviposition period. Data from marked weevils released at a field site indicate that this might also occur in nature.

Feeding Stage and Host Impact

Adult weevils feed on garlic mustard foliage. The most damaging stage is however the larval stage. In manipulative experiments, attack by *C. alliariae* caused a decrease in plant height and a reduction in seed output per plant. Larvae of the two stem-mining species, *C. alliariae* and *C. roberti*, cannot be distinguished morphologically and for this reason, attack from the range where both co-occur (sympatric range) almost certainly comprise both species. In field sites in the sympatric range, up to 30 larvae can be recorded in a

single shoot. Plants with such high attack levels show clear signs of damage: attacked shoots desiccated and do not produce any seeds (Figure 3-15). In some cases, the whole plant can die. Up to 100 percent of shoots can be attacked by the shoot miners at a site.

Figure 3-15. Garlic mustard plant heavily attacked by the stem-mining weevils (right) compared to plant with lower attack collected at the same field site. (Hariet L. Hinz and Esther Gerber, CABI)

Host Specificity

Host-range evaluation for this potential biocontrol agent has not been completed yet. Tests are being conducted in the native range of the weevil and so far covered 77 species and subspecies, 51 in the family Brassicaceae, and the remaining in 21 different families. Test results to date clearly show that plant species outside the family Brassicaceae are not at risk to be attacked by *C. alliariae*. Within the Brassicaceae, five test plant species allowed complete larval development.

- The same three plant species as for *C. scrobicollis*—field pennycress (*Thlaspi arvense*), garlic cress (*Peltaria alliacea*) and watercress (*Nasturtium officinale*)—also allowed development of *C. alliariae*. All three species are of European origin.
- In no-choice tests, adults emerged also from sweet alyssum (*Lobularia maritima* [L.] Desv.), an ornamental plant of European origin, and from spreading yellowcress (*Rorippa sinuata*), a native North American species. Tests are currently being carried out to investigate attack of these two species under more natural conditions.

Comments

Stem-feeding species have been used in biological control of weeds worldwide and several have contributed to reductions of weed populations. Overall, attack by both *C. alliariae* and *C. roberti* resulted in very similar damage patterns and experimental studies indicate that the overall impact of both species combined can be predicted by summing the impact of each species alone. Provided *C. alliariae* and *C. roberti* prove to be equally specific once host range tests are completed, both species are equally promising.

Both species can reduce plant height and/or seed output. As a strictly biennial plant relying solely on seeds for regeneration, garlic mustard should be particularly vulnerable to seed reduction.

While observed seed reduction by stem borers alone might not be sufficient to control garlic mustard across its full range of demographic variability, release of a stem borer in combination with *C. scrobicollis* could be successful in suppressing up to 88 percent of populations of the weed in its invasive range. In addition, a reduction in average stem height by weevil attack might further affect the competitiveness of garlic mustard with native species for light. When stems of garlic mustard were cut off at the base, native plants were able to grow and overtake the excised garlic mustard plants. It remains to be seen, however, whether the negative effect of stem miners on plant height will be sufficient to reduce the competitive ability of the weed in the invaded range.

Ceutorhynchus roberti Gyllenhal

Order: Coleoptera

Family: Curculionidae

Native Distribution

Scandinavia, from Western Europe (France) to eastern Europe (Russia). *Ceutorhynchus roberti* can be found in a wide range of habitats; some authors mention a higher preference of sunny habitats compared to the otherwise very similar *C. alliariae*, but this was not confirmed in a recent study. The two species occur both geographically isolated (allopatric) and associated (sympatric) in Europe.

Original Source

Ceutorhynchus roberti used in host-specificity tests originated from the Delémont region (Switzerland).

Description

Adult *C. roberti* are 2.8 to 3.7 mm long; they are on average slightly longer than the closely related *C. alliariae*, but size cannot be used reliably to separate the two species (Figure 3-16). Their body, including tarsi, are uniformly black; the latter allows distinguishing the species from the otherwise very similar *C. alliariae*. Eggs are 0.60 x 0.40 mm in size and pale yellow. The legless larvae have white bodies with clearly distinctive reddish brown head capsules. Mature 3rd instar larvae are 6-7 mm long.

Figure 3-16. Adult *Ceutorhynchus roberti*. (Tim Haye, CABI)

Life History

Ceutorhynchus roberti has one generation per year (Figure 3-13). The biology of this species is very similar to *C. alliariae*. Adults lay eggs into stems of bolting plants, occasionally also into large petioles of rosettes and subsequently cover the opening with secretion (Figure 3-17). Egg-laying occurs from mid-March to the beginning of June (Figure 3-13). Based on laboratory observations, females lay on average around 90 eggs over this time period. In contrast to *C. alliariae*, *C. roberti* frequently lays eggs in clusters of up to of eight eggs (Figure 3-18). In addition, several holes with eggs can be made into the same stem. Larvae mine within stems and pass through three instars before leaving the plants to pupate in the soil from early May onwards. Larval development requires approximately seven weeks from egg to mature larva. After emergence, *C. roberti* feed on garlic mustard leaves, and then remain mainly inactive until the following spring. From the end of February onwards, weevils become active again. In captivity, some adults survived for more than one year and had a second oviposition period. Data from marked weevils released at a field site indicate that this might also occur in nature.

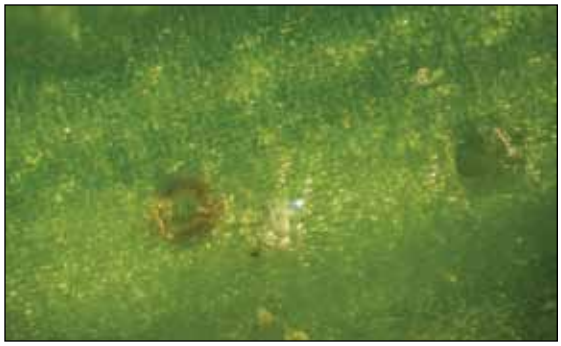

Figure 3-17. Feeding hole (right) and oviposition hole covered with secretion (left). (Tim Haye, CABI)

Figure 3-18. Eggs laid in clusters by *Ceutorhynchus roberti*. (Hariet L. Hinz and Esther Gerber, CABI)

Feeding Stage andHost Impact

Adult weevils feed on garlic mustard foliage. The most damaging stage is the larval stage. In manipulative experiments, attack by *C. roberti* caused similar plant responses as *C. alliariae*.

Host Specificity

Host-range evaluation for this potential biocontrol agent has not been completed yet. Tests are being conducted in the native range of the weevil and so far covered 69 species and subspecies, 51 in the family Brassicaceae, and the remaining in 14 different families. Test results so far clearly show that plant species outside the family Brassicaceae are not at risk to be attacked by *C. roberti*. Within the Brassicaceae, four test plant species allowed complete larval development.

- The same three plant species as for *C. scrobicollis* and *C. alliariae*—field pennycress (*Thlaspi arvense*), garlic cress (*Peltaria alliacea*), and watercress (*Nasturtium officinale*)—also allowed development of *C. roberti*. All three species are of European origin.
- In no-choice tests, adults emerged also from Farnsworth's jewelflower (*Streptanthus farnsworthianus* J.T. Howell), a native North American species. Tests are currently being carried out to investigate attack of this species under more natural choice conditions.

Comments

See information given for *C. alliariae*.

Although the impact on garlic mustard of both stem-mining weevils was overall very similar in a manipulative experiment carried out with both species, plants reacted differently in regard of the number of inflorescences produced. Attack by *C. roberti* increased the number of inflorescences, while attack by *C. alliariae* had no effect on this parameter. Aggregated feeding of first-instar *C. roberti* larvae might break the apical dominance and result in increased inflorescence production. However, the increase in inflorescences did not result in higher seed production.

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CHAPTER 4: THE BIOLOGICAL CONTROL COMPONENT OF AN INTEGRATED GARLIC MUSTARD MANAGEMENT PROGRAM

Introduction to Integrated Weed Management (IWM)

The successful management of an invasive species requires the integration of research findings, management goals, and available management tools. Indeed, findings of a recent web-based survey reaffirmed the need to integrate research efforts and knowledge with the needs of land managers (Renz et al. 2009). A holistic approach to managing invasive plant pests has its roots in the concept of Integrated Weed Management (IWM). The entomology-centric Integrated Pest Management (IPM) movement for agronomic cropping systems was set as a national policy goal by the Nixon administration in 1972. IWM soon followed with passage of the Federal Noxious Weed Act of 1974. IWM further refined key IPM concepts to accommodate the unique attributes of plant pests, offering improved focus and outcomes. By 1981 IWM was widely adopted in scientific circles, with specific relevance to biological control of weeds presented at an international symposium by Andres (1982). IWM, with its roots in agronomic systems, has since been tailored to fit the needs of forest managers (Ferguson et al. 2003; USDA Forest Service 2001, 2003).

IWM, as described in the Federal Noxious Weed Act, is a multidisciplinary, ecological approach to managing unwanted plants. It uses an interdisciplinary approach to contain or control undesirable plant species in an area being managed. The short-term objective of such a program is to implement the most effective combination of control methods available for the target weed(s). Concurrently, landowners and managers develop a long-term plan to manage undesirable plants and maintain desirable vegetation. The ultimate goal of an effective IWM program is to replace undesirable plants that cause resource, economic, habitat, or aesthetic losses with plants that are beneficial to the environment. Implementation of an effective biological control program for garlic mustard requires an IWM approach.

Integrating the Biology of Garlic Mustard into Control Strategies

Control of garlic mustard is most effective if its biology is taken into consideration. Garlic mustard is a biennial, meaning a plant lives for two years. During the first year garlic mustard

seedlings develop into rosettes with rounded leaves. The plant overwinters as a rosette and leaves remain green throughout the winter. Herbicides work best when applied to the seedling and rosette stages. In the following spring, garlic mustard rosettes "bolt" to produce flowering stems with plants flowering from May to June. This is the best stage for hand-pulling or cutting, or for mowing stands. By July, seedpods can be seen which are long, brown, and curved when mature, making them easy to identify. Mature seedpods readily open to disperse the seed. This is not the time to do any management practice other than removing the rare, isolated plant where the seed can be contained during removal. Once a plant produces seed, it dies. There is little reason to control dying plants with any method. Figures 4-1 through 4-4 show garlic mustard seedling, 1st year rosette, 2nd year bolting plant, and senescing 2nd year plant, respectively.

Figure 4-1. Cotyledons and two true leaves (top) and slightly older seedling with the 3rd true leaf starting to show the typical garlic mustard morphology (bottom). Garlic mustard seedlings emerge in the spring, and are very susceptible to prescribed burns or foliar herbicide application. (Roger Becker, University of Minnesota)

Figure 4-2. Close-up of an overwintered garlic mustard rosette in 2nd year (top), which appear as individual rosettes or coalesce into an indistinguishable carpet of rosettes at higher populations (middle). Leaves can vary widely in size in the rosette stage (bottom). Rosettes are susceptible to foliar herbicide application fall or early spring and can be suppressed with spring burns. (Roger Becker, University of Minnesota)

Figure 4-3. Garlic mustard 2nd year bolting plant (flowering shoot elongating) (top). This is the key staging target for mowing or hand-pulling. Bolting shoots can develop into flowering plants in days or weeks (bottom) so the window for control may be short. Mowing and pulling can be effective if seed pods are not yet visible. Large tracts are best suited to mechanical control such as mowing bolting plants up to the early flower stage. Otherwise, treat large infestations with spring burns to kill seedlings, or herbicides applied to rosettes before garlic mustard gets to this stage. (Top: Mark Renz, University of Wisconsin; bottom: Laura Van Riper, Minnesota Department of Natural Resources)

Figure 4-4. Senesced (mature) plants are distinct and are easy to spot on the landscape. By now, seeds are mature and dispersing, and stems and crowns are naturally dying (senescing), preempting the need for control efforts at this time. Control efforts attempted at this time often spread seed and only make the problem worse. (Roger Becker, University of Minnesota)

Producing seed is critical to perpetuate an infestation (Figure 4-5). Unlike perennial species such as invasive honeysuckles (*Lonicera* spp.) or common buckthorn (*Rhamnus cathartica*) that will survive despite preventing seed production for a few years, biennials like garlic mustard will not. Thus, manage garlic mustard to prevent seed production to deplete the seedbank, which in turn will control an infestation and prevent spread to adjacent areas. Scientists debate the time required to deplete garlic mustard seed in the seedbank, but most seed will not survive for more than five years. Preventing seed production for two to three years will dramatically reduce the density of a population, preventing seed production for 4 years will, for all intents and purposes, remove an infestation (Baskin and Baskin 1992). Thereafter, a minute fraction of the seed in the seedbank may manage to survive to produce a few scattered plants, but many sites would see no survival.

Integrating Biological Control Methods

Classical biological control has been applied to many invasive weed species with both single- and multiple-agent introductions successfully controlling target weeds. Historically, using biological agents as the sole control strategy has been effective in about 30 percent of attempts, and may take up to 20 years or more to reduce weed populations to acceptable levels (McFadyen 2000). Integrating other weed management strategies with biological control will improve the chance of control success and shorten the time required to reduce weed populations. Similarly, within

Figure 4-5. An open (dehisced) garlic mustard seed pod (silique) and close-up of an individual seed (insert). Individual seeds are approximately 3 mm in length. (Elizabeth Katovich, University of Minnesota; inset: Roger Becker, University of Minnesota)

biological control, integrating multiple biological control agents may be necessary to gain control of the target weed, as has been reported for garlic mustard (Landis et al., 2005; Davis et al., 2006). Gerber and Hinz (2005; see also Chapter 3) describe the use of multiple *Ceutorhynchus* spp. to deploy multiple sites of attack on garlic mustard. In weed management terminology, multiple sites of attack offer multiple modes of action to control garlic mustard. Host range testing of multiple *Ceutorhynchus* spp. has been conducted (Hinz and Gerber, 2005; Katovich et al., 2005) toward eventual release of multiple biological agents offering multiple modes of action and improved garlic mustard control.

Once *Ceutorhynchus* spp. are released in North America, research will be needed to determine which IWM control methods are most compatible with *Ceutorhynchus* weevils. Simultaneous use of other control methods likely will not harm adult weevils, but damage to the host plant may cause adults to disperse, and may kill developing larvae within the plant. Once released, researchers can begin to determine the impacts integrating management methods such as hand removal, mowing, herbicides, and fire may have on the establishment, survival, and dispersal of *Ceutorhynchus* spp. It is anticipated that damage inflicted by *Ceutorhynchus* spp. on populations of garlic mustard will slowly diminish the dominance of garlic mustard seed in the soil seedbank, allowing other desirable species to compete and eventually restore diversity on the landscape. Research and monitoring will be needed to determine if additional restoration management may be needed to prevent the replacement of garlic mustard with other invasive species.

Weed Control Methods Used to Manage Garlic Mustard

Education, Prevention and Early Detection, and Rapid Response

Education programs include literature and ad campaigns to build awareness of the problem, advise regarding action steps that can be taken to prevent the spread or control a plant pest, and provide additional resources and contact information about the plant pest. A recent example is the Play Clean Go campaign (www.playcleango.org; accessed Feb. 28, 2012), an education campaign to inform resources users about invasive pests, and to build awareness on topics such as transporting seeds on clothes or in dirt clinging to equipment and recreational vehicles. Programs that include demonstration plots, tours, workshops, and meetings often accompany these educational materials.

Prevention programs focus on reducing unintentional transport of garlic mustard seed from infested areas to uninfested areas. Prevention also includes maintaining forests in ways that minimize their susceptibility to invasion. For preventative maintenance, follow IWM practices that encourage and promote desirable species, minimize disturbance, minimize sources of seed introduction or movement, and give high priority to eradicating remote satellite populations when discovered. Finding and controlling satellite populations should be given priority by land managers over controlling large, entrenched populations of a given invasive species (Moody and Mack, 1988) to achieve the biggest management impact in a geographic region for the time, money, and labor invested.

If prevention fails, early detection and rapid response (EDRR) is needed to prevent new infestations from establishing in previously uninfested areas (Westbrooks 2004). Monitoring is critical to successful EDRR (Figures 4-6 and 4-7). Monitor known pathways of introduction for new infestations and eradicate populations when discovered. Pathways include rights-of-way, public access areas, roads or trials, and areas impacted by disturbance events such as blow-downs, lightning strikes, disease or insect outbreaks, and timber harvests (Figure 4-8). Monitoring is also necessary when infestations of garlic mustard have moved beyond EDRR, when populations are common on the landscape and have progressed to a stage where general management is needed. Before any population can be controlled it first must be found. Therefore, monitoring programs are an important first step in any phase of a control program. Beyond simply finding a population, it is important to monitor a population after a control program has been initiated to determine what effect the control program is having.

Biological Control

Biological control involves the use of living organisms, such as insects or pathogens, to control a weed infestation and recreate a balance of plant species with predators. Research has focused primarily on the introduction of natural predators from the garlic mustard's area of origin (see Chapter 2 for more details). This biological control section will be updated with information on how to plan a local biological control program, select release sites, obtain and disseminate weevils, and how to monitor establishment and any potential impacts following release once a *Ceutorhynchus* species is approved for release. Integration of biological control with other control methods will also be added.

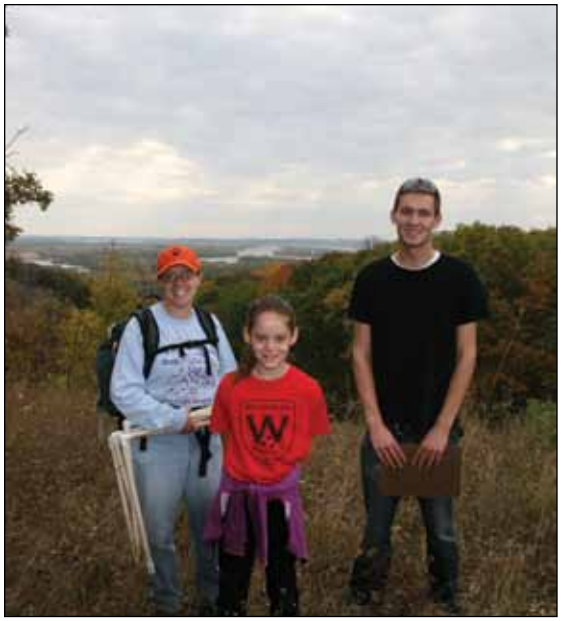

Figure 4-6. Monitoring crews can bring communities together and build support for control efforts. (Roger Becker, University of Minnesota)

Figure 4-7. Monitoring crew taking quadrat counts. Monitoring is a critical first step for most management efforts. (Roger Becker, University of Minnesota)

Figure 4-8. Access trail in a woodland. Trails and roads are common corridors of initial invasion from which invasive species spread into surrounding areas. (Roger Becker, University of Minnesota)

Hand-pulling or Cutting and Mowing to Control Garlic Mustard

For small populations, physically pulling or hand-cutting before flowering are effective control techniques (Figure 4-9). Pulling is easier if the soil is moist (e.g., after rain) to allow for the removal of the entire tap root. Pulling second-year plants is easier than pulling first-year rosettes. Alternatively, cut the entire taproot with a sharp shovel or spade 1 to 2 inches below the soil surface. With pulling or cutting, try to minimize soil disturbance to avoid exposing new seed and creating fresh germination sites. Immature seed can mature after cutting or pulling plants so if flowers are present when these control measures are applied, bag material and dispose of it in a landfill to avoid potential for seed spread. Disposal may be governed by local and state guidelines and regulations, which supersede any recommendations in this publication. If properly applied, cutting or pulling can control 90 to 100 percent of the population in the year treatment is applied. Plan on continuing management the following year as more than 50 percent of the controlled population can return, primarily from germinating seeds. New populations, termed satellite or nascent populations, lend themselves to control by hand cutting or pulling.

Mowing can be effective on infestations that are too large for pulling or cutting. Mowing controls garlic mustard by disrupting seed production. Mow 2-year-old plants as low as possible. Time the mowing after the plants have bolted, but before the emergence of flowers. Plants may resprout and flower, but will rarely have time to produce viable seed in the northern region of the Midwestern United States. Monitor populations and repeat mowing if plants resprout and flower in time to produce seed during the growing season. Care must be taken not to mow when mature seeds are present as this will spread the seed and do little to harm the existing population.

Figure 4-9. Garlic mustard "pulls" build awareness of the problem, bring communities together to manage invasive species, and can effectively control localized infestations. Hand-pulling works best when garlic mustard is bolting and the soil is moist. Then it is easy to grasp, and the rooting base of the plant is easily removed. (The Stewardship Network, Ann Arbor, MI)

Mowing will not eradicate first year seedling or rosette plants since their growing points are close to the soil surface, enabling them to resprout and survive the winter to complete their life cycle the following year. While mowing has been reported as an effective means of suppression of 2nd-year flowering plants, it is not known how many years of mowing are required to control a population by depleting the seedbank. If properly applied, mowing can control 70 to 90 percent of the population in the year treatment is applied. Plan on additional management the following year as without additional treatment, one can expect more than 50 percent of the population to return from seedlings and first year rosettes.

Prescribed Fire

Similar to mowing, prescribed fire is a management tactic that controls garlic mustard by disrupting seed production (Figure 4-10). Prescribed fire can either promote or reduce garlic mustard invasion, depending on how it is performed. Ideally, burn in the spring before desirable vegetation begins growing, but after garlic mustard seedlings have emerged. Burning at this time will control seedlings, but survival of second-year plants is variable depending upon fire intensity. Burning can stimulate germination of seedlings, but management of these seedlings after the burn can dramatically reduce the number of garlic mustard seeds in the soil seedbank. A hand-held propane torch can be effective for treating seedlings (Figure 4-11). If properly applied, fire can control 50 to 70 percent of the population in the year treatment is applied. Without treatment the following year, one can expect more than 50 percent of the controlled population to return, primarily from seedlings and first year rosettes.

Figure 4-10. A prescribed fire to control garlic mustard seedlings. (Thomas C. Croker, USDA Forest Service, Bugwood.org)

Figure 4-11. Using a hand-held propane torch to control small patches of garlic mustard. (Great Smoky Mountains National Park Resource Management Archive, USDI National Park Service, Bugwood.org)

Cultural Control

Forests that are healthy through the use of good cultural practices will resist invasion. Use cultural practices to keep a competitive ecosystem that favors the native species in that system, or in the case of disturbance, minimize the time to recovery of the native ecosystem. Disturbed forest canopies with increased light penetration tend to experience increased invasion of garlic mustard. Forested areas are particularly vulnerable to invasion during or after disease or insect outbreaks or timber harvests. These are critical periods that require management to prevent or minimize invasion. If the canopy of a forest becomes disturbed, plant new plants or manage species present to increase light interception and restore the canopy as quickly as possible. Plant species that are adapted to the site paying particular attention to site characteristics such as the dominant soil type, pH, organic matter, water holding capacity, fertility, slope and slope aspect. Focus other management activities (e.g., mechanical or physical control techniques) around these areas of canopy disturbance if invasion occurs for a rapid response. Cultural methods will not quickly control an existing population, but will slow the spread of the current population and potentially prevent future invasion. Other control methods need to be integrated with cultural practices to eradicate an existing population.

Herbicidal Control

Herbicides are effective at controlling garlic mustard, but applications must be timed to the appropriate stage of growth. While some soil-applied herbicides can kill seedlings as they emerge (pre-emergence activity), none are known to provide 100 percent control. Therefore, the most effective are foliar applications of herbicides when garlic mustard has emerged and is actively growing. The proper timing of an application is specific to the active ingredient of the herbicide being used, but typical foliar applications are made to rosette plants in the fall or in the spring before bolting (elongation of shoots that will eventually flower and set seed)

(Figure 4-12). If desirable plants are present, herbicides with no residual activity are often preferred (e.g., glyphosate). These are applied when garlic mustard rosettes are present but desirable plants have not yet emerged (spring) or have gone dormant (fall). Since garlic mustard emerges earlier and goes dormant later than most desirable vegetation, it provides an application window for improved selectivity. Be aware some herbicides have residual activity in the soil after a foliar application that may effect desirable vegetation through uptake by roots or emerging shoots.

These optimal spring and fall timings for garlic mustard control often occur when temperatures are suboptimal for herbicide performance. If daily air temperatures do not rise above 40 °F it is recommended that the maximum label rate be applied to obtain adequate control. Spring applications of non-residual herbicides, if broadcasted, can control emerged seedlings and second-year plants. However, fall applications of these herbicides provide no control of seedlings that emerge the following year. Fall applications of herbicides that have residual soil activity (e.g., metsulfuron) can provide some suppression of seedlings the following spring. Residual control has been highly variable among sites, however, and residual soil activity has never provided more than 80 percent seedling suppression. Applications of foliar herbicides made later in the growing season to bolting or flowering plants can still suppress garlic mustard, but typically higher herbicide rates are required and increased injury to desirable plants growing alongside garlic mustard often occurs. Do not apply an herbicide if immature seed are present

Figure 4-12. Garlic mustard control following a foliar application of glyphosate herbicide to rosettes in the spring (left) compared to an application of glyphosate herbicide to rosettes the previous fall (right). Garlic mustard can quickly reinvade an area treated with a herbicide without soil residual activity such as glyphosate, absent recruitment of a competitive cover of native species. (Mark Renz, University of Wisconsin)

since the herbicide will likely not work fast enough to prevent the seed from becoming viable, and the plant will naturally die on its own after flowering. Apply herbicides directly to individual plants or broadcast herbicide across an infested area. Broadcasted foliar applications are typically the most cost-effective treatment for dense infestations. Use lower rates of herbicide on smaller plants or less dense plant populations and higher rates of herbicide on larger plants or denser plant populations (Table 4-1). Always follow labeled instructions for the herbicide product used and wear appropriate protective clothing when applying (Figure 4-13). Figure 4-14 is a schematic diagram matching management of garlic mustard to the life-stages for best control.

Table 4-1. Application rates and timing, and characteristics of herbicides for control of garlic mustard.[1]

Active Ingredient	Broadcast Rate/Acre	Spot Treat Rate	Application Timing	Potential to Injure Emerged Plants at Application	Residual Activity
Bentazon	16-32 fl oz/A (0.5-1.0 lb a.e./A)	Equivalent to broadcast rates	Rosettes in the fall or spring, or to bolting plants	High, broadleaf plants	None
Glyphosate	0.75-1.5 lb a.e./A	1-3% (0.03-0.09 lb a.e./gal)	Rosettes in the fall or spring, or to bolting plants	High, all plants with green tissue (includes young trees with green bark)	None
Imazapic	10-16 fl oz/A (0.15-0.25 lb a.e./A)	0.25-1.0% (0.005-0.02 lb a.e./gal)	Rosettes in the fall or spring, or to bolting plants	High, cool season grasses and some broadleaf plants	1-6 months
Imazapyr	48-64 fl oz/A (0.75-1.0 lb a.e./A)	0.5-1.0% (0.01-0.02 lb a.e./gal)	Rosettes in the fall or spring, or to bolting plants	High, all herbaceous and woody plants	Can be >1 year
Metsulfuron	0.25-1.0 oz/A (0.15-0.6 oz a.i./A)	0.04 oz/gal (0.02 oz a.i./gal)	Rosettes in the fall or spring, or to bolting plants	High, some herbaceous and woody broadleaf plants	One to many months, depending on soil pH
Sulfometuron	0.25-1.0 oz/A (0.2-0.75 oz a.i./A)	Equivalent to broadcast rates	Rosettes in the fall or spring, or to bolting plants	High, some plants depending on rate	One to many months, depending on soil pH
Sulfosulfuron	1.0-2.0 oz/A (0.75-1.5 oz a.i./A)	0.01-0.02 oz/gal (0.008-0.02 oz a.i./gal)	Rosettes in the fall or spring, or to bolting plants	High, broadleaf plants and cool season grasses	Can be >1 year
Triclopyr	16-32 fl oz/A (0.5-1.0 lb a.e./A)	1-2% (0.04-0.08 lb a.e./gal)	Rosettes in the fall or spring, or to bolting plants	High, herbaceous and woody broadleaf plants	Weeks to a month
2,4-D	1-2 lb a.e./A	Equivalent to broadcast rates	Rosettes in the fall or spring, or to bolting plants	High, broadleaf plants	Days to a few weeks

[1] Reference to commercial products is made with the understanding that no discrimination is intended and no endorsement by the U.S. Forest Service or the authors of this chapter is implied. Always read and follow the herbicide label instructions for specific use recommendations and requirements.

Figure 4-13. Apply herbicides according to the label of the product used. Always read and follow label instructions for specific use recommendations and requirements. (Roger Becker, University of Minnesota)

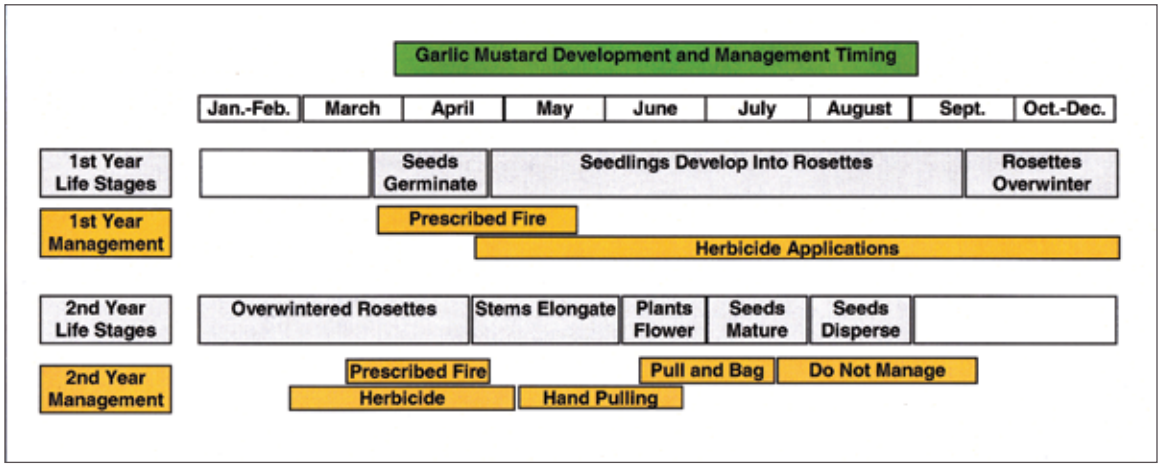

Figure 4-14. Schematic showing growth and development of garlic mustard and windows of opportunity for management. Note that many sites have predominately one life-cycle form present in a given year, and a few have both first and second year life-cycles present at the same time. Though best applied during the growing season, in the warmer regions of garlic mustard infestations in the Upper Midwest, herbicide applications have been successful during winter months providing sites are free of snow cover and air temperatures permit operation of spray equipment. Prescribed burns are most successful when seedlings are predominant in Year 1. If second year rosettes are predominant, prescribed burns have been variable in controlling garlic mustard.

Conclusion

Garlic mustard is found in the northeastern, midwestern, and western regions of the United States typically in disturbed woodlands, but also can be found in high quality woodlands, and in upland and floodplain forests. Native herbaceous cover can decline in invaded sites. Garlic mustard is regulated in several states, often requiring control. Control methods are available for small and larger infestations, but garlic mustard and the sites it invades are best suited for management with biological control agents. Research is underway to develop biological controls, but in the meantime, we have discussed the other options to control garlic mustard. Integrated control strategies will be required for success beyond eradicating isolated, local infestations. Eventually we anticipate biological control will be an essential component of these integrated control strategies.

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APPENDIX: MONITORING GARLIC MUSTARD BIOCONTROL AGENTS

Monitoring Garlic Mustard Biocontrol Agents

The purpose of monitoring is to evaluate how effective biocontrol insects are as a management tool for garlic mustard. Specifically, when land managers implement the monitoring protocol, they measure the number of seedling and adult plants, plant heights and number of seed capsules in the same plot over time. The number and abundance of other plant species are also recorded. These measurements document over time what is happening to garlic mustard and other plant species in the monitoring plots. The desired outcome is to see the population of garlic mustard decrease and the population of native species increase. Ideally, the monitoring plots should be established two to three years prior to the release of biocontrol insects to provide a "before and after comparison" of the effectiveness of the biocontrol insects.

Please refer to the following garlic mustard monitoring protocol for specific instructions on how to select monitoring sites and collect data. This standardized monitoring protocol was developed by the Ecology and Management of Invasive Plants Program at Cornell University. The protocol and accompanying forms are included in the subsequent pages. The protocol can be accessed at: http://www.invasiveplants.net.

Garlic Mustard Monitoring Protocol

Garlic Mustard Monitoring Protocol
June 2003

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122E Fernow Hall, Cornell University, Ithaca, New York 14853 USA
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with

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Contents:

Introduction

Garlic mustard (*Alliaria petiolata*) is a biennial European herb that invades forested communities in North America, especially in the central and eastern part of the US and adjacent Canada. A biological control program targeting garlic mustard was initiated in 1997. Four weevils (*Ceutorhynchus* spp.) including two stem-feeders, a seed-feeder, and a root-crown feeder, are under study, and releases of the first insects are anticipated to begin in 2004-2005. The following guidelines are intended to help monitor the abundance of both garlic mustard and the biocontrol insects, and assess the long-term impact of biological control. The protocol can also be used to detect change in herbaceous vegetation relative to change in garlic mustard. For maximum information, monitoring should ideally be initiated one or more years before biocontrol organisms are released: the resultant "pre-release" data will provide a baseline to assess "post-release" changes. For best results, monitoring should be conducted twice a year; in June to assess garlic mustard density and seed production, and in October to assess rosette abundance and external evidence of insect feeding.

Garlic mustard is an obligate biennial and can only spread by seeds; therefore the goal of biocontrol is population reduction, achieved by reducing total seed production. Garlic mustard seeds germinate in early spring, and form a basal rosette by June. Plants remain as rosettes through the winter, and produce flower stalks the following spring, usually blooming in April-May, depending on the location and temperature regime. Seeds are produced in siliques (linear pods) 4-8 weeks later, usually in June-July. Garlic mustard seeds live ≥5 years in the seedbank.

The four weevils are difficult to observe directly. Larvae induce most of the damage, but because they feed inside the plant (in seeds, stems, leaves, and root crowns) they are not usually observed. Adults are small (2mm) and black, and feed on stems and petioles, leaving a "scraping" mark. In addition, all four weevils produce a characteristic "window pane" feeding pattern that can be easily observed on the leaves. Under heavy attack by one or more of the weevil species, garlic mustard plants become shorter and less robust, often have tip dieback, and produce fewer flowers and siliques.

Site Selection and Quadrat Setup

Select a monitoring site that will be protected from other uses that may jeopardize your continued monitoring. It is imperative that the monitoring site be protected from all management that could damage the insects or the garlic mustard plants, in particular burning, herbicide application, and pulling of plants. We do not know how the weevils will respond to fire or flooding, and in the initial establishment phase a fire (which may burn the insects), flooding (which may drown the insects), or removal of garlic mustard plants (with the insect larvae hidden inside) could eradicate small populations. The study site should be sufficiently distant from a trail to limit vandalism.

The study site should contain a well-established garlic mustard population (≥0.5 ha). Garlic mustard does not need to form a continuous carpet, but should be present throughout the study area every year, as rosettes and/or adult plants. To determine response of the associated groundlayer vegetation to the anticipated reduction in garlic mustard, it would be beneficial to locate the study site in an area with native vegetation. Avoid establishing plots in a site where garlic mustard has been present for <3 years, as the population should be large enough with a well-established seed bank to maintain a reliable food source for the weevils.

We recommend an open-ended quadrat frame with the fourth side removable. Construct the quadrat frame from a 10' length of 1/2" diameter PVC or CPVC pipe, 4 right-angle elbows of the same diameter, and PVC or CPVC glue. The inside dimensions of the finished frame should measure 1 m by 0.5 m. After cutting the conduit to the correct lengths, glue two elbows to each 1 m long piece (make sure the elbows are perfectly aligned to each other). Set one piece aside (this will be the fourth side of the frame). Glue the elbows of the other 1 m long piece to two 0.5 m long pieces to form the open "U" shaped frame. Using a permanent marker, mark 1 dm intervals on each side to assist with estimating percent cover. In the field, slide the open-ended U-shaped frame along the ground to avoid disturbing the vegetation. Then, attach the fourth side to the frame.

Materials needed: 0.5 m^2 quadrat frame, permanent marker, GPS unit (if available), 50 m tape, conduit and hammer, Form 1, pencils and clipboard, camera.

We recommend a total of 20 permanent 0.5 m^2 (0.5 m x 1.0 m) quadrats, spaced ≥10 meters apart. This allows statistical analysis of the expected decline in garlic mustard, and provides sufficient locations to ensure that garlic mustard is present as adult or seedling in most quadrats each year. (In general, once garlic mustard is present, it will continue to be present almost every successive year in that location, although densities may vary significantly.)

Quadrats can be located in several ways: along two parallel transects, in 4 rows of 5 quadrats, or completely randomly. Relocating the quadrats is easier using parallel transects, and this method will be outlined here. Randomly establish two parallel transects, at least 100 m long and ≥10 meters apart. Locate quadrats at fixed intervals ≥10 meters apart along each transect. ALL quadrats must contain garlic mustard; if necessary, shift the location of the quadrat so that garlic mustard covers at least 25% of the quadrat. In sites where both age classes (adults and rosettes) are present, makes sure that these age classes are represented in the 20 quadrats. Record the position and numbers of quadrats on the vegetation map on Form 1. Use GPS coordinates for easy relocation in dense vegetation. Locate permanent photo-points and take photographs of study site, including one or more quadrats.

To establish the permanent quadrats, first locate the position of each quadrat, then place the quadrat frame on the ground, and mark the four corners by driving a 30-50 cm long and 1/2" diameter plastic or aluminum conduit into the ground. This will allow exact placement of the quadrat in future years. Write the quadrat number on each conduit with a permanent marker or other means. In areas with high public use and potential vandalism, conduits should be short and difficult to see. Obvious markings can attract vandalism and "helpful protectors" who remove the conduits. Avoid trampling vegetation in and near the quadrat.

Data Collection

Assessment of the plants and insects will occur twice each growing season. Four data forms are provided and described in detail on the following pages: Site location (Form 1); Summer monitoring (Forms 2a and 2b), and Fall monitoring (Form 3). In addition, "Quick Reference" sheets are provided to use in the field. To assess the growth and abundance of garlic mustard, and growth of other groundlayer species, a series of estimates are used. All estimates reflect the growth within each quadrat and NOT of the site as a whole, or plants near but not in the quadrat.

Form 1: Garlic Mustard Biocontrol Monitoring (Site Location)

FORM 1: GARLIC MUSTARD biocontrol monitoring (site location)

Site Name:_____ State:_____ GPS: N _____° _____'

Town: _____ County: _____ W _____° _____'

Date: _____ _____ _____
year *month* *day*

CONTACT PERSON:

Name: _____
Address _____
City: _____
State: _____
Phone: _____ - _____ - _____
e-mail: _____

LEGAL LANDOWNER:

Name: _____
Address _____
City: _____
State: _____
Phone: _____ - _____ - _____
e-mail: _____

SITE CHARACTERISTICS:

Habitat type: ___Upland forest ___Floodplain forest ___Field ___Roadside ___ Other _____

Road Map to Site

N

Site and Vegetation Map
Site

N

INSECT RELEASE HISTORY:

Date (year-month-day)	Species	Number and Stage (egg/larvae/adult)	Position of Release On Map (1,2,3,4…)

Form 1: Site Location, Background Information

Site Location:

Enter name of the site (for example: Fillmore Glen State Park, north unit; be as specific as possible); and the location (town, county, state, etc.). If Global Positioning System (GPS) coordinates are available, enter this information in the spaces provided.

Contact Person and Legal Landowner:

Provide the name, address, telephone number and email address of a contact person. This person can be the releaser or a local contact. If the contact person is not the legal landowner, please provide this information in addition.

Site Characteristics:

Check one of the options or provide specifics if none of the options are applicable.

Road Map:

Photocopy a road map (preferably a county road map) to the site from a road atlas or MapQuest and paste it into the space provided. Mark the location of the site. An arrow should indicate North on the map. If a written description of directions is needed, attach the description to this page. Be specific: assume the reader has never been to the locale. Attach additional pages if needed.

Site and Vegetation Map:

Provide a map of the area, or copy of an aerial photo, with access roads, approximation of garlic mustard infestation outlined, other vegetation types, trails, creek etc. An arrow should indicate North on the map. Paste map into space provided. Once insects are available for release, indicate with Arabic numerals (corresponding to numbers under Insect Release) points of single or multiple control agent releases.

Photographs of changes in vegetation over time are a powerful tool for presentations or to reinforce quantitative data. One or several permanent photo-points should be marked in the monitoring area using flagging tape or stakes driven into the ground. The position of these photo-points should be indicated on the vegetation map, and the direction in which the picture was taken should also be indicated with an arrow. Take pictures once a year at the same time of the year. The showy flowers of garlic mustard suggest taking pictures at the peak of the flowering period. Make sure to record which photos were taken from which location and when.

Insect Release History:

Document date, control agent species, life stage (adults, eggs or larvae), the number of individuals released, how individuals were released, time of day and weather conditions. Code each release with an Arabic numeral and insert number at the release point on the vegetation map (see above).

Form 2a: Garlic Mustard Biocontrol Monitoring (Summer)

FORM 2a:

GARLIC MUSTARD BIOCONTROL MONITORING (Summer)

SITE: _____ STATE: _____

DATE: _____ _____ _____ GPS: N ____° _____'

year month day W ____° _____'

please send a copy of *Investigators: Last name First name*

the completed form to: _____ _____

Dr. Bernd Blossey, _____ _____

Fernow Hall, Cornell Univ. _____ _____

Ithaca, NY 14853

Chart A: Percent cover & Leaf Attack

A	<1%
B	1-5%
C	6-25%
D	26-50%
E	51-75%
F	76-95%
G	>95%

Chart B: Estimated Density

I	1-10
II	11-25
III	26-100
IV	100-500
V	501 - 1000
VI	1001 - 2000
VII	>2000

Notes: 0.5 m^2 quadrat

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20
PERCENT COVER : (Use Chart A)																				
All Garlic Mustard																				
Adult GM																				
Seedling GM																				
All other vegetation																				
LEAF ATTACK: (Use Chart A)																				
% removed																				
Check if present: Leaf miner																				
Windowpane																				
Edge feeding																				
Holes																				
Spittle bug																				
Scale																				
Browse																				
Disease																				
Other																				
ROSETTE DENSITY: (use Chart B if too numerous to count)																				
PERCENT COVER OF: (Use Chart A or actual estimates that total 100%)																				
Soil																				
Wood																				
Leaf																				
Rock																				
LITTER DEPTH (cm)																				
SPECIES PRESENCE or % COVER (Use Chart A or actual estimates; total may exceed 100%)																				

Form 2b: Garlic Mustard Biocontrol Monitoring (Adult Height and Number of Siliques)

FORM 2B GARLIC MUSTARD BIOCONTROL MONITORING (Adult height and # siliques)

In each 0.5m quadrat, record:
Height (cm) and number siliques
of every adult garlic mustard
ex: 34-16 = 34 cm tall, 16 siliques

SITE: ____________________ STATE: __________

DATE: ________ ________ ________ GPS: N ______° ____________'
year *month* *day* W ______° ____________'

Notes:

Investigators: Last name *First name*

please send a copy of the completed form to:
Dr. Bernd Blossey, Fernow Hall,
Cornell University, Ithaca, NY 14853

QUADRAT #

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Instructions for Form 2a: Garlic Mustard Biocontrol Monitoring (Summer)

Materials needed: 1 meter stick; 0.5 m^2 quadrat frame; data sheets (Form 2a and several copies of Form 2b), pencils and a clipboard, camera, permanent marker to refresh quadrat numbers.

Summer data should be recorded when garlic mustard has completed flowering and has fully formed green siliques, but before the siliques turn brown and start to disperse seed. In northern locales this is usually in mid- to late June, while in southern locales this may be as early as mid-May. Begin with quadrat 1 and fill out both Form 2a, and then Form 2b (if adult garlic mustard are present), then move to the next quadrat. Use new data sheets each year. Summer monitoring is easier with two people, one to make the observations and the other to record data.

1. Before collecting data, please record in spaces provided: site name, date (year, month, day), and the names of the observers (last name, first name), as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. Take photographs at permanent photo points.
2. First, slide the frame into position. Standing over the frame, and looking straight down, estimate how much of the quadrat is covered by garlic mustard and, independently, how much is covered by all other vegetation. Use cover estimates in Chart A, or a finer scale (for example: Present; <1% cover; 2-5% cover, and in 10% increments thereafter; i.e., >5-15%, >15-25%, etc.). If both garlic mustard and other vegetation are abundant, these estimates may total >100%, due to layering. Next, focus only on garlic mustard. If adult garlic mustard plants are uncommon or small, or if only seedlings are present, you may need to carefully move vegetation to determine how much garlic mustard is actually present in each age class. Estimate the actual percent cover (using the cover classes in Chart A) of all garlic mustard; of only adult garlic mustard; and of only seedling garlic mustard. Often, adult garlic mustard will overtop seedling garlic mustard, and their combined cover will therefore exceed the "all garlic mustard" cover. That is okay, as we are interested in monitoring how much of each size class is present.
3. Next, scan the garlic mustard for any damage to the leaves, shoots, or siliques. After insect release, look especially for the "window pane" feeding pattern of the biocontrol weevils. Some windowpane feeding is already present but in low abundance. This may originate from native species or accidental introductions. Estimate the percent leaf area of garlic mustard removed by insect feeding integrated over the entire quadrat, using Chart A. Initially, this will be very low or non-existent. After weevil populations build up you may find as much as 50% of the leaves are damaged. Next, indicate what type of damage is visible, such as leaf miners, deer browse, disease, etc., using a "check" or "+" in the appropriate box. This may be omitted if feeding damage is very low (<1%) and not clearly discernible. Make a note if some other type of damage is present, and include a sketch or photograph of the damage.

   Estimating the amount of leaf area removed by insect feeding will initially be difficult because you need to scan through the vegetation, and leaves and plants will show different amounts of feeding damage, but you will get better over time. Experienced observers should introduce new personnel to the methods and to their assessments to increase the accuracy of reported results. We expect to observe large differences over time, especially following high abundance of *Ceutorhynchus* larvae and adults.

4. Count the number of seedlings. If seedling density is very high, count the number of seedlings in a section of the quadrat, and then use this density to estimate the total number of seedlings in the quadrat. If time does not allow counting individuals or a subset of the population, use Chart B to estimate seedling density. Estimations are never as accurate or powerful as actual counts, so count actual seedling density whenever possible.
5. Looking below all vegetation, estimate the cover of soil, wood, leaves, and rock using Chart A or actual percent cover: This should total 100%. Often, sites with abundant garlic mustard have little leaf litter.
6. Measure litter depth to the closest cm in the center of each half-quadrat.
7. If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat. Use cover estimates in chart A, or a finer scale (for example: Present; <1% cover; 2-5% cover, and in 10% increments thereafter; i.e., >5-15%, >15-25%, etc.).
8. Other Observations: Record any general observations or useful information about the site; windfall, flooding, deer herbivory, insects, etc. Most of this information will be difficult to evaluate, so do not spend too much time on this.

Instructions for Form 2b: Garlic Mustard Biocontrol Monitoring (Adult Height and Number of Siliques)

Use this form when adult garlic mustard are present in the quadrat. Write the quadrat number in the appropriate box at the top of the sheet. Then, beginning at one corner of the quadrat and working systematically across the quadrat, measure the height in cm, and count the number of siliques, of each garlic mustard stem. Record this information in the appropriate boxes below the quadrat number. Record each stem that originates from the ground as a separate stem, even if you suspect that some stems may originate from a single root. When a stem branches >2cm above the ground, then the branch is counted as part of the single stem. Also, look carefully for short, frequently sterile stems. These small plants are usually overlooked, but it is important to record their presence. Record every stem, using several columns if necessary, and writing the quadrat number above each column. To be counted, a stem must originate within the quadrat; if it originates under the frame, then it is not recorded.

If you see overt damage or anything unusual on a stem, you can record this in the same box, by using an asterisk, or a letter, or other symbol, and defining it in the box labeled “notes”. For example, if you see leaf mining on a stem 30 cm tall with 7 siliques, you could record this by writing “30-7 *” on the data sheet and writing in the notes box “* = leaf mining”.

It is important to measure every stem in the quadrat, even if some quadrats have numerous plants. We anticipate that under heavy insect attack garlic mustard plants will decrease in density, height, and silique production, and will also change in plant architecture and produce more small side branches. Therefore it is very critical to have accurate baseline data to compare to “post-release” data, and accurately assess the impact of the weevils on garlic mustard.

Forms 2a and 2b: Summer Monitoring Quick Reference Guide

Materials needed: 1 meter stick; 0.5 m^2 quadrat frame; data sheets (Form 2a and several copies of Form 2b); pencils and clipboard, camera

1. Take photos at permanent photo points.
2. Walk to quadrat 1. Slide quadrat frame into location. Fill out Form 2a first, then Form 2b.

Form 2a:

3. Write Site name, date, and names of investigators, state, and GPS coordinates if known.
4. Estimate Vegetation Cover: Use Chart A.
   a. Estimate total vegetation cover (maximum 100%). Write "0" if no vegetation present.
   b. Estimate total garlic mustard cover. Write "0" if no garlic mustard present.
   c. Estimate cover of adult garlic mustard. Write "0" if no adult garlic mustard present.
   d. Estimate cover of seedling garlic mustard. Write "0" if no seedling garlic mustard present.
5. Look for evidence of leaf attack.
   a. Estimate percent of garlic mustard leaf area removed by insect feeding, estimated over the entire quadrat (use Chart A).
   b. Indicate type of damage visible and/or insects present in quadrat: check or write "+" for each type present.
6. Count the number of garlic mustard seedlings present in the quadrat. If too many to count, estimate density using Chart B.
7. Measure litter depth to the nearest 0.5 cm in the center of each half-quadrat.
8. Looking below all vegetation, estimate percent cover of bare soil, leaf litter, down wood, and rock. Use Chart A or visually estimate so all 4 categories add up to 100%.
9. Optional: Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Chart A or other scale.
10. If adult garlic mustard are present in the quadrat, fill out Form 2b.

Form 2b:

11. Write Site name, date, and names of investigators, state, and GPS coordinates if known.
12. Write quadrat number at top of the column. Start at one end of the quadrat and for each adult garlic mustard in the quadrat, record the:
    a. Height (in cm) of stem, measured to the top of the growing point.
    b. Number of siliques (seedpods). Count only siliques that have at least one seed; do not count very small or empty siliques.
13. After completing Forms 2a and 2b for quadrat 1, proceed to quadrat 2, and repeat the process (steps 3-11, above). Continue until all quadrats have been located and recorded.

Form 3: Garlic Mustard Biocontrol Monitoring (Fall)

FORM 3:
GARLIC MUSTARD BIOCONTROL MONITORING (Fall)

SITE: ______________________ STATE: __________

DATE: ________ ________ ____ GPS: N _____°_________'
year *month* *day* W _____°_________'

please send a copy of the completed form to:
Dr. Bernd Blossey,
Fernow Hall, Cornell Univ.
Ithaca, NY 14853

Investigators: Last name *First name*

Chart A: Percent cover & Leaf Attack

A	<1%
B	1-5%
C	6-25%
D	26-50%
E	51-75%
F	76-95%
G	>95%

Chart B: Estimated Density

I	1-10
II	11-25
III	26-100
IV	100-500
V	501 - 1000
VI	1001 - 2000
VII	>2000

Notes: 0.5 m^2 quadrat

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20
PERCENT COVER : (Use Chart A)																				
Garlic Mustard																				
All other vegetation																				
WEEVILS (Use Chart B if too numerous to count in one minute)																				
Number observed																				
LEAF ATTACK: (Use Chart A)																				
% removed																				
Check if present: Leaf miner																				
Windowpane																				
Edge feeding																				
Holes																				
Spittle bug																				
Scale																				
Browse																				
Disease																				
Other																				
ROSETTE DENSITY: (use Chart B if too numerous to count)																				
PERCENT COVER OF: (Use Chart A or actual estimates that total 100%)																				
Soil																				
Wood																				
Leaf																				
Rock																				
LITTER DEPTH (cm)																				
SPECIES PRESENCE or % COVER (Use Chart A or actual estimates; total may exceed 100%)																				

Instructions for Form 3: Garlic Mustard Biocontrol Monitoring (Fall)

Materials needed: 1 meter stick; 0.5 m^2 quadrat frame; data sheet (Form 3), pencils, clipboard.

These are similar measures to those collected in summer, except that flower stem density and height are not measured. Because only one size class (rosette) is present, the autumn monitoring takes less time than the spring monitoring, and can be conducted by one individual. Monitoring should occur about the time deciduous trees lose their leaves. Indicate in the "notes" box whether trees have lost some, all, or none of their leaves (this helps with interpretation of leaf litter depth, and of garlic mustard percent cover, as small rosettes are often covered by new leaves and will be missed in sampling).

1. First, if insects have been released, approach the quadrat slowly and observe for weevils. Typically, only the rosette-feeder *C. scrobicollis* will be active at this time. You may see these small (2 mm) black insects near the center of a rosette.
2. Next, slide the frame into position. If insects have been released, count number of weevils observed in one minute. As long as you are able to count the exact number of weevils, please provide that number. If the allowed search time does not enable you to count all present individuals, use estimates in Chart B. Standing over the frame, and looking straight down, estimate how much of the quadrat is covered by garlic mustard and, independently, how much is covered by all other vegetation. Use cover estimates in Chart A, or a finer scale (for example: Present; <1% cover; 2-5% cover, and in 10% increments thereafter; i.e., >5-15%, >15-25%, etc.). If rosettes are uncommon or small, or tall vegetation is present, you may need to carefully move vegetation to determine how much garlic mustard is actually present. If both garlic mustard and other vegetation are abundant, these estimates may total >100%, due to layering. That is okay, as we are interested in monitoring how much of each is present.
3. Next, scan the garlic mustard for any damage to the leaves, shoots, or siliques. After insect release, look especially for the "window pane" feeding pattern of the biocontrol weevils. Some window pane feeding is already present but in low abundance. Autumn is when this feeding pattern is most distinct if the rootcrown feeder *C. scrobicollis* is present. Estimate the percent leaf area of garlic mustard removed by insect feeding integrated over the entire quadrat, using Chart A. Initially, this will be very low or non-existent. After weevil populations build up you may find as much as 50% of the leaves are damaged. Next, indicate what type of damage is visible, such as slugs (round holes >1 cm diameter), deer browse, disease, leaf miners, etc., using a "check" or "+" in the appropriate box. This may be omitted if feeding damage is very low (<1%) and not clearly discernible. Make a note if some other type of damage is present, and include a sketch or photograph of the damage.

   Estimating the amount of leaf area removed by insect feeding will initially be difficult because you need to scan through the vegetation, and leaves and plants will show different amounts of feeding damage, but you will get better over time. Experienced observers should introduce new personnel to the methods and to their assessments to increase the accuracy of reported results. We expect to observe large differences over time, especially following high abundance of *Ceutorhynchus* larvae and adults.
4. Count the number of rosettes. If rosette density is very high, count the number of rosettes in a section of the quadrat, and then use this density to estimate the total number of rosettes in the quadrat. If time does not allow counting individuals or a subset of the population, use Chart B to estimate rosette density. Estimations are never as accurate or powerful as actual counts, so count actual rosette density whenever possible.

5. Looking below all vegetation, estimate the cover of soil, wood, leaves and rock using Chart A. This should total 100%. Often, sites with abundant garlic mustard have little leaf litter.
6. Measure litter depth to the closest cm in the center of each half-quadrat.
7. If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat. Use cover estimates in chart A, or a finer scale (for example: Present; <1% cover; 2-5% cover, and in 10% increments thereafter; i.e., >5-15%, >15-25%, etc.).
8. Other Observations: record any general observations or useful information about the site; windfall, flooding, deer herbivory, insects, etc. Most of this information will be difficult to evaluate, so do not spend too much time on this.

Form 3: Fall Monitoring Quick Reference Guide

Materials needed: 1 meter stick; 0.5 m^2 quadrat frame; data sheet (Form 3); pencils and clipboard; stop watch (after insect release)

1. Write Site name, date, and names of investigators, state, and GPS coordinates if known, at the top of Form 3.
2. Walk to quadrat 1. If insects have been released:
   a. Approach the quadrat slowly and observe for weevils. Slide quadrat frame into location.
   b. Count number of weevils seen in the quadrat in one minute (use stopwatch). Record actual number of weevils seen, or use Chart B to estimate density.
3. Slide quadrat frame into location.
4. Estimate Vegetation Cover: Use Chart A.
   a. Estimate total vegetation cover (maximum 100%). Write "0" if no vegetation present.
   b. Estimate total cover of rosette garlic mustard. Write "0" if no garlic mustard present.
5. Look for evidence of leaf attack.
   a. Estimate percent of garlic mustard leaf area removed by insect feeding, estimated over the entire quadrat (use Chart A).
   b. Indicate type of damage visible and/or insects present in quadrat: check or write "+" for each type of damage or insect seen.
6. Count the number of garlic mustard rosettes present in the quadrat. If too many to count, estimate density using Chart B.
7. Measure litter depth to the nearest 0.5 cm in the center of each half-quadrat.
8. Looking below all vegetation, estimate percent cover of bare soil, leaf litter, down wood, and rock. Use Chart A or visually estimate so all 4 categories add up to 100%.
9. Optional: Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Chart A or other scale.
10. After completing Form 3 for quadrat 1, proceed to quadrat 2, and repeat the process (steps 2-9). Continue until all quadrats have been located and recorded.

Report 2010–12

Biological control of common buckthorn, *Rhamnus cathartica*

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Minnesota Department of Natural Resources

Table of Contents

Summary

1. Following a reassessment of the potential for biological control of *Rhamnus cathartica*, work in 2010–12 focussed on assessing the feasibility of using the psyllid *Trichochermes walkeri* and the seed-feeding midge *Wachtiella krumbholzi* as biological control agents, and determining the causes of the high levels of seedling mortality and post-dispersal seed mortality of *R. cathartica* observed in Europe as compared to North America.

2. In Europe, *R. cathartica* trees were found to be infected with '*Candidatus* Phytoplasma rhamni' ('*Ca.* Phytoplasma rhamni') at almost all surveyed localities, confirming previous reports of host association of this phytoplasma with *R. cathartica,* although the presence of witches' broom symptoms were not observed. Phytoplasma was not detected in any of the other *Rhamnus* species analysed, which suggests a very specific host association of this phytoplasma with its plant host, and also a very specific relationship between the insect vector of the pathogen and its host plant.

3. Work on '*Ca.* Phytoplasma rhamni' in North America has been carried out by Dr Roger Becker and Dr Dimitre Mollov, University of Minnesota, St Paul, USA. '*Ca.* Phytoplasma rhamni' has not been detected in 75 *R. cathartica* populations from North America suggesting either that the phytoplasma has not been introduced in the exotic range of its host plant, or that the absence of a suitable vector for phytoplasma propagation constrained its establishment in North America.

4. The absence of symptoms on all phytoplasma-infected trees could be an indication of a commensal relationship between the phytoplasma and its plant host, i.e. the absence of negative effects which would lead to the development of a disease in the host plant. Plants with asymptomatic presence of phytoplasma are considered to be a wild reservoir of the pathogen, since they are not affected by its presence.

5. *Trichochermes walkeri* proved to be infected with '*Ca.* Phytoplasma rhamni' at a very high rate in almost all sampled localities. Transmission trials strongly suggest that *T. walkeri* is not a vector of '*Ca.* Phytoplasma rhamni'. *Trichochermes walkeri* acquires the phytoplasma during feeding on infected plants, but it is not capable to re-inject the phytoplasma during feeding. The presence of phytoplasma in *Cacopsylla rhamnicola* and *Trioza rhamni* adults is reinforcing the need for elucidating the potential role of these psyllids in phytoplasma infection of *R*. *cathartica*.

6. To date, we have recorded the seed-feeding midge *W. krumbholzi* at most *R. cathartica* sites where we have looked for its presence; we found it at ten sites in Serbia, six sites in Austria, three sites in western Switzerland and two sites in southern Germany. Midge larvae have also been discovered in the fruits of *R*. *saxatilis* ssp. *tinctorius* at one site in Serbia, where *R. cathartica* also occurs. Based on the mitochondrial COI (cytochrome c oxidase) gene, midges from *R. cathartica* and *R. saxatilis* ssp. *tinctorius* are clearly two closely related but distinct species. This further confirms the likely high degree of host specificity of *W. krumbholzi.* Since we have not succeeded so far in obtaining even reproducing trees of the host, *R.*

cathartica, when grown in pots, we are doubtful whether it will be feasible to successfully screen *W. krumbholzi* in the near future.

7. An impact study of the effect of leaf galling by *T. walkeri* on eight-month-old *R. cathartica* seedlings was set up in August 2011. A total of 714 eggs were laid on infected trees. However, in 2012, no galls were recorded and the test was terminated without having obtained conclusive results.

8. We did not find evidence of negative plant–soil feedback by mature *R. cathartica* on conspecifics that could explain low seedling numbers of *R. cathartica* in the native range. There was however a positive plant–soil interaction in the rate of seedling emergence. A small difference in the number of days to seedling emergence probably explains most of the variation in seedling growth.

9. Due to the difficulties surrounding currently studied agents and the low probability of finding additional potential agents, it has been decided that the project will be stopped. A publication summarizing main results will be prepared until 2013.

1. Introduction

Rhamnus cathartica L. (common buckthorn) is a shrub or small tree native to much of Europe and western Asia that has successfully invaded many habitats in North America including abandoned agricultural fields, hedgerows, forest, field and wetland edges, and occasionally contiguous forest habitats (Kurylo *et al.*, 2007).

Rhamnus cathartica was introduced to North America as an ornamental shrub in the early 1800s and was originally used for hedges, farm shelter-belts and wildlife habitats (Gourley, 1985; Randall and Marnelli, 1996; Gale, 2001). It is now naturalized throughout the upper mid-western and north-eastern USA and the maritime provinces of Canada.

Rhamnus cathartica is bird-dispersed and dioecious (Godwin, 1943). It has a wide habitat tolerance but grows most quickly in areas with more light if moisture is not limiting (Knight *et al.*, 2007). The positive association between availability of light and seedling density in North American forest habitats also shows the importance of canopy openings in colonization by *R. cathartica* (McCay *et al.*, 2009).

Fruit production by *R. cathartica* in North America has been described as “very prolific” and “aggressive” (Knight *et al.*, 2007). As expected from the prolific fruit production and high germination rates of *R. cathartica*, high densities of seedlings may be found near parent shrubs in invaded areas (see (Knight *et al.*, 2007). While average number of seedlings was greater than 100/m^2 beneath a dense *R. cathartica* stand in Saskatchewan, Canada, we have only observed very low seedling density in Europe. A study in a plantation in England, where all mature *R. cathartica* shrubs were known to be reproducing yearly, found only 6.2 seedlings/m^2 under conspecific shrubs (Kollmann and Grubb, 1999).

Research to develop biological control for buckthorns was initiated in 1964 and preliminary screening tests were conducted in 1966–67 (Malicky *et al.*, 1970). A new programme was started in 2001 and has taken into consideration increasing concerns over potential non-target impacts of biological control agents and greater demands for high levels of specificity (Louda *et al.*, 1997; Pemberton, 2000).

Over 30 specialized arthropod species have been recorded on *R. cathartica* in Europe, including 21 Lepidoptera, six Hemiptera, two Diptera, one Coleoptera and three Acari. Less is known about fungal pathogens associated with this species. Based on a literature search and evaluation of herbarium records, a couple of potentially specific fungal pathogens are recorded on *R. cathartica,* which may too cause considerable damage to their host plant in the native range (Gassmann *et al*., 2001).

A literature review has indicated that Lepidoptera have been one of the least successful taxonomic groups for the biological control of shrubs and trees (Gassmann *et al*., 2010). In addition, the seven Lepidoptera we have investigated so far are either not sufficiently specific or very difficult to test (Gassmann *et al.*, 2008). Also, the only specialized beetle known on

buckthorn in Europe, the stem-boring longhorn beetle, *Oberea pedemontana* (Coleoptera: Cerambycidae), is not specific at the genus level.

Based on the results to date, the next best group to consider is the sap suckers. Nine species have been recorded on *R. cathartica* in Europe including three psyllids – *Trichochermes walkeri* (Hemiptera: Triozidae), *Cacopsylla rhamnicola* (Hemiptera: Psyllidae) and *Trioza rhamni* (Triozidae) – one Miridae (Hemiptera) and three Eriophydidae (Acari). With the exception of one species inducing leaf erinea on *R. cathartica*, none of the eriophyid mites has been observed on buckthorn in past surveys. The leaf-margin gall psyllid *Trichochermes walkeri* is currently being evaluated.

The detection of '*Candidatus* Phytoplasma rhamni' ('*Ca.* Phytoplasma rhamni') in *T. walkeri* adults in 2009 raises several questions that need to be addressed before further considering sap suckers for biological control of *R. cathartica*: (i) is the phytoplasma '*Ca.* Phytoplasma rhamni' common on *R. cathartica* in Europe, (ii) does '*Ca.* Phytoplasma rhamni' already occur in North America, and if yes, what is the vector, (iii) does the phytoplasma occur on other *Rhamnus* species in Europe, (iv) does *T. walkeri* transmit the phytoplasma, and if not, what is the vector, and (v) is '*Ca.* Phytoplasma rhamni' specific to *R. cathartica* as is suggested in the literature?

Another important group of potential agents, given the high seed output of *R. cathartica* in North America, are the seed feeders. Two midge (Diptera: Cecidomyiidae) species and two Lepidoptera are known to attack the fruits of *R. cathartica* in Europe (Gassmann *et al.*, 2001). One midge, *Wachtiella krumbholzi*, is under evaluation. We have not found the second midge species, *Lasioptera kosarzewskella*, or the two lepidopteran species, *Sorhagenia rhamniella* (Cosmopterigidae) and *Hysterosia sodaliana* (Tortricidae), which in addition do not appear to be genus specific according to the literature. *Wachtiella krumbholzi* is therefore the only available potential seed feeder for biological control of *R. cathartica* but the feasibility of host-range testing still needs to be addressed.

Following recommendations from an external group of experts, the project focussed in 2010–12 on (i) continuing to assess the feasibility of using the psyllids, in particular *T. walkeri*, and the seed-feeding midge *W. krumbholzi* as biological control agents (this includes additional studies of the phytoplasma '*Ca.* Phytoplasma rhamni'), and (ii) determining the causes of the high levels of seedling mortality and post-dispersal seed mortality of *R. cathartica* observed in Europe as compared to North America as a step towards identifying additional potential biological control agents including pathogens.

Work on '*Ca.* Phytoplasma rhamni' in North America has been carried out by Dr Roger Becker and Dr Dimitre Mollov, University of Minnesota, St. Paul, USA.

2. Phytoplasma '*Candidatus* Phytoplasma rhamni'

2.1. Introduction

Plant-pathogenic phytoplasmas are non-culturable, insect-transmitted, wall-less prokaryotes of the class *Mollicutes* that are associated with diseases in

several hundred plant species, including many woody shrubs or small trees (Marcone *et al*., 2004; Weintraub and Beanland, 2006). Based on conventional and computer-simulated RFLP (restriction fragment length polymorphism) analyses of 16S rRNA (ribosomal RNA) gene sequences, all phytoplasmas identified to date are classified within 30 main groups (designated 16SrI to 16SrXXX) and over 100 subgroups which are designated with a letter suffix (Zhao *et al*., 2010).

A lethal witches' broom disease of *R. cathartica* was observed for the first time in the Rhine Valley in south-western Germany in the 1990s (Mäurer and Seemüller, 1996). This disease, characteristic symptoms of which we have never observed, is caused by buckthorn witches' broom (BWB) phytoplasma, which belongs to the 16SrXX – BWB phytoplasma group (Wei *et al*., 2007), subgroup -A. BWB phytoplasma was previously classified within the 16SrX – apple proliferation group (AP) as subgroup -E (Lee *et al.*, 1998), due to the closer phylogenetic relatedness of BWB to the phytoplasmas of this group than to other phytoplasma subclades (see Marcone *et al.,* 2004 for references). Following a recently updated classification scheme, the 16SrX group of phytoplasmas currently includes the AP (16SrX-A), pear decline (16SrX-C), Spartium witches' broom (16SrX-D) and European stone fruit yellows (16SrX-F) phytoplasmas. On the other hand, the BWB phytoplasma is, on the basis of low RFLP pattern similarity with all known phytoplasmas in group 16SrX and other groups, assigned to the 16SrXX group (Wei *et al.*, 2007).

To resolve the taxonomic position of the phytoplasmas, a provisional taxonomic system for uncultured bacteria (Murray and Schleifer, 1994) was recently adopted for naming phytoplasma species candidates, within a genus-level taxon '*Candidatus* Phytoplasma' ('*Ca*. Phytoplasma') (IRPCM, 2004). So far, 32 '*Ca.* Phytoplasma' species have been formally described (Zhao *et al*., 2010 and references therein; Lee *et al.*, 2011; Malembic-Maher *et al.*, 2011; Davis *et al.*, 2012; Martini *et al.*, 2012). For uncultured phytoplasmas, a novel putative species may be described when its 16S rRNA gene sequence (>1200 base pairs) has ≤ 97.5% similarity to any previously described '*Ca.* Phytoplasma' species (IRPCM, 2004). The BWB phytoplasma shares < 97.5% 16S rDNA sequence similarity with other known phytoplasmas, including the AP group phytoplasmas. Thus Marcone *et al*. (2004) proposed the BWB phytoplasma as a novel '*Ca.* Phytoplasma' species, i.e. '*Ca.* Phytoplasma rhamni'. According to these authors, the BWB phytoplasma has clearly distinct molecular and biological properties, and in particular a different and unique field host plant, *R. cathartica*.

The single most successful group of insect vectors of phytoplasmas are the Hemiptera. Phytoplasmas are phloem-limited; therefore, only phloem-feeding insects can potentially acquire and transmit these pathogens. However, within the phloem-feeding Hemiptera only a small number, primarily in a very few taxonomic groups, have been confirmed as vectors of phytoplasmas (Weintraub and Beanland, 2006). The main group of known vectors is the Cicadellidae, although 15 species in another seven families are also known to be vectors of phytoplasmas (Weintraub and Beanland, 2006).

The importance of psyllids as possible vectors of phytoplasma diseases has been recognized only recently and comprehensive research on their role as vectors has been carried out in the past few years (reviewed in Jarausch and Jarausch, 2010). All confirmed and recognized psyllid vectors to date belong to a single genus, *Cacopsylla*. Five species of *Cacopsylla* are confirmed vectors and transmit AP group (16SrX) phytoplasmas on apple, stonefruit and pear trees: *C. picta*, *C. melanoneura*, *C. pruni*, *C. pyri* and *C. pyricola* (Jarausch and Jarausch, 2010). Another three species within this genus are considered as possible vectors: *C. pyrisuga*, *C. qianli* and *C. chinensis*; these latter *Cacopsylla* species were found to be infected with AP group phytoplasmas, but their vector role and transmission efficacy has yet to be clarified. Additionally, there are two reports of psyllid vectors belonging to genera other than *Cacopsylla,* both transmitting a phytoplasma to carrots. *Bactericera trigonica* was found to transmit a stolbur (16SrXII) phytoplasma and *Trioza nigricornis* to transmit the aster yellows (16SrI) phytoplasma. Nonetheless, since the vector role of these psyllids in phytoplasma transmission is not confirmed, at present they are treated only as tentative vectors.

In 2009 the presence of the phytoplasma '*Ca.* Phytoplasma rhamni' (16SrXX-A subgroup) was detected in *Trichochermes walkeri* adults from two localities in western Switzerland. Our goal in 2010–12 was (1) to sample *R. cathartica*, other *Rhamnus* species, *Frangula alnus* and the psyllids *T. walkeri*, *C. rhamnicola* and *Trioza rhamni* from a number of sites in western and south-eastern Europe and checkthem for the presence of phytoplasma, and (2) to carry out transmission trials with *T. walkeri*.

2.2. Materials and methods

2.2.1. Plant and insect collections

Characteristic symptoms of witches' broom, which would indicate the presence of the phytoplasma, were not observed at any of the surveyed buckthorn sites. At some localities discrete leaf yellowing and/or small leaves were present on a few trees and these were sampled individually and treated as possibly symptomatic. All other sampled *R. cathartica* trees were asymptomatic.

When possible, five trees (samples) were sampled at each buckthorn site and 15–20 leaves collected per tree. One constraint was that plant material collected for the detection of phytoplasma should be neither dry nor mouldy. Leaf tissue was cut approximately 3 mm either side of the mid vein. For each sample, mid veins and petioles were put together in a plastic vial (8 cm long; 1.5 cm diameter). A small hole was made in the lid for ventilation. Collections were always sent within 24 hours to the Institute for Plant Protection and Environment in Belgrade, Serbia, for processing. Delays were encountered with some of the shipments, but the plant material was still in good condition even 8–10 days after collection when stored as described above.

For *R. cathartica*, five leaves from each tree sampled for phytoplasma detection were also sampled for molecular identification of plant genotype. For this purpose the leaves were placed in silica gel.

In July–August 2010, all buckthorn sites were carefully inspected for *T. walkeri* galls. Enough galls were collected at each site to give about 20 L4–L5 *T. walkeri* nymphs. Development of *T. walkeri* was delayed by at least ten days compared to previous years, and in Austria only L2–L4 could be collected. All samples were stored in 95% ethanol before being processed.

In June 2011 leaf samples of *R. cathartica* with galls of *T. walkeri* were collected at Šušara in Serbia and tested for phytoplasma presence. Phytoplasma-positive trees were selected for the collection of 100 *T. walkeri* adults in August 2011, with the assumption that these adults had harboured the phytoplasma. These specimens were used for transmission trials (see section 2.5), and were all subsequently analysed for phytoplasma presence.

In addition a few *C. rhamnicola* and *Trioza rhamni* adults were collected in 2010–11 at Griessheim, Germany and at Beranje, Serbia for phytoplasma detection.

2.2.2. Molecular detection and characterization of 'Ca. Phytoplasma rhamni*'*

Total nucleic acids from plant midribs and petioles were extracted using a previously reported CTAB (cyltrimethylammonium bromide) protocol (Angelini *et al*., 2001). To identify phytoplasmas in *Trichochermes walkeri*, collected specimens were analysed in pools of 3–5 nymphs (depending on the stage) or individually in the case of adults. For *C. rhamnicola* and *Trioza rhamni* all collected specimens were adults and were analysed individually. DNA extraction from insects was performed using a modified CTAB method (Gatineau *et al*., 2001).

Phytoplasmas were detected in plant and insect DNA samples by polymerase chain reaction (PCR) amplification of the 16S rRNA gene using the universal phytoplasma and group specific primer pairs. Amplification was performed in nested PCR with P1/P7 primers (Deng and Hiruki, 1991; Smart *et al.*, 1996) followed by an F2n/R2 universal primer pair (Gundersen and Lee, 1996) or R16(X)F1/R1 primers specific for amplification of 16SrX group and related phytoplasmas (Lee *et al*., 1995). Amplicons obtained with F2nR2 primers were subjected to RFLP analyses with *Mse*I, *Alu*I and *Hpa*II endonucleases, following the previously described procedure of Lee *et al*. (1998). '*Candidatus* Phytoplasma rhamni' DNA isolated from naturally infected *R. cathartica* from a location between Neuhofen and Ludwigshafen in Rheinland-Palatinate, Germany (type locality of '*Ca.* Phytoplasma rhamni'; provided by Bernd Schneider, Institut für Pflanzenschutz im Obstbau, Dossenheim, Germany) was used as a reference positive control in all reactions.

Characterization of detected phytoplasmas was performed by sequence analysis of the 16S rRNA gene and ribosomal protein gene operon consisting of the *rpl*22 and *rps*3 genes encoding ribosomal proteins L22 and S3. For sequence analysis of the 16S rRNA gene, amplification was conducted using P1/P7 primers in direct PCR, followed by nested PCR with the P1A/P7A primer pair, with reaction conditions according to Lee *et al*. (2004). Amplification and sequence analysis of ribosomal protein genes *l*22 and *s*3 was performed as described by Martini *et al*. (2007), with rpL2F3/rp(I)R1A primers used for direct PCR and followed by nested PCR with the rpF1C/rp(I)R1A primer pair.

2.3. Results and discussion

2.3.1. Plants

'*Candidatus* Phytoplasma rhamni' was detected in 25% of all *R. cathartica* samples, at several sites in all countries surveyed, except for Montenegro, but not in any of the other three *Rhamnus* species sampled or in *F. alnus* (Jović *et al*., 2011) (Table 1; Annex 1).

Table 1. Geographic origin and number of *Rhamnus* spp. and *Frangula alnus* samples analysed with PCR results on '*Candidatus* Phytoplasma rhamni' presence.

Country	**Number of plant samples positive/analysed**				
	R. cathartica	***R. alpina***	***R. saxatilis***	***R. rupestris***	***F. alnus***
Switzerland	14/35	0/20	0/0	0/0	0/19
Germany	3/25	0/0	0/0	0/0	0/0
Austria	11/30	0/0	0/3	0/0	0/0
Serbia	6/41	0/10	0/15	0/5	0/0
Montenegro	0/2	0/5	0/0	0/5	0/0
Total	34/133	0/35	0/18	0/10	0/19

We did not observe the witches' broom disease found previously on one occasion in Germany in the 1990s (Mäurer and Seemüller, 1996) and at present we cannot associate the presence of the phytoplasma with any particular symptoms. Our results have revealed a much wider geographic distribution of '*Ca.* Phytoplasma rhamni' than was previously known, which reinforced the need for a more detailed characterization of geographically distant isolates (Fig. 1). This is particularly important for elucidating '*Ca*. Phytoplasma rhamni' epidemiology, since it can be expected that different strains are transmitted by different vectors and with different rates of efficacy.

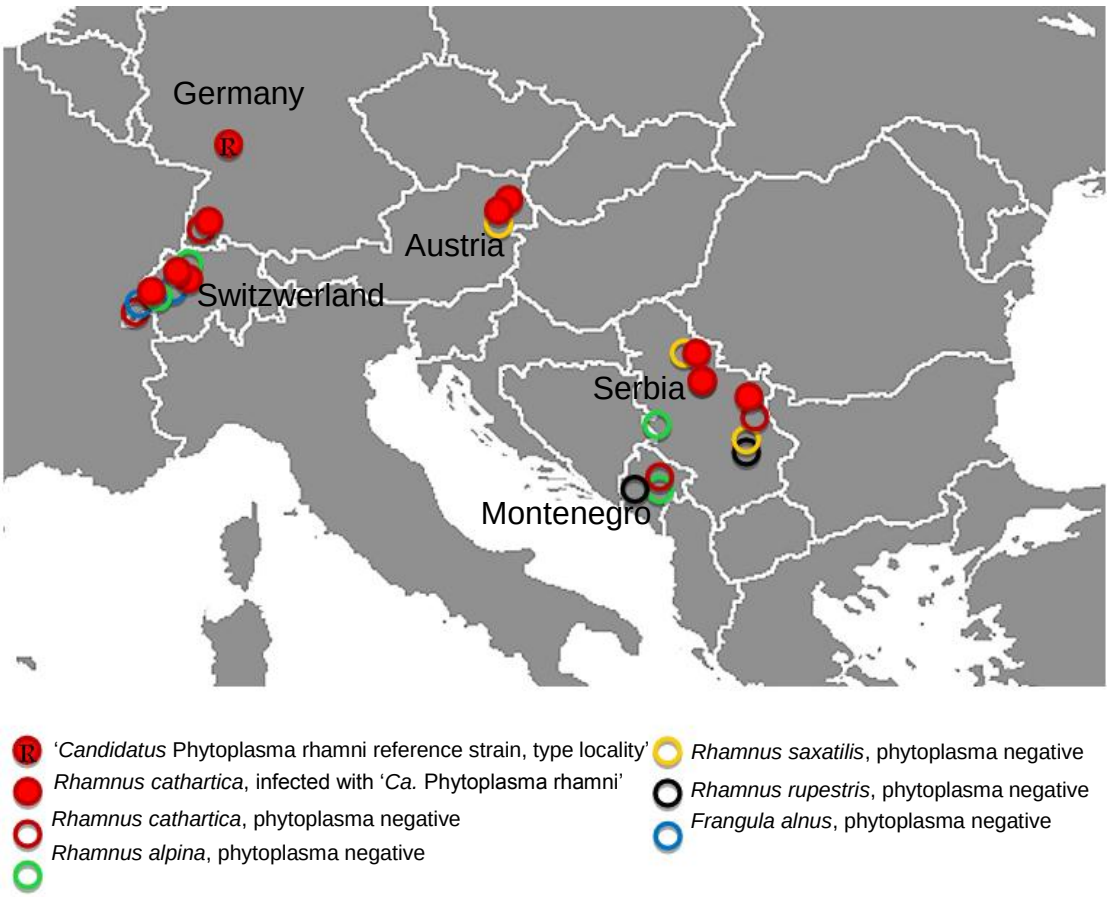

Fig. 1. Occurrence and geographical distribution of '*Candidatus* Phytoplasma rhamni' on *Rhamnus* spp. and *Frangula alnus* in Europe.

In order to trace the molecular variability of the phytoplasma and to identify possible strain differences we sequenced two phytoplasma gene fragments (16Sr RNA and *rpl22–rps3*) of selected '*Ca*. Phytoplasma rhamni' isolates which were geographically the most distant (Table 2). Comparison of 16Sr RNA gene sequences among isolates from Switzerland, Germany, Austria and Serbia showed that they share 100% identical nucleotide composition. Comparison with the reference BWB strain of '*Ca.* Phytoplasma rhamni' showed 99% identity, which was the consequence of the low quality of sequence read of this historical isolate which had several ambiguous nucleotide positions (Table 2, N positions). Phylogenetic analyses of '*Ca.* Phytoplasma rhamni' relatedness with closest relatives from AP group phytoplasmas confirmed the previously determined clear phylogenetic separation of these *Candidatus* species (pairwise distance ranged from 3.4% to 4.6%), which supports its biological uniqueness and specific host-plant association (Fig. 2).

Table 2. Nucleotide differences in 16S rRNA gene sequences in newly obtained and historical sequences of '*Candidatus* Phytoplasma rhamni' reference strain.

Isolate	Locality	16S rRNA nucleotide position [a]						
		553	573	944	945	1077	1104	1246
BWB [b]	S Germany	N [c]	-	A	T	N [c]	N [c]	N [c]
172-08-10	SW Switzerland	C	C	-	-	C	A	G
48-07-10	NW Switzerland	C	C	-	-	C	A	G
42-08-10	SW Germany	C	C	-	-	C	A	G
14-07-10	NE Austria	C	C	-	-	C	A	G
174-09-10	NE Serbia	C	C	-	-	C	A	G
207-09-10	CE Serbia	C	C	-	-	C	A	G
55-06-10	E Serbia	C	C	-	-	C	A	G

[a] Bases according to BWB (buckthorn witches' broom) reference strain (Marcone *et al*., 2004).
[b] Reference '*Ca.* Phytoplasma rhamni' strain BAWB (Marcone *et al*., 2004) GenBank Acc. number X76431 is highlighted in grey.
[c] N represents any nucleotide.

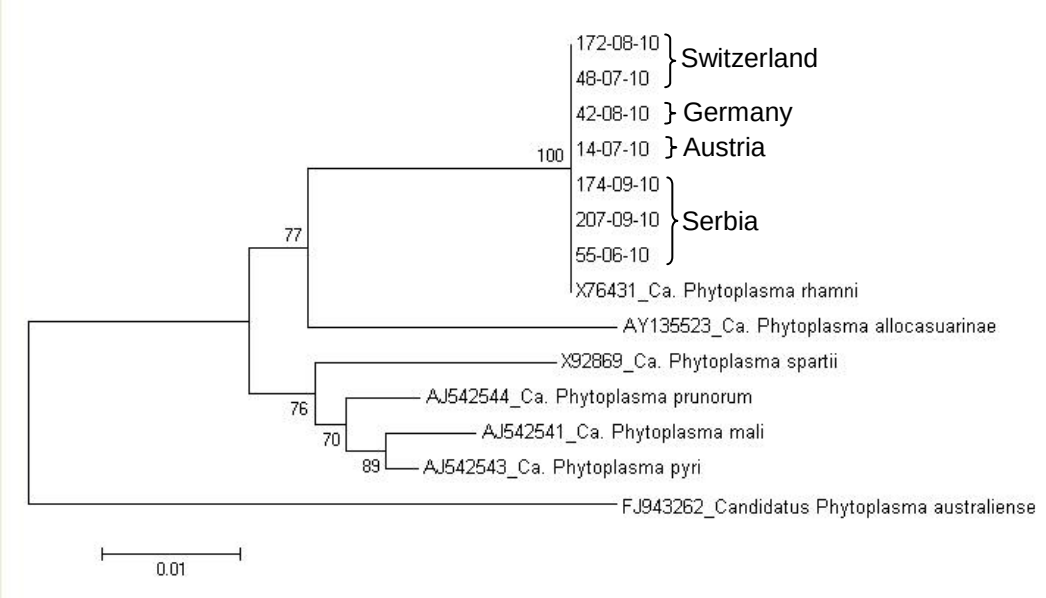

Fig. 2. Phylogenetic tree constructed by neighbour-joining method (p-distance model) inferred from 1358 base pairs of 16S rRNA gene fragments for seven '*Candidatu*s Phytoplasma rhamni' isolates from *Rhamnus cathartica* and reference strains of the 16SrX phytoplasma group ('*Ca.* Phytoplasma mali', '*Ca.* Phytoplasma pyri', '*Ca.* Phytoplasma prunorum', '*Ca*. Phytoplasma spartii', '*Ca*. Phytoplasma allocasuarinae'). '*Candidatus* Phytoplasma australiense' (16SrXII-B subgroup) was used as an outgroup to root the tree. Bootstrap values for 500 replicates are shown on branches. GenBank accession numbers of reference strains are indicated.

Sequence analyses of a ribosomal protein gene operon (*rpl*22–*rps*3 genes) of eight '*Ca*. Phytoplasma rhamni' isolates from different parts of western and south-eastern Europe (including the isolate from the species type locality in Germany) revealed a low level of intraspecific variability with only a single nucleotide change present in the sequence of isolates from north-west Switzerland and north-east Austria (Fig. 3). In contrast, interspecific

differences with reference sequences of 16SrX (AP group) phytoplasmas (‘*Ca.* Phytoplasma mali’, ‘*Ca.* Phytoplasma pyri’, ‘*Ca.* Phytoplasma prunorum’) confirmed the clear separation of ‘*Ca*. Phytoplasma rhamni’, with pairwise distances ranging from 16.3% to 16.6%. This high genetic divergence clearly confirms the independent evolution of ‘*Ca.* Phytoplasma rhamni’ from related phytoplasmas probably due to its specific adaptation to its environment (host plant). High genetic divergence also confirmed that ribosomal genes are genetic markers with higher resolution potential than the 16S rRNA gene which is the reason why they are useful in identification and separation of closely related strains. In the case of ‘*Ca*. Phytoplasma rhamni’, analysis of these marker genes revealed very low genetic variability.

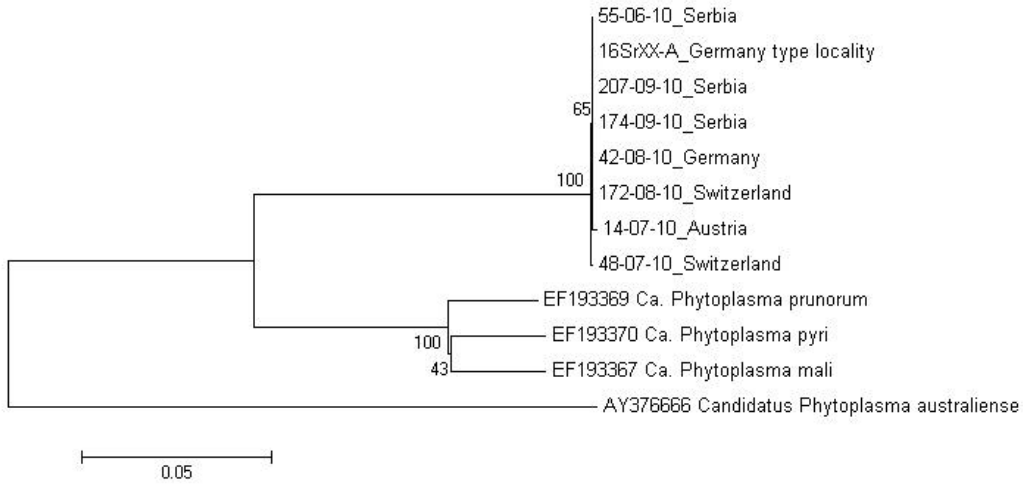

Fig. 3. Phylogenetic tree constructed by neighbour-joining method (p-distance model) inferred from 1048 base pairs of *rpl*22–*rps*3 genomic loci for eight ‘*Candidatus* Phytoplasma rhamni’ isolates from *Rhamnus cathartica* and reference strains of 16SrX phytoplasma group phytoplasmas (‘*Ca.* Phytoplasma mali’, ‘*Ca.* Phytoplasma pyri’, ‘*Ca.* Phytoplasma prunorum’). ‘*Candidatus* Phytoplasma australiense’ (16SrXII-B subgroup) was used as an outgroup to root the tree. Bootstrap values for 500 replicates are shown on branches. GenBank accession numbers of reference strains are indicated.

2.3.2. Insects

Samples of *Trichochermes walkeri* from all collection sites in 2010 were positive for ‘*Ca.* Phytoplasma *rhamni’* except those from a very small site in Switzerland (CH10, see Annex 1) where neither *R. cathartica* nor *T. walkeri* tested positive. Interestingly, *T. walkeri* tested positive at site CH19, but the phytoplasma was not detected in any of the five heavily galled *R. cathartica* analysed. This is probably the consequence of an uneven distribution of phytoplasma bodies in the phloem of the infected trees, their low concentration (known for woody hosts in particular) and variations in titre according to the season and plant organ (reviewed in Firrao *et al.*, 2007), leading to false-negative results from the analysed plant samples.

From nine adult *C. rhamnicola* collected at a single site in Griessheim, Germany in 2010, one adult was found to be infected with ‘*Ca.* Phytoplasma

rhamni'. This is the first record of *C. rhamnicola* being infected with a phytoplasma (Table 3). In 2011, one adult *Trioza rhamni* from Serbia tested positive for '*Ca.* Phytoplasma *rhamni*'. Thus, all three psyllid species recorded from *R. cathartica* are tentative vectors of '*Ca.* Phytoplasma rhamni'.

Table 3. Detection of '*Candidatus* Phytoplasma rhamni' in three psyllid species recorded from *Rhamnus cathartica* in Europe.

Country	**Date of collection**	**'*Candidatus*' phytoplasma rhamni' analysis (No. of positive pulls / No. tested)**		
		Trichochermes walkeri	***Cacopsylla rhamnicola***	***Trioza rhamni***
Austria	July 2010	34 / 36	-	-
Switzerland	July–Aug 2010	84 / 98	-	-
Germany	July 2010	10 / 10	-	-
Germany	June 2010	-	1 / 9	-
Germany	April 2011	-	0 / 2	-
Serbia	July 2010	3 / 11	-	-
Serbia	April 2011	-	0 / 3	1 / 7
Serbia	August 2011	70 / 100	-	-

2.4. Transmission trials

Trichochermes walkeri proved to be infected with '*Ca.* Phytoplasma rhamni' at a very high rate in almost all localities sampled in 2010 (see Table 3). However, *T. walkeri* infection with phytoplasma only shows that this psyllid is acquiring the phytoplasma during feeding on infected plants, but not that it is capable of re-injecting the phytoplasma during feeding.

In 2011-2012, we carried out trials to test whether *T. walkeri* is capable of transmitting the phytoplasma to its host plant *R. cathartica.*

METHODS. Between 21 and 23 August 2011, a total of 100 *T. walkeri* adults were collected at Susara (South Banat, Serbia) from *R. cathartica* trees which had previously proven to be infected with '*Ca.* Phytoplasma rhamni'. Twenty *T. walkeri* adults were set up on each of three potted *R. cathartica* seedlings (eight-leaf stage). In addition 20 *T. walkeri* adults were set up on two other European *Rhamnus* species: *R. saxatilis* and *R. rupestris*. All adults were re-collected from the plants after 48 hours and subsequently preserved in 96% ethanol for detection of '*Ca.* Phytoplasma rhamni'. The exposed plants as well as two control plants of each of the species tested were kept in a mesh cage outdoors. All plants were analyzed by PCR for the presence of the phytoplasma on 7 April, 22 May and 3 September 2012.

RESULTS. Analyses of the *T. walkeri* adults used in the transmission trials confirmed the presence of phytoplasma in 70% of all specimens (Table 4). Twelve months after exposure to phytoplasma-infected *T. walkeri* adults, no symptoms could be observed on either of the *Rhamnus* tested. Control plants

were also asymptomatic. The repeated analysis in 2012 of all three *Rhamnus* species exposed to feeding by *T. walkeri* in 2011 did not reveal the presence of the phytoplasma. These results strongly suggest that *T. walkeri* is not a natural vector of '*Candidatus* Phytoplasma rhamni'.

Table 4. Results of transmission trials with phytoplasma infected *Trichochermes walkeri* adults on *Rhamnus* spp.

		Trichochermes walkeri		**Phytoplasma detection in *Rhamnus* spp.**
	No. of replicates	**No. of adults released / replicate**	**Adult infection rate / replicate**	
R. cathartica	3	20	65-70%	Negative
R. saxatilis	1	20	75%	Negative
R. rupestris	1	20	70%	Negative

2.5. Detection of '*Ca.* Phytoplasma rhamni' in *Rhamnus cathartica* from North America (Roger Becker and Dimitre Mollov, University of Minnesota)

The potential use of *T. walkeri* as a biological control agent of *R. cathartica* in North America is complicated by the presence of '*Ca.* Phytoplasma rhamni'. It was therefore necessary to determine whether the phytoplasma already occurs in the introduced range of *R. cathartica*.

METHODS The leaf sampling protocol was similar to that used in Europe. In contrast to work done in Europe, a composite sample of several trees per site was used to detect the presence of the phytoplasma in buckthorn. Most samples were from Minnesota, with a few from Indiana, Michigan, Iowa and Wisconsin. All samples were processed using the Qiagen kit DNA extraction protocol and nested PCR (Lee *et al*., 1995; Smart *et al*., 1996). Two rounds of PCR reactions, (i) general phytoplasma primers and (ii) phytoplasma group X specific primers, were performed.

RESULTS None of the 75 buckthorn sites tested was found to have the buckthorn phytoplasma while a positive control sample obtained from Jelena Jović (Institute for Plant Protection and Environment, Serbia) was included each time and gave the expected size (positive) band. It can therefore be concluded that '*Ca.* Phytoplasma rhamni' does not occur in North America.

2.6. General discussion

Rhamnus cathartica trees were found to be infected with '*Ca.* Phytoplasma rhamni' at almost all surveyed localities, confirming previous reports of a host association between this phytoplasma and *R. cathartica*, although witches' broom symptoms were not observed. Phytoplasma was not detected in any of the other *Rhamnus* species analysed, nor in *F. alnus*, which could indicate a very specific host association between this phytoplasma and its host plant, as well as a very specific relationship between the insect vector of the pathogen and its host plant.

'*Ca.* Phytoplasma rhamni' has not been detected in *R. cathartica* populations from North America suggesting either that the phytoplasma has not been introduced in the exotic range of its host plant, or that the absence of a suitable vector for phytoplasma propagation constrained its establishment in North America.

The absence of symptoms on all phytoplasma-infected trees could be an indication of a commensal relationship between the phytoplasma and its plant host, i.e. the absence of negative effects which would lead to the development of a disease in a host plant. Plants with asymptomatic presence of phytoplasma are considered to be a wild reservoir of the pathogen, since they are not affected by its presence.

Trichochermes walkeri proved to be infected with '*Ca.* Phytoplasma rhamni' at very high rates in almost all sampled localities. Even young instars (L2–L3) collected in Austria were found to be infected with phytoplasma. Our transmission trials strongly suggest that *T. walkeri* is not a natural vector of the phytoplasma, and that the high infection rate detected in this species is the result of a very close host–plant association of this psyllid with *R. cathartica. Trichochermes walkeri* acquires the phytoplasma during feeding on infected plants, but it is not capable to re-inject the phytoplasma during feeding. No psyllid of the genus *Trichochermes* has previously been found to even harbor a phytoplasma, let alone transmit it. In addition, all confirmed psyllid vectors of phytoplasmas belong to the genus *Cacopsylla*, and in our field survey one *C. rhamnicola* adult was found to be infected with '*Ca.* Phytoplasma rhamni'. Although the limited number of specimens analysed does not yet allow us to draw conclusions, the presence of the phytoplasma in *C. rhamnicola* reinforces the need for elucidating its possible role as a vector of '*Ca.* Phytoplasma rhamni'.

Recently, '*Ca*. Phytoplasma rhamni' was also detected in *Cacopsylla myrthi* Puton during a survey for vectors of '*Ca*. Phytoplasma trifolii' (16SrVI group) on solanaceous crops in Lebanon (Choueiri *et al*., 2007). *Cacopsylla myrthi* was collected on *R. cathartica* (X. Foissac, pers. comm., 2011) and one of 13 analysed pulls of this psyllid was infected with '*Ca*. Phytoplasma rhamni'. This further suggests that species in the genus *Cacopsylla* could be the major vectors of '*Ca*. Phytoplasma rhamni' on *R. cathartica*. We could not find any record of *T. walkeri* in Lebanon.

Finally the phytoplasma has also been detected in *Trioza rhamni* thus showing that the three psyllid species associated with *R. cathartica* are able to acquire the phytoplasma during feeding. Previous to our research, only one species in the genus *Trioza*, *T. nigricornis,* had been found to harbour a phytoplasma, aster yellows phytoplasma (16SrI group), but the vector role of this species was not confirmed and it is treated only as a tentative vector. In a system where three psyllid species are probably associated with one unique host plant carrying a phytoplasma, it is unlikely that all three species would be the vector of the pathogen, at least not within the same epidemiological cycle. Given the known and well-documented vector ability of *Cacopsylla* spp. to transmit phytoplasmas from the AP group and the evolutionary relatedness of '*Ca*. Phytoplasma rhamni' with the AP group phytoplasmas, it can be

expected that feeding by *Cacopsylla* species on *R. cathartica* might play a major vector role in the transmission of the phytoplasma.

3. Impact of Leaf Galling by *Trichochermes walkeri* on the Growth of *Rhamnus cathartica* Seedlings

METHODS To determine the potential impact of *T. walkeri* on young buckthorn plants, ten eight-month-old *R. cathartica* were exposed to three field-collected pairs of *T. walkeri* on 17 August 2011. Another ten plants were used as controls. The number of eggs laid by the psyllids was recorded in late October 2011. The number of galls and the impact of leaf galling on plant growth were assessed in spring 2012.

RESULTS Two plants without eggs were discarded. The number of eggs recorded on each replicate is shown in Table 5. Previous oviposition experiments indicated that about 10% of eggs laid resulted in gall and larval development the following year, however no galls were recorded in 2012 and the impact experiment was terminated without having obtained conclusive results.

Table 5. Impact study with *Trichochermes walkeri* in 2011–12.

Plant	Shoot height (cm)	No. of leaves	No. of eggs	No. of adults alive, 14 October 2011	Gall and larval development in 2012
Test plants					
No. 1	26	14	107	1 ♀ / 1 ♂	0
No. 10	29	13	34	0	0
No. 11	29	14	15	0	0
No. 16	32	14	62	1 ♀	0
No. 22	37	17	57	0	0
No. 23	36	16	53	1 ♀	0
No. 26	36	14	188	2 ♂	0
No. 29	36	16	198	1 ♂ / 2 ♀	0
Mean ± SD	32.6 ± 4.2	14.8 ± 1.4			
Control plants					
No. 2	34	11	-	-	-
No. 7	29	14	-	-	-
No. 9	32	14	-	-	-
No. 13	28	12	-	-	-
No. 17	35	15	-	-	-
No. 21	38	14	-	-	-
No. 24	28	12	-	-	-
No. 25	33	12	-	-	-
No. 28	40	19	-	-	-
No. 30	41	16	-	-	-
Mean	33.8 ± 4.8	13.9 ± 2.4			

4. *Wachtiella krumbholzi* (Diptera: Cecidomyiidae)

4.1. Background

Little is known about this insect, which was identified by Dr M. Skuhrava (Czech Republic). Interestingly, with the exception of a few specimens reared from *R. cathartica* in the Czech Republic, Skuhrava has not found this species during 50 years of investigations on cecidomyiids in 1800 European localities (Simova-Tosic *et al*., 2000, 2004; Skuhrava *et al.*, 2005).

The main characteristics of fruits attacked by *W. krumbholzi* resemble premature fruit maturation in terms of changes in colour, but the fruits are larger in size and irregularly shaped. Attacked fruits become dark-red/black while healthy fruits remain green (Plate 1). Casual observations revealed up to nine midge larvae per fruit and three larvae in one seed. Once mature, the midge larva leaves the fruits and enters the soil to prepare a larval cocoon made of silk and debris.

Plate 1. Healthy fruits of *Rhamnus cathartica* (left) and attacked fruits (with mature larvae) (right); Griessheim, Germany, 20 July 2009.

Preliminary oviposition tests in 2009 indicated successful oviposition and larval development by *W*. *krumbholzi* in the very young developing fruits of *R. cathartica*. In contrast, no oviposition occurred in the one-month-older well-developed fruits.

4.2. Molecular characterization

To date, we have recorded *W. krumbholzi* in most *R. cathartica* sites where we have looked for it, i.e. we found it at ten sites in Serbia, six sites in Austria, three sites in western Switzerland and two sites in southern Germany. Midge larvae have also been discovered in the fruits of *R. saxatilis* ssp. *tinctorius* at one site in Serbia, where *R. cathartica* also occurs. Based on the mitonchondrial COI (cytochrome c oxidase) gene, midges from *R. cathartica* and *R. saxatilis* ssp. *tinctorius* are clearly two closely related but distinct species (Fig. 4). The mean genetic differences between the two species ranged between 2.7% and 3.7%. Strict host association with high genetic divergence between the two midge species has thus been confirmed at this sympatric site where both *Rhamnus* species co-occur.

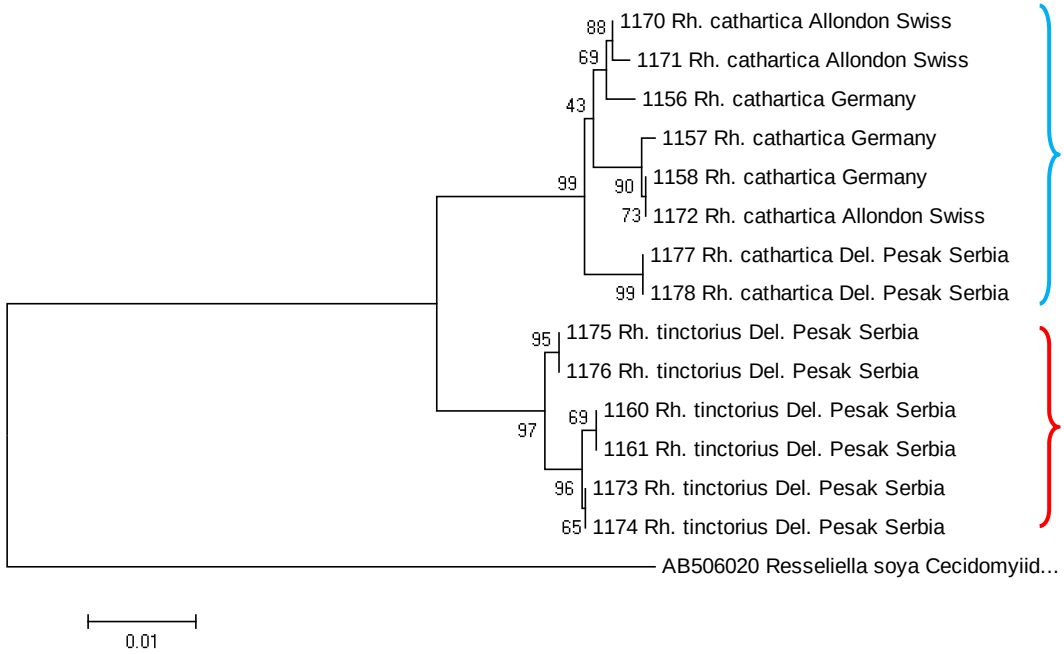

Fig. 4. Neighbour-joining phylogenetic tree (p-distance model) inferred from 626 base pairs of the mitochondrial cytochrome c oxidase gene for 14 midge specimens from *Rhamnus cathartica* and *R. saxatilis* ssp. *tinctorius*. The percentages of replicate trees in which taxa clustered together from a bootstrap analysis (500 replicates) are indicated (values lower than 40% are omitted).

No midge larvae were reared from the fruits of *F. alnus* collected in 2008 at one site in Austria and one site in Switzerland, where *R. cathartica* and *F. alnus* co-occur.

4.3. Adult emergence

In the past few years, emergence of gall midge adults in an outdoor shelter started about mid-May and was completed by early June. Adults started to emerge from gall midge cocoons, which had been stored at 3°C until late spring, two weeks after being moved to either the outdoor shelter or to controlled conditions of 20°C and emergence was completed within eight days. In 2009, adult emergence from larval cocoons stored at 1°C until early June started three weeks after they were returned to outdoor conditions and was completed within ten days. This indicates that we have the capacity to manipulate adult emergence to a certain extent. We also observed that cold treatment (10°C) was lethal for adults ready to emerge.

In 2011, 30 adult *W. krumbholzi* emerged in our outdoor shelter between mid-May and mid-June from about 200 fruits of *R. cathartica* collected in southern Germany in late summer 2010. Unfortunately, none of our potted *R. cathartica* flowered or fruited, thus no oviposition tests could be carried out.

4.4. Conclusions and outlook

Host-range tests with this fruit-attacking gall midge species relies entirely on oviposition tests. Synchronization between adult midge emergence and the availability of fruits in a very early phenological stage suitable for oviposition is therefore paramount. Our main difficulty is, however, to obtain fruits on potted *Rhamnus* species, which are mostly dioecious (i.e. male and female flowers are on separate plants). In addition, it takes several years for these trees/shrubs to reproduce. Since we have not so far succeeded in obtaining even reproducing trees of the host, *R. cathartica,* when grown in pots, we are doubtful whether it will be feasible to successfully screen *W. krumbholzi* in the near future.

5. Post-dispersal Seed and Seedling Mortality

5.1. Introduction

The interactions between plants and their soil community can result in dynamic feedbacks (Reinhart *et al*., 2003; McCarthy-Neumann and Kobe, 2010). Positive or negative soil feedbacks occur when a plant promotes a soil community that in turn benefits or inhibits conspecific plant performance compared with heterospecifics. Differences in interactions between native versus exotic plants and resident soil may play an important role in biological invasions and the persistence of exotic species (Nijjer *et al*., 2007). For example, the invasion of *Prunus serotina* in Europe is facilitated by the associated soil community (Reinhart *et al*., 2003). In the native range in the USA, the soil community that develops near *P. serotina* inhibits the establishment of neighbouring conspecifics and reduces seedling performance. This mechanism does not exist in the invaded range in Europe where the soil community enhances the growth of *P. serotina* seedlings.

The effect of mature *R. cathartica* shrubs on the growth and survival of seedlings is controversial (Knight *et al*., 2007). Some studies have shown a positive or neutral effect of mature conspecifics. Other studies have shown a negative effect presumably due to a strong shade effect of dense mature thickets. However, the potential accumulation of species-specific microbial communities in soil associated with the roots of adult trees has not been studied and the potential benefit of mycorrhizal colonization in the native versus invaded ranges of *R. cathartica* and the relative effects on *R. cathartica* and native plants remains unknown (Knight *et al*., 2007).

In 2011, we tested the hypothesis that seedling emergence and seedling performance of *R. cathartica* in Europe is affected by negative plant–soil feedbacks. We have conducted a greenhouse experiment on seedling emergence and seedling performance of *R. cathartica* using sterile versus non-sterile soils from two buckthorn sites in Switzerland.

5.2. Materials and methods

Approximately 1000 mature fruits of *R. cathartica* were collected at Griessheim in Germany on 3 September 2010 and at Cheyres and La Sauge in Switzerland on 9 September 2010. Fruit tissue was removed within three

days, seeds air-dried for 24 hours and stored in a paper bag at 4°C. A germination trial was set up on 19 November 2010 and 14 January 2011, 2.3 and 4.2 months after vernalization, respectively. Seeds from Griessheim germinated very poorly and were excluded from the experiment.

Soil samples were collected on 10 May 2011 at the two Swiss sites. At each site, a 5-litre soil sample was taken from the tree base of five mature female *R. cathartica* trees (the so-called tree effect) (Plate 2). A control soil sample was taken at a distance of 5 m from each sampled tree, making sure that no other *R. cathartica* tree was within 5 m of where the control sample was taken. All control samples were taken in the same habitat as the sample tree (i.e. forest or forest margin) with one exception, where the control sample was collected in the neighbouring wetland a couple of metres away from the forest edge. Vegetation cover was usually high around all trees and no seedlings could be seen within a distance of 5 m of any of the sampled trees. Each soil sample was air-dried for 24 hours, and then hand mixed and prepared by removing living macro-invertebrates, large organic particles and stones. In order to homogenize the samples, we sifted them through a 2-mm mesh sieve.

Each soil sample was air-dried for another 24 hours before being split into two equal parts. Soil to be sterilized was taken on 12 May to LEONI Studer Hard AG in Switzerland for gamma sterilization (max 50kGray, min 29kGray, Dosimeter Type Alanine 01/11). The non-sterilized soil was kept under the same conditions as the sterilized soil, i.e. in closed plastic boxes in the laboratory until being used in the germination trays. The experiment with non-sterile soil was set up on 24 May 2011 and with sterilized soil on 27 May, due to problems with the sterilization process.

Germination trays (48 × 25 × 6 cm) were separated into three parts by two pieces of wood (Plate 3). The two external parts (germination compartments) were used for the experiment. The middle part was left empty to avoid exchanges of particles between the germination compartments. The size of each germination compartment was about 15 × 25 × 6 cm containing approximately 2.2 litres of soil.

Seeds were surface-sterilized, the day before being sown, in a 7% sodium hypochlorite (Javel) solution for 3 min, and then rinsed with tap water for 30 sec before being soaked twice for 30 sec in sterilized (boiled) water (Chen, 2010). Forty seeds and 36 seeds respectively for soil from Cheyres and La Sauge were planted in four rows in each germination compartment (within the top 0.5 cm of soil) and covered with a fine layer of sand from a calcareous gravel pit obtained from a local commercial supplier.

The trials were set up as a full-factorial experiment with three factors: (i) site (Cheyres and La Sauge), (ii) *Rhamnus* presence (soil samples collected from underneath mature *Rhamnus* trees vs. control samples), and (iii) sterilization (sterilized vs. non-sterilized soil), resulting in eight treatment combinations. Each treatment combination was replicated five times, resulting in a total of 40 germination compartments. Each replicate was randomly assigned to each of the 40 germination compartments. Germination trays were gently watered as necessary and their position in the greenhouse changed randomly twice a week.

Seedling emergence was recorded 4–5 times every week and each seedling tagged using a toothpick (Plate 3). Between 12 and 15 September, seedling height was measured and the number of leaves (excluding cotyledons) counted. Seedlings were then harvested, dried for 24 hours at 80°C, and the aboveground and belowground biomass then measured immediately. The number of days to seedling emergence from the non-sterile soil was modified to take into account the three days' difference between set up of the experiment with sterile and non-sterile soils. Dead seedlings were excluded from the calculation of the mean dry weight.

Plate 2. M. Bennett, M. Penic and A. Leroux collecting soil samples within buckthorn stands at La Sauge, 10 May 2011.

Plate 3. Seedlings of *Rhamnus cathartica* in germination trays on 11 August 2011, 11 weeks after seeds were sown.

On 18 July, soil samples were collected in each germination compartment for mineral nitrogen (NO_3^--N and NH_4^+-N) analysis. Each sample consisted of two cores of soil giving about 20 g of fresh weight. Cores were taken without disturbing seeds or seedlings and placed in plastic bags. Holes in the germination compartments were then refilled with the sand used to cover the

surface of the trays. On 19 July, the samples were taken to the Swiss Federal Institute of Technology in Zurich (ETHZ) to extract the mineral nitrogen. Analyses were carried out in the laboratory of the plant ecology group (Prof. P.J. Edwards) with the assistance of S. Güsewell and B. Jahn. About 10 g of soil was taken from each sample, weighed and placed in a glass jar together with 40 ml of calcium chloride extraction solution, closed with a lid and placed in a mechanical shaker for 60 min. The content of each jar was then filtered for nearly one hour and stored at 4°C. The total NO_3^--N and NH_4^+-N content was measured on 20 July in a photometric analyser. Two additional blank jars were added as a control.

The experiment was analysed as a nested split-plot design using a linear mixed-effects model with site, soil and sterilization as fixed factors (see above) and replicate as a random factor. Differences between treatment combinations were assessed using Tukey's HSD. The relationship between the number of days for seedling emergence and the total biomass of the plant at the end of the experiment was analysed using linear regression. The nitrogen content and plant biomass data were $\log_{10}$-transformed prior to analysis. All analyses were done in R 2.13.0 (R Development Core Team, 2011).

5.3. Results and discussion

Non-sterile soil had a higher mineral nitrogen content than sterilized soil (19.9 ± 3.4 versus 11.8 ± 1.2 mg kg $soil^{-1}$), which was mainly due to a higher mineral nitrogen content at one of the sampling spots at Cheyres (Fig. 5; Annex 2). No correlation was found between the mineral nitrogen content and any of the plant parameters measured, thus the variation in the mineral nitrogen between sites or between sterile and non-sterile soils does not explain any of the results obtained.

On average, percentage of emerged seedlings was lower in sterile soil than in non-sterile soil (60.6 ± 1.2% versus 70.6 ± 2.1%) and higher at La Sauge than at Cheyres when soil was not sterilized (Fig. 5; Annex 2). This is probably because of the negative effect of sterilization on soil microbes that degrade the seed coat and thus facilitate seed germination and seedling emergence. Our data do not provide any evidence for pathogenic microorganisms causing pre-emergence seedling mortality, as shown for example with *Prunus serotina* in some North American sites (Reinhart *et al*., 2005).

Time to seedling emergence was not different in sterile and in non-sterile soils (52.1 ± 0.7 versus 54.6 ± 0.9 days) (Fig. 6; Annex 2). Times to seedling emergence or germination time are seldom considered in plant–soil feedback studies. Andonian *et al.* (2011) found no effect of soil sterilization on germination time of *Centaurea solstitialis* but a significant effect of soil regions. In contrast, de la Pena *et al.* (2010) did not find a significant effect of site or soil biota on the germination of the ground-hugging succulent perennial *Carpobrotus edulis*. In its exotic range in North America, seeds from *R. cathartica* trees growing in oak (*Quercus* spp.) woods germinated two weeks faster and had higher germination rates than seeds from neighbouring wetlands (Gourley 1985, in Knight *et al.*, 2007).

All plant parameters were greater in sterile soils than in non-sterile soils (Fig. 6; Annex 2). A negative correlation between time to seedling emergence and plant biomass was found (Fig. 7). This correlation suggests that plants in sterile soils were larger and had more leaves because they had slightly longer to grow than plants in non-sterile soils. It is likely that this apparent negative plant–soil feedback on seedling growth would be reduced should the seedlings be allowed to grow for a longer time before being harvested.

Finally no tree effect could be seen, indicating that the microbial communities were similar in buckthorn areas and buckthorn-free areas and the plant–soil interactions found at the two study sites are not the result of microorganisms associated with *R. cathartica*.

In summary, a positive plant–soil interaction was found in the rate of seedling emergence. The small, non-significant difference in time to seedling emergence probably explains most of the variation in seedling growth within the growing period under study.

Thus, we did not find evidence of negative plant–soil feedback of mature *R. cathartica* on conspecifics that could explain low seedling numbers of *R. cathartica* in its native range. However, this study suggests a balance of positive and negative interactions between *R. cathartica* and the soil biota that may contribute to give buckthorn a competitive advantage in a changing environment. Novel interactions between *R. cathartica* and resident soil organisms in the introduced range could generate benefits for the invader compared to the native plants.

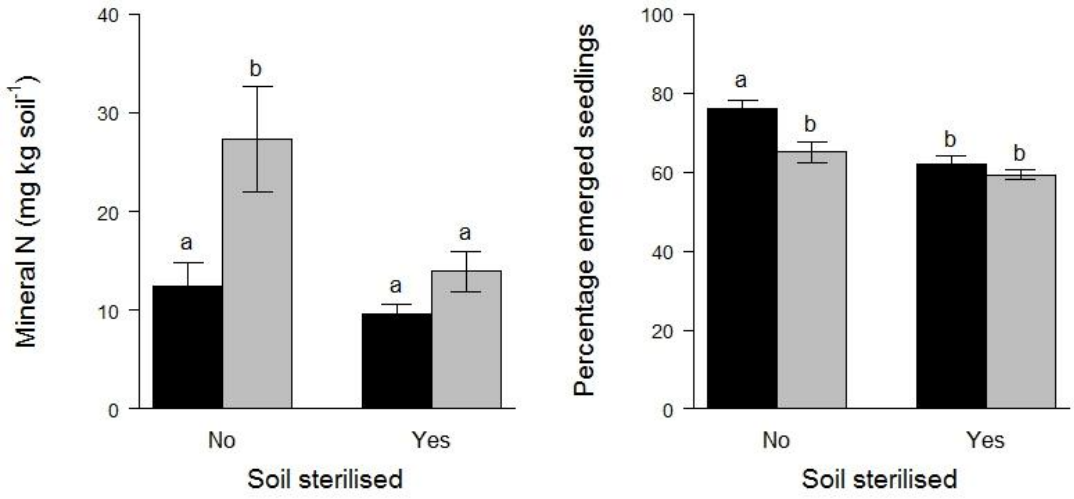

Fig. 5. Total mineral nitrogen content (left) and percentage of emerged seedlings of *Rhamnus cathartica* (right) in sterile and non-sterile soils from two Swiss sites in 2011. Grey and black bars indicate means for Cheyres and La Sauge, respectively. Error bars indicate one SE and small characters above the bars indicate significant differences (Tukey's HSD, $P < 0.05$).

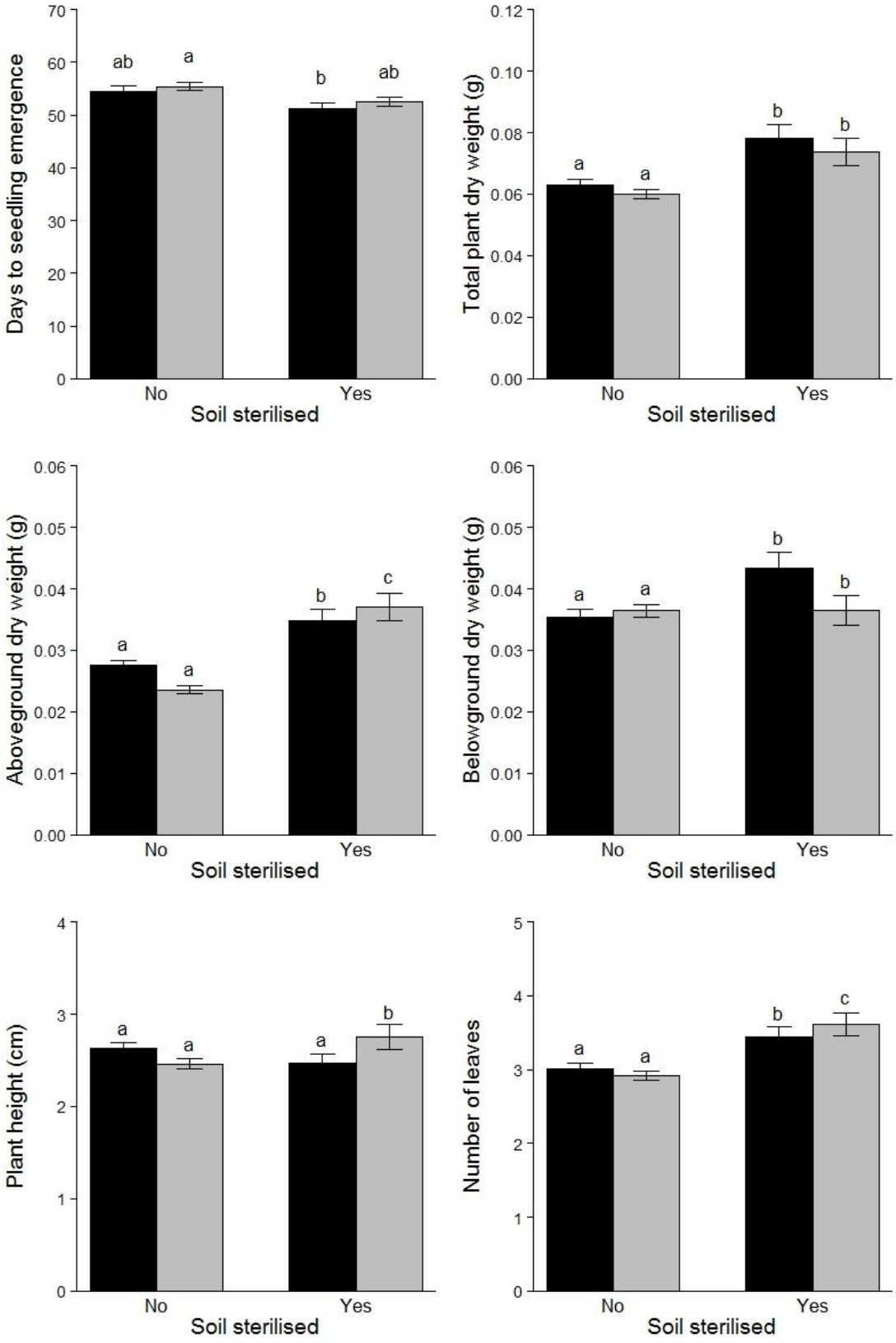

Fig. 6. Time to seedling emergence and seedling growth of *Rhamnus cathartica* in sterile and non-sterile soils from two Swiss sites in 2011. Shading as in Fig. 5. Error bars indicate one SE and small characters above the bars indicate significant differences (Tukey's HSD, $P < 0.05$).

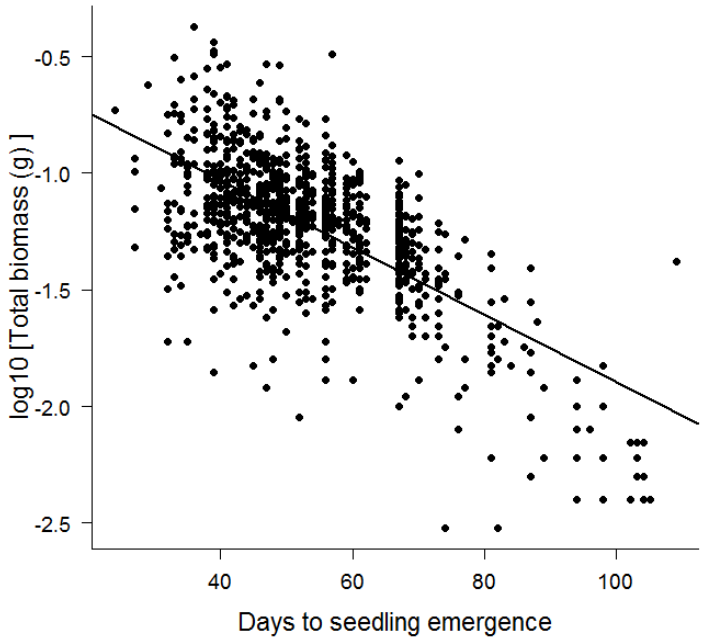

Fig. 7. Total dry weight of *Rhamnus cathartica* seedlings grown in soils from two Swiss sites in 2011 as a function of the number of days to seedling emergence (R^2 = 0.43, *n* = 916, *P* < 0.001).

6. Discussion

Although phytoplasma-infected trees have been identified over a wide geographic area within the natural range of *R. cathartica*, the phytoplasma has not been detected in any of the sampled buckthorn populations covering a wide geographical area from North America. One question is whether the phytoplasma has not been introduced into North America or whether the absence of a specific transmission mechanism prevented the pathogen to establish and spread in buckthorn populations in the introduced range.

Although all nymphal stages, as well as adults of *T. walkeri*, were found to harbour '*Ca.* Phytoplasma rhamni', this finding only indicates intensive feeding by *T. walkeri* on *R. cathartica* (which was infected at these locations) and their very close association. Transmission trials strongly suggest that *T. walkeri* is not a vector of '*Ca.* Phytoplasma rhamni'. No psyllids from the genus *Trichochermes* have ever been found to transmit any phytoplasma, while all currently recognized psyllid vectors belong to the genus *Cacopsylla*. The presence of '*Candidatus* Phytoplasma rhamni' in *Cacopsylla rhamnicola* and *Trioza rhamni* reinforces the need to elucidate the epidemiology of the phytoplasma, especially the vector role and transmission efficacy of these two psyllids as well as the host-plant specificity of '*Ca.* Phytoplasma rhamni' to *R. cathartica* and its congeners. Phylogenetic analyses of 16S rRNA and *rpl*22–*rps*3 genes of '*Ca*. Phytoplasma rhamni' and related phytoplasmas, further support its uniqueness and the clear separation of this phytoplasma from its

relatives, probably due to the specific transmission route and host-plant association. Given that this phytoplasma was described as causing a witches' broom disease in its host, its impact on *R. cathartica* and other *Rhamnus* species would need to be tested under controlled conditions. The absence of symptoms in transmission trials and in infected trees on all surveyed sites, suggests a commensal association between the phytoplasma and *R. cathartica*, with *R. cathartica* serving as a wild reservoir of the phytoplasma.

The occurrence of '*Ca.* Phytoplasma rhamni' in the three psyllids associated with *R. cathartica* makes the use of any of these potential agents more complicated. However, because our transmission trials indicate that *T. walkeri* does likely not transmit the phytoplasma, we believe that this probably very specific insect still has potential as an agent for *R. cathartica*.

Assessing the host specificity of *T. walkeri* relies on oviposition and larval development tests. Adult feeding and oviposition of *T*. *walkeri* are restricted to species in the genus *Rhamnus*. The likelihood of *T. walkeri* accepting a non-target species for oviposition in containment that would not be accepted in the field (a false positive) is considered high. *Trichochermes walkeri* has been recorded exclusively on *R. cathartica* in Europe. However, it must be noted that only a few *Rhamnus* species occur in its native range in Europe. Specific requirements for host acceptance and suitability will probably be related to stage of developing leaf bud, leaf shape and toughness as well as habitat. There are indications that larvae of *T. walkeri* will not complete development on small tough or thick evergreen leaves such as those of *R. alaternus*. Therefore, the native North American *Rhamnus* species *R. crocea*, *R. ilicifolia*, *R. serrata* and *R. smithii* are unlikely to be suitable for development of *T. walkeri* nymphs to the adult stage. Critical native non-target North American species for *T. walkeri* are *R. alnifolia* and *R. lanceolata* because of their leaf shapes and leaf smoothness, and their geographical distributions which partially overlap that of *R. cathartica.*

The challenges in working with the seed-feeding midge *Wachtiella krumbholzi* will be obtaining reproductive trees, pollination of female buckthorn flowers and synchronizing fruit development with midge oviposition and larval development. Since we have not so far succeeded in obtaining even reproducing trees of the host, *R. cathartica,* when grown in pots, we are doubtful whether it will be feasible to successfully screen W*. krumbholzi* in the near future. More generally, one current constraint in developing biological control of buckthorns is the difficulty of obtaining seeds for a number of test plant species and/or growing plants from seeds.

Finally, we did not find evidence of a negative plant–soil feedback of mature *R. cathartica* with conspecifics in its native range that could explain at least in part the invasiveness of *R*. *cathartica* in its introduced range. This indicates that the chances are slim of finding a specific soil-borne fungal pathogen with biocontrol potential.

Due to the difficulties surrounding currently studied agents and the low probability of finding additional potential agents, it has been decided that the project will be stopped. A publication summarizing main results will be prepared until 2013.

8. Acknowledgements

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Distribution list

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Annexes

Annex 1. Detection of '*Candidatus* Phytoplasma rhamni' in *Rhamnus* spp. and *Trichochermes walkeri* in 2010.

		Plants		*Trichochermes walkeri*	
Collection site	**Date of collection**	**Plant species sampled (# samples = trees)**	***'Ca.* Phytoplasma rhamni' analysis (# positive / # tested)**	***Trichochermes walkeri* galls present (and collected)**	***'Ca.* Phytoplasma rhamni' (# positive pulls / # tested)**
Austria					
A17 - Traiskirchen	20 July 2010	*Rhamnus cathartica* (5)	**2/5**	yes	4/4 (18 nymphs)
A19 - Oberwaltersdorf	21 July 2010	*Rhamnus cathartica* (5)	**2/5**	yes	2/4 (18 nymphs)
A48 - Truman	21 July 2010	*Rhamnus cathartica* (5)	**2/5**	no	-
A21 - Unterwaltersdorf	21 July 2010	*Rhamnus cathartica* (5)	**2/5**	yes	4/4 (18 nymphs)
A25 - Purbach	22 July 2010	*Rhamnus cathartica* (5)	**2/5**	yes	24/24 (103 nymphs)
A26 - St Margarethen	21 July 2010	*Rhamnus cathartica* (5)	**1/5**	no[a]	-
		Rhamnus saxatilis (3)	**0/3**	no	-
Switzerland					
CH1 - Allondon	4 August 2010	*Rhamnus cathartica* (5)	**0/5**	no[a]	-
CH3 - Satigny	4 August 2010	*Rhamnus cathartica* (2)	**0/2**	no[a]	-
		Frangula alnus (5)	**0/5**	no	-
CH6 - Chatillon	3 August 2010	*Rhamnus alpina* (5)	**0/5**	no	-
CH7 - La Combe	3 August 2010	*Rhamnus cathartica* (2)	**1/2**	yes	5/5 (17 nymphs)
	3 August 2010	*Frangula alnus* (2)	**0/2**	no	-
CH10 - Courroux	10 August 2010	*Rhamnus cathartica* (3)	**0/3**	yes	0/3 (9 nymphs)
CH11 - Delémont	5 August 2010	*Rhamnus cathartica* (3)	**1/3**	yes	5/6 (19 nymphs)

		Plants		*Trichochermes walkeri*	
Collection site	**Date of collection**	**Plant species sampled (# samples = trees)**	**'*Ca.* Phytoplasma rhamni' analysis (# positive / # tested)**	***Trichochermes walkeri* galls present (and collected)**	**'*Ca.* Phytoplasma rhamni' (# positive pulls / # tested)**
CH14 - Cheyres	11 August 2010	*Rhamnus cathartica* (5)	**5/5**[b]	yes	24/24 (82 nymphs)
		Frangula alnus (5)	**0/5**	no	-
CH17 - La Sauge	11 August 2010	*Rhamnus cathartica* (5)	**4/5**	yes	26/27 (90 nymphs)
		Frangula alnus (5)	**0/5**	no	-
CH19 - Vermes	6 August 2010	*Rhamnus cathartica* (5)	**0/5**	yes	4/10 (2 adults + 29 nymphs)
		Rhamnus alpina (5)	**0/5**	no	-
		Frangula alnus (2)	**0/2**	no	-
CH30 - Soulce	26 July 2010	*Rhamnus cathartica* (5)	**3/5**[b]	yes	10/13 (5 adults + 33 nymphs)
CH31 - Courcelon	10 August 2010	*Rhamnus alpina* (5)	**0/5**	no	-
CH32 - Haute-Borne	10 August 2010	*Rhamnus alpina* (5)	**0/5**	no	-
Germany					
D8 - Zienken	28 July 2010	*Rhamnus cathartica* (5)	**0/5**	no[a]	-
D20 - Griessheim	28 July 2010	*Rhamnus cathartica* (5)	**2/5**	yes	10/10 (2 adults + 28 nymphs)
D21 - Griessheim	11 August 2010	*Rhamnus cathartica* (5)	**0/5**	no[a]	-
D22 - Griessheim	11 August 2010	*Rhamnus cathartica* (5)	**0/5**	no[a]	-
D23 - Griessheim	11 August 2010	*Rhamnus cathartica* (4)	**1/4**	no[a]	-
Serbia					
Deliblatski pesak	7 June 2010	*Rhamnus cathartica* (5)	**0/5**	no[a]	-

		Plants		*Trichochermes walkeri*	
Collection site	**Date of collection**	**Plant species sampled (# samples = trees)**	**'*Ca.* Phytoplasma rhamni' analysis (# positive / # tested)**	***Trichochermes walkeri* galls present (and collected)**	**'*Ca.* Phytoplasma rhamni' (# positive pulls / # tested)**
		Rhamnus saxatilis spp. *tinctorius* (5)	**0/5**	no	-
Sicevo	8 June 2010	*Rhamnus saxatilis* spp. *tinctorius* (5)	**0/5**	no	-
		Rhamnus rupestris (5)	**0/5**	no	-
Rajac, East Serbia	26 Jun 2010	*Rhamnus cathartica* (5)	**1/5**	no[a]	-
Deliblatski pesak, Susara	30 July 2010	*Rhamnus cathartica* (5)	**1/12**	yes	3/11 (11 adults + 1 nymph)
Deliblatski pesak, Susara	30 July 2010	*Rhamnus saxatilis* spp. *tinctorius* (5)	**0/5**	no	-
Deliblatski pesak, exit	30 July 2010	*Rhamnus cathartica* (5)	**0/5**	no[a]	-
Mitrovac na Tari	1 August 2010	*Rhamnus alpina* (5)	**0/5**	no	-
Tara	1 August 2010	*Rhamnus alpina* (5)	**0/5**	no	-
Cerovica, kanjon iza Knjazevca	13 August 2010	*Rhamnus cathartica* (5)	**0/5**	no	-
Beranje	8 Sept. 2010	*Rhamnus cathartica* (5)	**1/5**	no	-
Rajac	9 Sept. 2010	*Rhamnus cathartica* (4)	**3/4**	no[a]	-
Montenegro					
Kolasin	17 August 2010	*Rhamnus cathartica* (2)	**0/2**	no	-
Kolasin	17 August 2010	*Rhamnus alpina* (5)	**0/5**	no	-
Nudo	18 August 2010	*Rhamnus rupestris* (5)	**0/5**	no	-

[a] *Trichochermes walkeri* was recorded in previous years.

[b] Phytoplasma-positive samples of *T. walkeri* were collected in 2009.

Annex 2. Results of a mixed-effect model on the influence of site (La Sauge vs Cheyre), soil (*Rhamnus cathartica* area vs *R. cathartica*-free area) and sterilization (non-sterile vs. sterile soil) on seedling emergence and seedling growth of *Rhamnus cathartica.* Significant *P*-values are in bold; ndf and ddf denote numerator and denominator degrees of freedom, respectively. Mineral N (nitrogen) values and plant biomass data were $\log_{10}$-transformed prior to analysis.

Tray Data:		**Mineral N**		**Percentage seedling emergence**	
	ndf,ddf	F	*P*	F	*P*
Site	1,4	9.60	**0.036**	7.68	**0.050**
Soil	1,8	0.10	0.764	0.04	0.849
Sterile	1,16	5.50	**0.032**	24.02	**<0.001**
Site × Soil	1,8	1.56	0.246	1.34	0.281
Site × Sterile	1,16	1.91	0.186	4.29	0.055
Soil × Sterile	1,16	0.23	0.639	0.09	0.764
Site × Soil × Sterile	1,16	0.55	0.468	0.85	0.371

Plant Data:		**Days to seedling emergence**		**Plant height**		**No. of leaves**	
	ndf,ddf	F	*P*	F	*P*	F	*P*
Site	1,4	0.49	0.522	1.62	0.272	2.24	0.209
Soil	1,8	2.23	0.174	2.57	0.148	3.94	0.082
Sterile	1,16	3.55	0.078	9.69	**0.007**	39.15	**<0.001**
Site × Soil	1,8	0.25	0.629	0.00	0.949	0.00	0.997
Site × Sterile	1,16	0.00	0.967	11.73	**0.004**	6.59	**0.021**
Soil × Sterile	1,16	0.29	0.598	1.27	0.276	0.42	0.525
Site × Soil × Sterile	1,16	1.32	0.268	0.74	0.404	0.63	0.440

		Total biomass		**Belowground biomass**		**Aboveground biomass**	
	ndf,ddf	F	*P*	F	*P*	F	*P*
Site	1,4	0.00	0.961	0.04	0.854	0.07	0.803
Soil	1,8	3.55	0.096	3.88	0.084	2.72	0.138
Sterile	1,16	17.37	**<0.001**	6.67	**0.020**	27.53	**<0.001**
Site × Soil	1,8	0.06	0.818	0.13	0.731	0.00	0.981
Site × Sterile	1,16	1.09	0.312	0.35	0.562	5.58	**0.031**
Soil × Sterile	1,16	1.68	0.214	3.09	0.098	0.52	0.482
Site × Soil × Sterile	1,16	0.12	0.733	0.00	0.955	0.39	0.542

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Garlic Mustard Biological Control

Developing Biological Control Insects, Working Towards Field Release

June 6, 2014

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Report to the Legislative-Citizen Commission on Minnesota Resources

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Executive Summary

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

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

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

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

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

***Ceutorhynchus scrobicollis* in containment.**

Elizabeth J. Stamm Katovich, Roger. L. Becker, Esther Gerber and Hariet L. Hinz and Richard C. Reardon*

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

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

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

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

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

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

RESULTS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[a]Photoperiod and dark period total 24 hours.

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

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

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

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

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

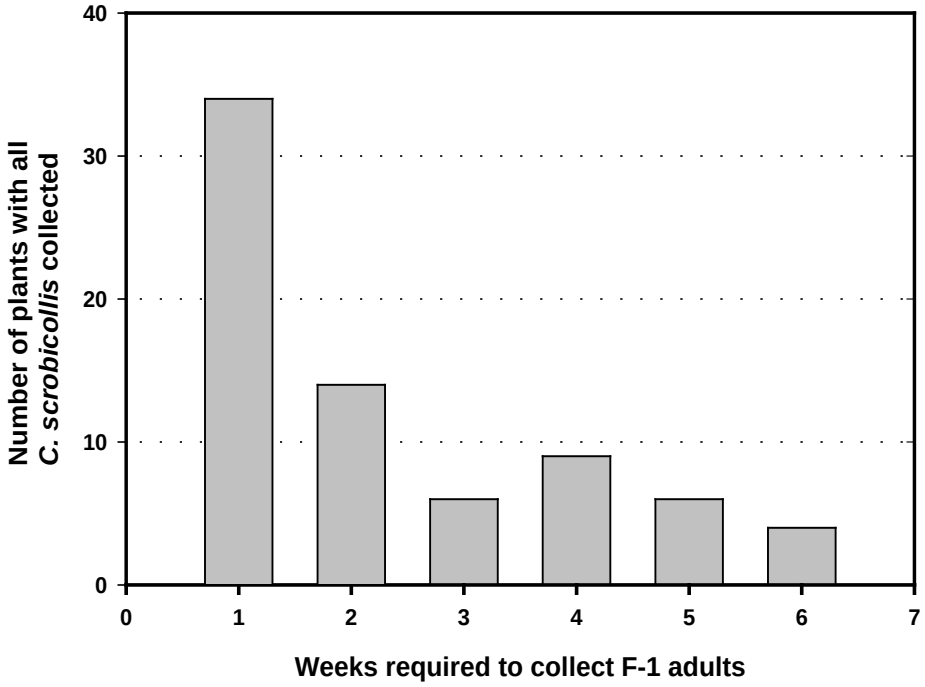
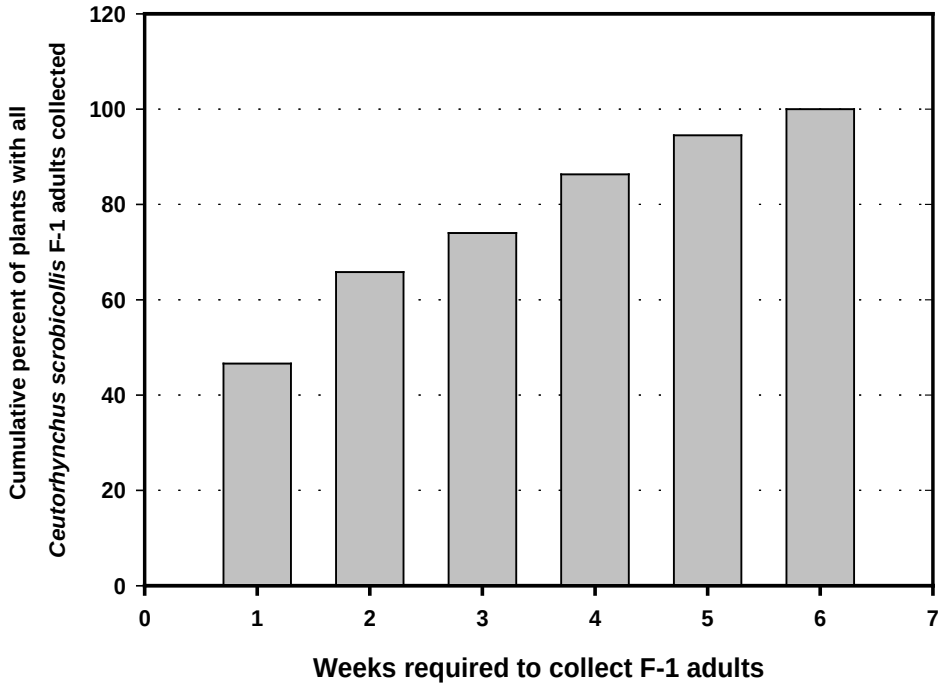


Figure 2b. Cumulative percent of plants with all *Ceutorhynchus scrobicollis* F-1 adults collected



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ORIGINAL CONTRIBUTION

Biological control of *Rhamnus cathartica*: is it feasible? A review of work done in 2002–2012

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Abstract

Rhamnus cathartica (common buckthorn) is a shrub (or small tree) of Eurasian origin, which has become invasive in North America. Internal feeders and sap suckers were prioritized for biological control from over 30 specialized insects identified from the target plant in its native European range. Five leaf-feeding moths were also considered for further investigations. Field observations and preliminary host range tests with the stem-boring beetle *Oberea pedemontana*, the root-boring moth *Synanthedon stomoxiformis*, the shoot-tip-boring moth *Sorhagenia janiszewskae* and the leaf-feeding moths *Ancylis apicella*, *A. unculana*, *Triphosa dubitata*, *Phileremetransversata* and *P. vetulata* confirmed that all of these species were lacking host specificity in no-choice conditions. Choice oviposition tests carried out with most of the prioritized species to assess their ecological host range yielded unreliable results. Three psyllids, *Trichochermes walkeri*, *Cacopsylla rhamnicolla* and *Trioza rhamni* are promising in terms of host specificity, but are infected with the plant disease '*Candidatus* Phytoplasma rhamni'. Fruit- or seed-feeding insects may present the best potential for biological control of buckthorn in directly reducing seed set and thus seedling establishment. However, it was not possible to obtain adult fruiting trees of native North American *Rhamnus* species for testing. It is concluded that there are no promising arthropod agents based on what is known to date. Pathogens could offer new opportunities for biological control of *R. cathartica* in North America.

Introduction

Rhamnus cathartica L. (common buckthorn) (Rhamnaceae) is a shrub or small tree of Eurasian origin that has become invasive in North America. The species was deliberately introduced in the late 1800s into north-eastern North America primarily as an ornamental hedge plant and shelterbelt tree and was then brought to Saskatchewan for the same purposes in the 1930s (Gourley 1985; Randall and Marnelli 1996; Archibold et al. 1997). It has escaped cultivation and has spread into most Canadian provinces and 34 states predominantly in the North-eastern and Midwestern United States (Zouhar 2011; USDA, NRCS 2013). *R. cathartica* is declared as noxious in six U.S. states and two Canadian provinces (USDA, NRCS 2013; http://www.omafra.gov.on.ca/english/crops/facts/info_buckthorn.htm).

The most effective management strategies of common buckthorn involved a combination of cutting or girdling with applications of glyphosate or picloram/2,4-D (Qaderi et al. 2009). However, cutting trees near the base provides temporary control only because the plant is able to regrow from the stump (Maw 1984). *R. cathartica* can be controlled by annual or biennial prescribed burns for 5 or more years, but this may be inappropriate because of damage to native species (Heidorn 1991), and burning may also enhance populations (Zouhar 2011).

A project was initiated in 1964 to investigate the possibilities for biological control of *R. cathartica* in Canada (Malicky et al. 1970). The project was halted due to lack of funding 2 years later despite the fact that host-specific and effective herbivores were found (Malicky et al. 1970). In 2001, a new project was started to continue the work initiated by Malicky et al. (1970) and to reassess the potential for biological control of common buckthorn, especially considering the new paradigm shift towards recognizing the value of 'non-useful' native plants from a conservation and ecological perspective. This paper reports field observations and host specificity work done in 2002–2012 on selected biological control arthropod agents. Factors that limit the feasibility of biological control of common buckthorn in North America are discussed.

The target plant

Rhamnus cathartica is found throughout most of Europe, absent only in the extreme south, the area north of southern Sweden and also from most parts of the Iberian Peninsula (Tutin 1968; Anderberg 1998). Common buckthorn is a dioecious shrub or small tree 4–8 m tall, grey to black, 0.5- to 2.2-cm-long thorns grow at the tips of branches or in the forks of two branches. Leaves are toothed and may be arranged both alternately and oppositely on the same branch. Winter buds have dark scales. *R. cathartica* reproduces by seeds and regenerates by sprouting from cut or damaged stems or from the root crown following complete or partial stem removal (Zouhar 2011).

Rhamnus cathartica is adapted to a wide range of climatic and habitat conditions. In Western Europe, the species prefers mesophile to meso-xerophile open or half-shaded habitats on calcareous alkaline or neutral soils, but it can also be found in swampy areas (Rameau et al. 1989). In North America, *R. cathartica* seems to have an affinity for disturbed, fertile, calcium-rich, moist areas, open woods and woodland edges, but it can tolerate both dry and partially flooded conditions. It avoids extreme shading and drought (Qaderi et al. 2009). In North America, common buckthorn can become the dominant understory vegetation, displacing native vegetation through the formation of a dense canopy, thus creating a major threat to native biodiversity (Heidorn 1991; Catling 1997; Moffatt and McLachlan 2004). One of the most important impacts of *R. cathartica* is the alteration of ecosystem processes. Heneghan et al. (2006) found that soil in woodland areas where buckthorn dominates has higher percentage of nitrogen (N) and carbon (C), modified nitrogen mineralization rates, elevated pH and higher soil moisture than those areas where buckthorn was not present. Indirect economic damage results from *R. cathartica* being an alternate host of the pathogenic fungus causing crown rust and leaf rust of oats, *Puccinia coronata* Corda. f. sp. *avenae* Eriks. & Henn. (Maw 1984), and the primary overwintering host plant for the soybean aphid *Aphanes glycines* Matsumura (Zhu et al. 2006).

Natural enemies

In its native European range, the feeding guild of the 36 specialized arthropods reported by Gassmann et al. (2008) on *R. cathartica* is dominated by leaf feeders (17 spp), followed by sap suckers (12 spp), fruit or seed feeders (4 spp) and shoot/root borers (3 spp). Most of the 150 host associations between *R. cathartica* and fungal species are reported from Europe (Farr and Rossman 2012). Also, the cucumber mosaic virus was detected in *R. cathartica* in Germany (Kegler et al. 1994), and the occurrence of '*Candidatus* phytoplasma rhamni' in *R. cathartica* in Europe was confirmed by Jović et al. (2011).

In North America, the soybean aphid and other Hemiptera, such as the green stink bug, *Acrosternum hilare* (Say), or the Say's stink bug, *Chlorochroa sayi* Stal., have been commonly observed feeding on *R. cathartica* in Ontario (Qaderi et al. (2009). In a 2-year study, Yoder et al. (2008) recorded a total of 32 herbivorous arthropod species representing 20 families and six orders from common buckthorn in Minnesota. Only generalists were found and more Hemipterans were encountered than Lepidopterans. There are therefore much fewer insect and fungal species associated with *R. cathartica* in the introduced range in North America than in the native European range, and none appear to be at minimum genus specific. This is another possible reason for the invasiveness of common buckthorn in North America (Knight et al. 2007).

Prioritization of biological control agents for *Rhamnus cathartica*

A meta-analysis of results published after 2000 confirmed previous analyses that Chrysomelidae and Curculionidae families are the most effective weed biological control agents (Clewley et al. 2012). Reviews of successes and failures in 25 programmes against invasive trees and shrubs as of 2010 concluded that Curculionidae are the most effective agents against woody perennials followed by sap-sucking

species in the Phlaeothripidae (Thysanoptera) and Psyllidae (Hemiptera) families (Moran et al. 2004; Gassmann et al. 2010). Internal feeders and sap suckers were therefore prioritized for biological control of buckthorn. From 17 leaf-feeding moths known from buckthorn in Europe, five species were considered in an early stage of the project to confirm their field host records. Fruit- or seed-feeding insects may also present a good potential for biological control of buckthorn through directly reducing seed set and thus seedling establishment. In contrast to the lepidopterous species, seed-feeding midges seem to be potentially host specific enough for biological control of buckthorn.

Test plants are selected using criteria based on phylogenetic relatedness, biogeographic overlap and ecological similarity (Briese 2003). Although much disputed historically, the separation of *Frangula* from *Rhamnus* is now widely accepted, being supported by recent genetic data (Bolmgren and Oxelman 2004) with *Rhamnus* and *Frangula* being predominant in the Old Word and New World, respectively (Grubov 1949; Johnston and Johnston 1978). Ten native *Rhamnus* taxa and 20 native *Frangula* taxa are known in North America (USDA, NRCS 2013). The native North American species *R. alnifolia* L'Hér., *R. lanceolata* Pursh and *F. caroliniana* (Walt.) Gray have a broad habitat range, and their geographical distribution overlaps most with common buckthorn. These are therefore key species in preliminary host range studies of potential biological control agents for *R. cathartica*.

Studied biological control candidates

Stem and root borers (3 species)

Three internal root/shoot borers are known on *R. cathartica* in Europe:

The stem-boring beetle *Oberea pedemontana* Chevrolat (Cerambycidae) is the only specialized beetle known from buckthorn in Europe. Although Contarini and Garagnani (1980) observed beetles in Italy to infest *F. alnus* and to avoid adjacent *R. cathartica* bushes, we found larvae in the branches of both buckthorn species in two neighbouring sympatric sites in northern Italy. In Serbia, we sampled *O. pedemontana* larvae in seven *R. cathartica* sites and one *F. alnus* site, but no adults could be collected on the host trees in five collection trips made in early summer perhaps due to cryptic or nocturnal adult behaviour. Field records confirmed that the beetle lacks specificity at the genus level, and it was rejected as a potential biological control agent of buckthorn in North America.

The root-boring moth *Synanthedon stomoxiformis* (Hübner) (Lep., Sesiidae) is widely distributed in the Palaearctic region (Doczkal and Rennwald 1992). There are three subspecies which are all associated with *Rhamnus* and *Frangula* species in different geographical areas in Europe and Asia Minor (Spatenka et al. 1999). *Synanthedon stomoxyformis* ssp. *stomoxiformis*, which was observed from *R. cathartica* and *F. alnus* between central-southern Europe to the Urals, has been found relatively commonly at several *R. cathartica* sites in Serbia, where its presence has been confirmed by the use of the pheromone lure SYMY *Synanthedon myopaeformis* (*PHERO*BANK®). *S. stomoxyformis* ssp. *stomoxiformis* has a biennial life cycle (Spatenka et al. 1999) and oviposits on the trunk and branches of buckthorn. Newly hatched larvae crawl down or fall from the oviposition site and start mining in the stem base or root. During the second year, larvae move further down, boring into the roots. In the autumn of that year, the larva builds a long and visible reddish exit tube aboveground, made out of scraps of organic material, sawdust and silk, in which pupation occurs and from which the adult emerges the following spring.

Larval development tests

Methods

Because mating and oviposition could not be achieved in confinement, we used eggs laid by two females mated under field conditions for no-choice larval development tests. The tests were carried out on potted plants in 3–10 replicates of 6 or 12 larvae on 15 plant species and six plant families.

Results and conclusion

The moth completed development in 1 year on all buckthorn species (table 1). Optimal larval development was observed on the European species, *F. alnus* and *R. alpina*. Larval survival was lower on the target plant *R. cathartica*, and similar to that recorded on the native North American species *R. alnifolia* and *F. caroliniana*. No larvae were found on any of the other 10 species tested outside the genera *Rhamnus* and *Frangula*. Larval development tests confirmed that *S. stomoxiformis* ssp. *stomoxiformis* lacks specificity at the genus level. The difficulty of achieving mating

Table 1 Larval survival and development of *Synanthedon stomoxiformis* in no-choice conditions

	2004			2005			
	No. of larvae/ replicate	No. of replicates	Total no. of larvae	Total no. of pupae	No. of larvae	Total% survival	Per cent of plants attacked
Rhamnaceae							
Rhamnus cathartica L.	6	15	90	0	15	16.7	60
R. alpina L.	6	10	60	17	6	38.3	90
R. alnifolia L'Hér.*	6	10	60	6	6	20.0	70
Frangula alnus P. Mill.	6	10	60	8	19	45.0	100
F. caroliniana (Walt.) Gray*	6	10	60	3	9	20.0	30
Hovenia dulcis Thunb.	6	10	60	0	0	0	0
Ziziphus ziziphus (L.) Karst	6	4	24	0	0	0	0
Elaeagnaceae							
Hippophae rhamnoides L.	12	5	60	0	0	0	0
Elaeagnus commutata Bernh. ex Rydb.	12	5	60	0	0	0	0
E. angustifolia L.	6	3	18	0	0	0	0
Vitaceae							
Parthenocissus tricuspidata (Sieb.&Zucc.) Planch.	12	5	60	0	0	0	0
Ampelopsis aconitifolia Bunge	6	5	30	0	0	0	0
Grossulariaceae							
Ribes rubrum L.	15	4	60	0	0	0	0
Rosaceae							
Sorbus aucuparia L.	12	5	60	0	0	0	0
Caprifoliaceae							
Lonicera xylosteum L.	12	5	60	0	0	0	0

*Native North America species.

and oviposition in confinement makes it difficult to determine the ecological host range of this species.

The stem-boring moth *Sorhagenia janiszewskae* Riedl (Lep., Cosmopterigidae) is found in most parts of Europe, except south of the Alps (Malicky and Sobhian 1971). The larvae mine the current year's growing shoots of *F. alnus* and more rarely those of *R. cathartica* and *R. alpina* (Malicky and Sobhian 1971; Gassmann et al. 2008). The species lacks therefore specificity at the genus level. Attempts of oviposition tests failed to provide reliable results for determining the ecological host range of this species.

Sap suckers (3 species)

The leaf-margin curl galler *Trichochermes walkeri* (Foerster) (Hom., Triozidae) is known only from *R. cathartica* in Europe (Ossiannilsson 1992). It is one of the most common insect species on *R. cathartica* and certainly one of the most conspicuous. Adults emerged in August. In a biology study, females started ovipositing 3–4 weeks after emergence. Eggs were laid on leaf bud axils. The nymphs hatched in spring from overwintered eggs and migrated to developing leaves, fed and induced rolling of the leaf margin. Host specificity was assessed using oviposition tests and subsequent larval and gall development.

No-choice adult survival and oviposition tests with newly emerged adults

Methods

Five buckthorn species and one no-plant control were individually tested with one newly emerged pair of *T. walkeri* in 12–20 replicates in small ventilated plastic cups (Ø 7.0 cm, height 8.5 cm) fixed on branches of potted plants. Adult mortality and oviposition were checked every 3-5 days, and males replaced. All plants were kept outdoors beneath a suspended tarpaulin, protected from rain and sun.

Results

Oviposition occurred only on the target plant, *R. cathartica* (table 2). First, eggs were recorded on *R. cathartica* about 30 days after set-up. Nearly 50% of the

Table 2 No-choice adult survival and oviposition tests with newly emerged *Trichochermes walkeri* adults

Test plants	No. of replicates (female)	No. of replicates with eggs	Total no. of eggs	Mean ± SD of eggs/ replicate (female)	Mean adult longevity ± SD (days)
Rhamnus cathartica	20	11	1493	74.7 ± 90.3	♂: 32.0 ± 28.1 ♀: 43.2 ± 27.0
*R. alnifolia**	15	0	0	0	♂: 5.6 ± 1.8 ♀: 8.2 ± 2.7
R. alpina	15	0	0	0	♂: 5.1 ± 2.2 ♀: 6.7 ± 3.2
Frangula alnus	15	0	0	0	♂: 6.7 ± 1.5 ♀: 9.9 ± 3.6
*F. caroliniana**	15	0	0	0	♂: 5.2 ± 1.4 ♀: 8.4 ± 3.4
No plant	12	0	0	0	♂: 4.5 ± 1.2 ♀: 6.4 ± 2.2

*Native North America species.

females on *R. cathartica* died before starting to oviposit. Females lived longer than males (overall means 14.0 ± 1.37 and 9.9 ± 1.37 days, respectively; $F_{1,174} = 4.56$, $P = 0.034$). Adult longevity was significantly higher on *R. cathartica* than on the other plant species ($F_{5,162} = 32.12$, $P < 0.001$). There was no significant difference in adult longevity between non-target plants and the no-plant control (Tukey HSD, $P > 0.45$) suggesting that little feeding occurred on these plants.

No-choice adult survival and oviposition tests with a 3-week feeding and pre-oviposition period on R. cathartica

Methods

Newly emerged adults were kept for 3 weeks on *R. cathartica* until they were ready to oviposit, as described above. Plant species were then individually tested with one pair of *T. walkeri* in 9–10 replicates as described above.

Results

Very little oviposition was recorded on non-target *Rhamnus* species with 3-week-old females (table 3). Females lived longer on *R. cathartica* than on the other plant species ($F_{4,43} = 31.46$, $P < 0.001$). Occasionally, the native North American species *R. alnifolia* sustained prolonged adult feeding: at the most, one female lived up 26 days but did not lay any eggs. There was no significant difference in female longevity among the non-target plants.

Sequential no-choice oviposition tests

Because very little oviposition occurred on non-target *Rhamnus* species in no-choice tests, we tested oviposition in sequential no-choice tests. This was under the assumption that females would survive on native North American *Rhamnus* species long enough in a post-release environment (before having the possibility to feed again on *R. cathartica*), to oviposit on perhaps less preferred but acceptable plant species.

Table 3 No-choice adult survival and oviposition tests with *Trichochermes walkeri* (after a 3-week feeding and pre-oviposition period on *Rhamnus cathartica*)

Test plants	No. of replicates	No. of replicates with eggs	Total no. of eggs	Mean ± SD of eggs/ replicate (female)	Mean female longevity ± SD (days)
Rhamnus cathartica	10	9	1164	116.3 ± 75.4	38.5 ± 15.6[a]
*R. alnifolia**	9	3	20	2.2 ± 4.2	9.8 ± 6.6[b]
R. alpina	9	1	1	0.1 ± 0.3	7.1 ± 2.0[b]
Frangula alnus	10	0	0	0	7.2 ± 2.2[b]
*F. caroliniana**	10	0	0	0	6.4 ± 1.0[b]

*Native North America species.
Letters indicate significant differences between test plants (Tukey HSD, $P < 0.05$).

Methods

Females and males were first exposed to *R. cathartica* for 4 weeks in groups of three pairs in ventilated plastic cylinders as described above. After this period, pairs of *T. walkeri* were transferred individually onto potted test or target plants as described above. Because previous no-choice adult feeding and survival tests showed that *T. walkeri* usually survives at least 3–4 days on non-target hosts, adult survival and oviposition were recorded every 3-4 days, and the plants were sequentially altered between the test plant and the target plant, *R. cathartica*. For each test plant, about 50% of the replicates started with the test plant and 50% with the target plant.

Results

Oviposition was high on the target plant, *R. cathartica* and the European species *R. alaternus* L. Oviposition was negligible on *R. alpina* and the native North American species *R. alnifolia* (table 4). Compared to the no-choice tests, oviposition on *R. cathartica* and female longevity were also reduced in the sequential no-choice oviposition tests, suggesting that the adults need to feed continuously on their field host to allow normal survival and reproductive output. In the *R. cathartica–F. alnus* series, all females died during the first exposure to *F. alnus*.

Single-choice tests

Because some oviposition occurred on *R. alnifolia* in sequential no-choice tests, single-choice tests were conducted to check whether this non-target North American species was attacked in the presence of the target weed.

Table 4 Sequential no-choice oviposition tests with *Trichochermes walkeri* (after a 3-week feeding and pre-oviposition period on *Rhamnus cathartica*)

	Mean no. of eggs/♀ (SD)	No. of ♀ × days of exposure	Mean female longevity in the series + SD (days)
Series 1 (N = 29)			
Rhamnus cathartica	11.7 ± 13.1	325	20.1 ± 12.7
*R. alnifolia**	0.6 ± 1.4	339	
Series 2 (N = 16)			
R. cathartica	10.2 ± 22.4	161	19.8 ± 13.5
R. alpina	1.3 ± 2.7	143	
Series 3 (N = 5)			
R. cathartica	49.0 ± 28.6	107	21.4 ± 8.3
R. alaternus	60.4 ± 40.1	85	
Series 4 (N = 11)			
R. cathartica	0.5 ± 1.2	40	9.0 ± 4.4
Frangula alnus	0	59	

*Native North America species.

Methods

Single-choice oviposition tests were carried out in five replicate 40 × 40 × 70 cm (l × w × h) cages each containing one potted *R. cathartica*, one potted *R. alnifolia* and three newly emerged *T. walkeri* pairs. All cages were kept outdoors beneath a suspended tarpaulin, protected from rain and sun.

Results

A total of 557 eggs were recorded on *R. cathartica* (mean = 111.4 ± 102.9; n = 5) and 24 eggs on *R. alnifolia* (mean = 4.8 ± 5.2; n = 5). On *R. alnifolia*, over 90% of the eggs were laid atypically on the trunk and branches. In contrast, on *R. cathartica*, over 60% of the eggs were laid on leaf bud axils, thus facilitating gall development in spring.

Larval and gall development

Methods

Branches with eggs of *T. walkeri* were marked with colour threads, and the pots were protected from contamination under a large gauze tent in a greenhouse until the end of November. All pots were then kept outdoors until late spring when the number of galled leaves, galls and larvae was counted.

Results

On *R. cathartica*, 13.7% of 2527 eggs developed into larvae in 2005–2006 and 30.5% of 855 eggs in 2008–2009. No galls and larvae were recorded from 24 eggs laid on *R. alnifolia* and from 302 eggs laid on *R. alaternus*.

Conclusions

Trichochermes walkeri is likely to be monophagous on *R. cathartica*. Some atypical oviposition without gall and larval development has been recorded on non-target hosts in the presence of the target weed. Because oviposition usually starts 3–4 weeks after adult emergence, oviposition on non-target hosts can be excluded in field situations where *R. cathartica* does not occur as *T. walkeri* females will die long before oviposition starts. *Frangula alnus* is not a suitable host for adult feeding and survival even in the alternate

presence of the target host. If feeding attempts occur, it is possible that *F. alnus* is lethal to the adults. By contrast, *R. alaternus* might provide a suitable food source for *T. walkeri*. In the sequential no-choice oviposition tests, the females laid a similar number of eggs on *R. cathartica* and *R. alaternus* although the leaf buds of the later species are smaller and tougher than those of *R. cathartica*. However, the leaf structure of *R. alaternus* is not suitable to allow gall and larval development of *T. walkeri*. More eggs laid on the native North American species *R. alnifolia* would be needed to ascertain that this species is not suitable for gall and larval development.

The discovery of a phytoplasma infection in *T. walkeri* adults and larvae (J. Jović unpublished results), however, renders this species problematic for biological control. '*Candidatus* Phytoplasma rhamni' was also detected in 25% of all *R. cathartica* samples at 12 sites in Switzerland, Germany, Austria and Serbia, but not in samples of *R. alpina*, *R. saxatilis* Jacq., *R. rupestris* and *F. alnus* (Jović et al. 2011). In contrast, the phytoplasma was not detected in a composite sample of several trees from 75 *R. cathartica* sites in Minnesota, USA (Becker and Mollov, unpubl. results). The presence of the phytoplasma could not be associated with any particular symptoms although a lethal witches' broom disease of *R. cathartica* was observed for the first time in the Rhine Valley in south-western Germany in the 1990s (Mäurer and Seemüller 1996). Non-destructive phytoplasma detection and clean mass rear of *T. walkeri* would be theoretically possible by feeding the adults with artificial media for at least 48 h to insure that infection rate is zero (Landi et al. 2013). However, adult mortality would likely be high and fitness of survivors much reduced because of inadequate food source. Another option would be to expose adults to healthy *R. cathartica* plants. However, plants could not be screened with 100% confidence. For example, if the defence mechanisms disabled propagation of the phytoplasma in the plant sieve elements, then the psyllid could be a vector, but the plant would show a negative reading of the phytoplasma. Additionally, single adult psyllids inject a low amount of phytoplasma bodies, which require time to multiply to a level which is detectable.

'*Candidatus* Phytoplasma rhamni' was also detected in *Cacopsylla rhamnicolla* (Scott) (Hom., Psyllidae) and *Trioza rhamni* (Schrank) (Hom., Triozidae), two other Psyllid species associated with *R. cathartica* in Europe (J. Jović unpublished results), making these species problematic for biological control of common buckthorn.

Defoliators (5 species)

The leaf-feeding moth *Ancylis apicella* (Denis & Schiffermüller) (Lep., Tortricidae) is widely distributed in Europe from the British Islands to Scandinavia and Asia Minor (Razowski 2003). Malicky et al. (1970) found *A. apicella* on *F. alnus*, *R. cathartica*, *R. saxatilis*, *R. alaternus* and *R. alpina*. Early larval instars develop singly within a folded leaf, later spinning two leaves flatly together, eating parenchyma and blanching the leaves in irregular patches. *A. apicella* is bivoltine and overwinters as a larva in a silk web in the soil.

Larval development tests

Methods

Adults reared from field-collected larvae bred easily in captivity. Eggs were usually laid on the lower leaf surface close to the veins. Preliminary no-choice larval development tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Five plant species were included in the test in 15–25 replicates.

Results and conclusions

Ancylis apicella larvae completed development on *F. alnus*, *R. cathartica*, *R. alpina* and the native North American species *R. alnifolia* and *F. caroliniana* (table 5). The pupae produced on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* ($F_{4,44}$=12.11, P = 0.036). Field observations and preliminary host range tests indicate that this species lacks specificity at the genus level. The difficulty of carrying out reliable oviposition tests in confinement makes it difficult to determine the ecological host range of *A. apicella*.

The geographical distribution of the congeneric leaf-feeding moth *Ancylis unculana* (Haworth) (Lep., Tortricidae) is similar to that of *A. apicella* (Razowski 2003). Unlike *A. apicella,* the species is found more commonly on *R. cathartica* than on *F. alnus* (Gassmann et al. 2008). No other field host is known in Europe. The biology of *A. unculana* and *A. apicella* are similar.

Larval development tests

Methods

Adults reared from field-collected larvae bred easily in captivity. Preliminary no-choice larval development

Table 5 Larval survival and development of *Ancylis apicella* and *A. unculana* on cut leaves in no-choice conditions

Test plant	No. replicates (L1)	Per cent larval development to the pupal stage	Pupal weight (mg) (mean ± SD) (N)
Ancylis apicella			
Rhamnus cathartica	25	72	11.1 ± 1.7[a] (13)
R. alpina	10	40	9.1 ± 2.2[ab] (3)
*R. alnifolia**	25	76	8.5 ± 1.5[b] (15)
Frangula alnus	15	53	9.6 ± 1.3[ab] (8)
*F. caroliniana**	25	40	9.6 ± 3.3[ab](10)
Ancylis unculana			
Rhamnus cathartica	30	37	9.6 ± 1.6 (11)
R. alpina	20	10	8.9 ± 3.4 (2)
*R. alnifolia**	30	40	9.3 ± 1.8 (12)
Frangula alnus	30	3	7.2 (1)
*F. caroliniana**	30	7	4.8 (1)

*Native North America species.
Letters indicate significant differences between test plants (Tukey HSD, $P < 0.05$).

tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Five plant species were included in the test in 20–30 replicates.

Results and conclusion

Rhamnus cathartica and *Frangula* species are less suitable hosts than *R. cathartica* and the native North American species *R. alnifolia* (table 5). Similar to *A. apicella*, this species lacks specificity at the genus level, and the difficulty of carrying out reliable oviposition tests in confinement makes it difficult to determine its ecological host range.

The leaf-feeding moth *Triphosa dubitata* L. (Lep., Geometridae) is widely distributed in Europe, but it is rare in most Northern Europe (Forster and Wohlfahrt 1981). *T. dubitata* larvae were found in small numbers on *R. cathartica* and *R. alpina* in nearly all surveyed areas in Austria, Germany, Switzerland and the Czech Republic (Gassmann et al. 2008). There is one record of *T. dubitata* on *F. alnus* (Malicky et al. 1970). The species overwinters as an adult in natural caves (Jacobi and Menne 1991), and females mate prior to hibernation (Malicky et al. 1970). Eggs and first-instar larvae can be found in late April. The species is univoltine.

Larval development tests

Methods

Preliminary no-choice larval development tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Neonate larvae were reared from eggs collected from *R. cathartica* and *R. alpina* and tested separately. Three and five plant species were included in the test, respectively, each with 15–35 replicates.

Results and conclusion

Larval survival to the pupal stage was higher on the native North American species *R. alnifolia* than on *R. cathartica* and *R. alpina* in both populations (table 6). No larvae developed to the pupal stage on *F. alnus* and the native North American species *F. caroliniana*. Time to pupation was significantly higher on *R. alpina* than on *R. cathartica* and the native North American species *R. alnifolia* for both populations. Pupal weight was significantly affected by both the

Table 6 Larval survival and development of *Triphosa dubitata* on cut leaves in no-choice conditions

Test plant	No. of replicates (L1)	Per cent larval development to the pupal stage	Time to pupation (days) (mean ± SD) (N)	Pupal weight (mg) (mean ± SD) (N)
From *R. cathartica*				
R. cathartica	25	48	40.2 ± 2.6 (12)[a]	140.0 ± 19.7 (12)[a]
R. alpina	29	38	46.5 ± 6.7 (11)[b]	116.5 ± 18.7 (11)[b]
*R. alnifolia**	21	81	40.6 ± 4.7 (17)[a]	135.3 ± 12.5 (17)[ab]
From *R. alpina*				
R. cathartica	35	49	40.5 ± 4.4 (17)[a]	127.7 ± 22.7 (17)[a]
R. alpina	35	37	48.9 ± 6.4 (13)[b]	100.0 ± 25.1 (13)[b]
*R. alnifolia**	30	70	41.3 ± 3.8 (21)[a]	127.4 ± 23.3 (21)[a]
*F. caroliniana**	19	0	0	0
F. alnus	15	0	0	0

*Native North America species.
Letters indicate significant differences between test plants (Tukey HSD, $P < 0.05$).

test plant and the field host plant (ANOVA: $F_{2,85} = 9.84^{***}$ and $F_{1,85} = 8.02^{**}$ for test plant and field host, respectively). *T. dubitata* is likely to be specific to the genus *Rhamnus,* but the native North American species *R. alnifolia* is a more suitable host for *T. dubitata* from either field host (*R. cathartica* and *R. alpina*) in no-choice larval development tests. Oviposition preference tests would be needed to assess the potential ecological host range of *T. dubitata*. However, this is not practical, given the adult biology of the species. These tests do not confirm species in genus *Frangula* as suitable host plants for larval development of *T. dubitata*.

The leaf-feeding moth *Philereme transversata* Hufnagel (Lep., Geometridae) is reported to be common across Europe (Carter 1987). *P. transversata* larvae were found in small numbers on *R. cathartica* and also very occasionally on *R. saxatilis, R. orbiculata* Bornm. and *F. alnus* (Malicky et al. 1970; Gassmann et al. 2008). The species is univoltine and hibernates in the egg stage.

Larval development tests

Methods

Adults reared from field-collected larvae did not breed easily in captivity. Preliminary no-choice larval development tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Four plant species were included in the test, each with 20–55 replicates.

Results and conclusions

The native North American species *R. alnifolia* was a less suitable host than *R. cathartica* (table 7). *F. alnus* and the native North American species *F. caroliniana* were not suitable host plants for larval development of this species. *P. transversata* is likely to be specific to the genus *Rhamnus*. In confinement, eggs of *P. transversata* were laid mostly on the cage frame making it difficult to determine the ecological host range of this species.

The congeneric leaf-feeding moth *Philereme vetulata* Denis & Schiffermüller (Lep., Geometridae) is widely distributed in Europe where it can be locally abundant (Forster and Wohlfahrt 1981). *P. vetulata* is associated exclusively with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965). Larvae feed within young folded leaves. *P. vetulata* is univoltine and overwinters in the egg stage on the bark of its host plant.

Larval development tests

Methods

Adults reared from field-collected larvae bred easily in cardboard cylinders (Ø 10 cm, height 27 cm). Preliminary no-choice larval development tests consisted of (i) one neonate larva offered cut shoots of four plant species with young folded leaves in individual Petri dishes and (ii) 5–10 neonate larvae transferred each onto potted plants of five species with newly developed leaf buds.

Results and conclusion

Drying of cut plant material resulted in a much higher larval mortality in Petri dishes than on potted plants (tables 7, 8). On cut shoots, larval development to the pupal stage was much higher on *R. cathartica* than on

Table 7 Larval survival and development of *Philereme transversata* and *P. vetulata* on cut leaves in no-choice conditions

Test plant	No. of replicates (L1)	Per cent larval development to the pupal stage	Time to pupation (days) (mean ± SD) (N)	Pupal weight (mg) (mean ± SD) (N)
Philereme transversata				
Rhamnus cathartica	40	23	35.7 ± 3.4 (9)	83.7 ± 24.5 (9)
*R. alnifolia**	20	5	36	47
Frangula alnus	55	0	0	0
*F. caroliniana**	40	0	0	0
Philereme vetulata				
Rhamnus cathartica	55	24	35.5 ± 2.1 (13)	53.9 ± 6.2 (13)
*R. alnifolia**	50	6	40.7 ± 5.1 (3)	39.5 ± 23.2 (3)
Frangula alnus	60	0	0	0
*F. caroliniana**	60	0	0	0

*Native North America species.

Table 8 Larval survival and development of *Philereme vetulata* on potted plants in no-choice conditions

Test Plant	No. of L1 transferred (No. of potted plants)	Per cent larval development to the pupal stage	Time to pupation (days) (mean ± SD) (N)	Pupal weight (mg) (mean ± SD) (N)
Rhamnus cathartica	119 (25)	72	32.8 ± 3.7 (86)[a]	0.055 ± 0.012 (86)[a]
R. alpina	80 (9)	60	37.3 ± 3.9 (40)[c]	0.051 ± 0.010 (48)[ab]
*R. alnifolia**	58 (5)	69	34.5 ± 2.4 (48)[b]	0.046 ± 0.001 (40)[b]
F. alnus	80 (12)	0	0	0
*F. caroliniana**	75 (10)	0	0	0

*Native North American species.
Letters indicate significant differences (Tukey HSD, $P < 0.05$).

the native North American species *R. alnifolia*. On potted plants, larval development to the pupal stage was similar on *R. cathartica*, *R. alpina* and *R. alnifolia*. However, pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica*. The time to pupation was shortest on the field host *R. cathartica*. No larval establishment or damage was observed on *F. alnus* and *F. caroliniana*. *P. vetulata* appears to be specific to the genus *Rhamnus*. No oviposition was recorded on *R. cathartica* in confinement, thus making it difficult to determine the ecological host range of this species.

Fruit and seed feeders (1 species)

From the two midge species known on *R. cathartica* (*Wachtliella krumbholzi* Stelter and *Lasioptera kozarzewskella* Mar.), only the former has been collected during surveys for potential biological control agents of buckthorn (Gassmann et al. 2008). No midges were reared from the fruits of *F. alnus* collected at two sites in Austria and in Switzerland where *R. cathartica* and *F. alnus* co-occur. The main characteristics of fruits attacked by *W. krumbholzi* are a change in colour resembling premature fruit maturation, fruits larger in size and irregular shape. Once mature, the midge larva leaves the fruits and enters the soil to prepare a larval cocoon made of silk and debris. Field records suggest that *W. krumbholzi* is specific to *R. cathartica*. Due to the inability to obtain fruits on potted target and non-target *Rhamnus* species, no host specificity testing could be carried out with *W. krumbholzi*.

Discussion

In 2001, a new research programme to develop biological control for common buckthorn was initiated, taking into account increasing concerns about the safety of native plants in the potential release areas of biological control agents. Candidate biological control agents would need to be monospecific to *R. cathartica* or their host ranges restricted to a few non-native species in the genus *Rhamnus*. Over 30 specialized insects were identified from *R. cathartica*, most of them with a likely lack of specificity at the species or genus level (Gassmann et al. 2008). Field observations and preliminary host range tests confirmed that the three internal feeders associated with buckthorn in Europe lack specificity at the genus level. Literature records of 12 leaf-feeding Lepidoptera known from buckthorn in Europe (Gassmann et al. 2008) combined with our work on another five species suggest that specificity requirements will not be met with those species as well.

Three psyllids, *T. walkeri*, *C. rhamnicolla* and *T. rhamni* are promising in terms of host specificity, but are infected with the plant disease '*Candidatus* Phytoplasma rhamni'. Transmission trials with *R. cathartica* were negative twelve months after exposure to phytoplasma-infected *T. walkeri* adults (J. Jović unpublished results). Due to the limited research on this disease and as it is not known to be present in the United States, there is low potential that the psyllids would be approved for release in the United States.

There is increasing evidence from studies of biological control of invasive trees in South Africa that reduction in the levels of seeding and hence of seedling recruitment by biological control agents greatly facilitate the management of invasive woody plants (Moran et al. 2004). Seed-feeding midges seem to be potentially host specific enough for further research on biological control of buckthorn. However, attempts to work with *W. krumbholzi* proved to be difficult in a research setting as it was not possible to obtain adult fruiting trees of native North American *Rhamnus* species for testing in Switzerland.

All arthropods considered for biological control of buckthorns so far have been discarded from further

consideration because of either a lack of specificity at the species or genus level, the occurrence of a phytoplasma disease or the lack of feasibility of host range testing. For example, there are a few potentially genus-specific Lepidoptera, that is *P. vetulata*, *P. transversata* and *T. dubitata*, however, it was not possible to achieve oviposition in confinement and to assess their ecological host range. Given that Lepidoptera have not shown to contribute significantly to the successful control of invasive trees and shrubs (Moran et al. 2004; Gassmann et al. 2010), it is questionable to pursue host range testing with lepidopteran species to demonstrate a hypothetical specific host range. Other species known from buckthorns such as the mirids, *Heterocordylus erythrophtalmus* Hb and *Lygocoris rhamnicola* Reuter and the free-living or erineum gall mites, *Aceria rhamni* Roiv., *Tetra rhamni* Roiv., *Eriophyes rhamni* (Pgst) and *Phyllocoptes annulatus* (Nal.) were not considered in this project because of either their lack of visible impact on the target plant or the lack of feedback in using such organisms in biological control of weeds. Also, species in the genus *Rhamnus* are dioecious making plant breeding to the reproductive stage difficult for testing fruit- or seed-feeding candidate agents.

After 11 years of searching for biological control arthropods that are host specific and damaging to buckthorn, we conclude that we do not have any promising agents based on what is known to date. It is rarely the case in weed biological control that a project is terminated without field releasing any agents. One further recent example, however, includes biological control of *Potentilla recta* L. (sulphur cinquefoil) (Cortat et al. 2013).

Pathogens have not yet been considered for biological control of buckthorn. Based on literature and herbarium records from the Royal Botanical Gardens, Kew, UK, a few pathogens show potential as biological control agents, for example *Coniothyrium rhamnigenum* (Sacc.) Bubák (leaf spot damage), *Septoria rhamni-cathartica* Ces (leaf spot damage), *Mycosphaerella vogelii* (Syd.) Tomilin (leaf spot damage, host record on *R. alnifolia* needs to be confirmed) and *Phyllosticta rhamnicola* Desm. (leaf spot damage). We believe that pathogens could therefore potentially offer new opportunities for biological control of *R. cathartica* in North America.

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SESSION 8: SOCIAL AND ECONOMIC ASSESSMENTS OF BIOLOGICAL CONTROL

The Garlic Mustard (*Alliaria petiolata*) Case, What Makes a Good Biological Control Target: The Intersection of Science, Perspectives, Policy and Regulation

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Abstract

In this paper, we present an overview of our shared experiences from a thirteen-year discovery and testing period in search of effective biological control agents for garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande). Our experiences during this time reflect much of the dialog, debate, dilemmas, and policy discussions occurring in biological control of weeds today. For example, in the last decade, the values that underpin biological control, as well as standard requirements and stakeholder perspectives have been in a state of flux. Many research programs fail to sustain funding for such long pre-release periods. Policy goals and acceptable safety criteria have changed. Moreover, the fundamental perception of garlic mustard as a pest is shifting, leading some to question whether garlic mustard is a driver of change in invaded habitats or rather a symptom of habitat disruption. If it is a symptom, this can shift the perception of the risks of biocontrol. In this shifting scientific and social milieu, land managers are still challenged by stakeholder demands for management of garlic mustard. Land managers have a responsibility to manage their sites for the purposes for which the land is preserved and have limited control, or no control over potential higher-level drivers such as earthworms, deer, climate change, and human population pressures. The intent of this presentation is to discuss these and other issues common to many who work in biological control, framing the discussion within our garlic mustard experience as the basis for dialog.

Background

Awareness and public interest in garlic mustard invasion of hardwood forests in the Midwest and Northeastern USA gained significant momentum in the 1980s with a series of key publications by Nuzzo (1991; 1996) and Nuzzo et al. (1996), culminating in the effort to develop a biological control program that started in 1998 with a broad base of support. At that time, available assessments 'indicated that the only viable long-term option for successful management of garlic mustard is classical biological control' (Blossey et al., 2001a). As part of the biological control project, a test plant list was developed, and CABI in Delémont, Switzerland contracted to conduct surveys and host specificity testing of candidate agents. Additional host specificity testing began in 2003 at the University of Minnesota, USA on plant species that were difficult to obtain or grow in Switzerland. Throughout this process stakeholders were engaged through various workshops (e.g. Skinner, 2005) and in the development (Blossey, 1999) and implementation of a long-term monitoring protocol for garlic mustard. The availability of pre-release data would allow us to gauge the impacts of anticipated biological control agent release(s) (Evans and Landis, 2007; Van Riper et al., 2010).

Gerber et al. (2009) summarized the biology and host-specificity results for the root-crown mining weevil (*Ceutorhynchus scrobicollis* Nerensheimer and Wagner) based on which, a petition for field release of the species was submitted in 2008 to the USDA APHIS TAG (United States Department of Agriculture, Animal and Plant Health Inspection Service, Technical Advisory Group). Based on the comments of reviewers, additional host testing was conducted from 2009 through 2011. Responses to reviewer comments to the 2008 petition and the results of additional host specificity testing were re-submitted to TAG in September of 2011.

Discussion

How safe is safe enough?

Typical of many weed biocontrol endeavors, the effort to release a biological control insect for garlic mustard in North America has been long and arduous. Thirteen years after officially initiating the research, we are awaiting TAG review of our latest submission. Much has changed during this time. For example, phylogenetic relationships among tribes within the Brassicaceae were redefined (Al-Shehbaz et al., 2006), necessitating continuous adaptation of our test plant list. Also, during this lengthy testing period, the concept of acceptable risk has changed. As common in risk assessments, "safe enough" is rarely achieved to the satisfaction of all stakeholders. One might conclude that agreement is rarely achieved now compared to biocontrol programs in decades past, as seen in papers presented at this conference. Lincoln Smith *(in press*) discussed an insect which has broad support for yellow starthistle (*Centaura solstitialis* L.) control, but ultimately was not approved for release. A retrospective review of past agents approved for release was presented by Hinz et al. *(in press)*, exploring the possibility that most of these agents would not be approved in today's regulatory climate in the USA.

In our case, *C. scrobicollis* did develop on the commercially grown watercress (*Nasturtium officinale* Ait. f.). Adult development on watercress was not consistent throughout tests conducted in different years. Moreover, *C. scrobicollis* development was only found when watercress was grown in artificial dryland mesocosms. Cultivated watercress is grown under water-saturated conditions (e.g., in running water). In refined host-specificity tests altered to simulate these growing conditions, *C. scrobicollis* was not able to complete its development on watercress. Additionally, *C. scrobicollis* has not been recorded as an economic pest, nor even in association with watercress in its native range where both co-exit, arguably the most comprehensive specificity testing possible.

While the overall host specificity package for *C. scrobicollis* on garlic mustard in North America suggests the ecological host range will be narrower than the physiological host range with the latter defined as development under highly artificial laboratory conditions, such data points could prove troublesome for the approval process. 'Troublesome' data points refer to data generated under circumstances which render the data suspect upon further scientific scrutiny. Once generated, however, these 'troublesome' data points do not go

away. Despite subsequent work that more accurately reflects scientifically valid outcomes, the initial data remains in the body of evidence submitted for approval and often result in lingering concerns, particularly at the policy level, rather than at the scientific review process for biological control agents within USDA APHIS.

Is garlic mustard really that bad?

Another phenomenon that has evolved during our lengthy testing period is the notion that yesterday's demonized pest may become today's ecosystem services star. Apropos the papers presented at this symposium by Dudley et al. *(in press)* and Norton et al. *(in press)* discussed litigation over biological control of saltcedar (*Tamarix* spp.) impacting the southwestern willow flycatcher (*Empidonax traillii extimus* A.R. Phillips). Campaigns to disparage target invasives are common, prompting critical reviews reflecting on the fear-based language used with the public to generate support for control efforts (Gobster, 2005). Indeed, we used the Good, the Bad, and the Ugly campaign effectively in Minnesota to generate support for the biological control of purple loosestrife (*Lythrum salicaria* L.). Paradoxically, this terminology is now being used by opponents of biological control to describe the biological control agents. Warner & Kinslow (2011) explored this phenomenon more broadly in the context of manipulating risk communication to the public in the case of biological control of the strawberry guava tree (*Psidium cattleianum* Sabine) in Hawaii, resulting in an outcome different than intended by the scientific and conservation communities.

In the thirteen years since our effort began on garlic mustard, views of how we view this plant are evolving. Some studies have shown negative impacts of garlic mustard in invaded ecosystems while others found no impacts. Is garlic mustard a principal driver of detrimental impacts? Research showed that garlic mustard competition for light negatively impacted tree seedlings and annual herbaceous species (Anderson et al., 1996; Cipollini and Enright, 2009; Meekins and McCarthy, 1999), altered nutrient levels (Rodgers et al., 2008), and was toxic to arbuscular mycorrhizal fungi which could result in altered nutrient and water acquisition by many native species (Callaway et al., 2008; Cipollini and Gruner, 2007; Roberts & Anderson, 2001). Of concern to the forest industry, research suggested garlic mustard negatively impacted desirable tree seedlings (Stinson et al., 2006).

Alternatively, is the presence of garlic mustard merely a symptom of a response to higher-level changes? Indeed, garlic mustard often is observed in disturbed areas that lack native cover (Trimbur, 1973; Nuzzo, 1991; Van Riper et al., 2010). Recently it has been proposed that the action of deer and earthworms facilitate garlic mustard invasion (Blossey et al., 2005; Knight et al., 2009; Nuzzo et al., 2009). Deer herbivory on natives can create disturbed microsites that promote dispersal of garlic mustard seeds (Anderson et al., 1996). Loss of native plants may create suitable conditions for garlic mustard invasion through increased light levels, moisture, and nutrient availability (Anderson et al., 1996) and decreased litter levels (Trimbur, 1973), as well as through anthropogenic effects such as erosion.

Who is the driver?

If garlic mustard is not the principal driver of negative impacts, some on our team propose that we should focus efforts on the higher-level drivers (e.g., deer and earthworms), not the symptoms (e.g., garlic mustard). Such ideas are gaining support in the ecological literature where for example, Davis (2011) argued that species such as garlic mustard do not pose as big a threat as scientists think. Some are finding evidence that native insects impacted by garlic mustard may be adapting to it (Keeler and Chew, 2008). As a result, after a decade of testing, we have reached the juncture where our group is discussing whether we should release *C. scrobicollis* even if approved by TAG.

Exotic earthworms are widely discussed relevant to invasion in forest ecosystems (Nuzzo et al., 2009) and once established, few, if any management options exist to remove them. There has long been evidence about the negative impacts of deer on native plants (e.g., Hough, 1965; Tilghman, 1989; Diamond, 1992). However, limiting deer populations is difficult. State natural resource agencies both promote deer for hunting and as an income generator via hunting permits, while concomitantly expending resources to remove deer or to install exclusion devices to promote regeneration of tree species impacted

by deer browse. Neither of these factors is likely to change significantly in the near term. Also, it is not clear how the public would react to deer herd reductions to the low level required to reduce disturbance to a degree that may stop the invasion of plants like garlic mustard.

What is involved if land managers were to shift from managing garlic mustard to instead managing higher-level drivers? Figure 1 shows the relative geographic scale and management difficulty of several drivers that impact invasive species. This concept was adapted from a CABI Biosciences schematic depicting the centrifugal phylogenetic method. This driver schematic assumes garlic mustard as a symptom, not a driver. As we move out from the center, the geographic scale of the potential negative impact of the driver, and concomitantly, the difficulty in altering that impact increases. Earthworms are problematic, but at present are less widely distributed in the Midwest USA compared to deer. As we move to a wider geographic scale, anthropogenic effects such as pollution (e.g., nutrient loading, sediment runoff, etc.) and more broadly, climate change are clearly drivers of negative environmental change. Managing drivers such as climate change is distinctly long-term and the outcome uncertain. Ultimately, it is people and the resultant impact of our lifestyles and actions that is the overarching driver. Changing any of these on a scale to reduce negative impacts to ecosystems is a daunting endeavor, especially for a land manager.

Will garlic mustard go away?

During the time invested to find a biological control agent for garlic mustard, some members of our team have observed a decline in long-standing populations of garlic mustard absent the introduction of a biological control agent (Blossey and Nuzzo, *in press)*. Perhaps we are just seeing the beginning of a decline in garlic mustard populations in North America, or are these population density fluctuations, related to climate cycles reflecting the natural ebb and flow of invasive species? If populations do significantly decline, will they resurge and expand to a point where we have populations of garlic mustard that are even more widely dispersed?

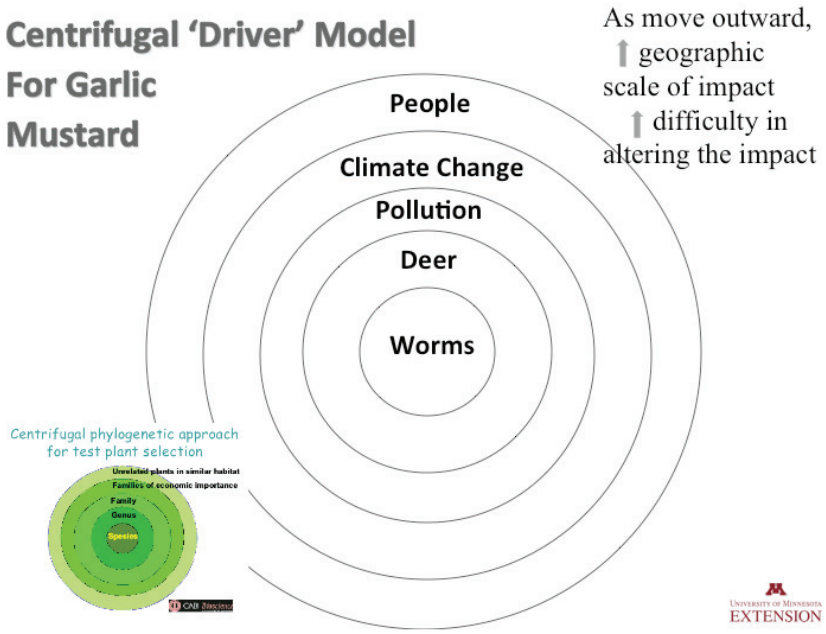

Figure 1. The centrifugal driver model. An adaptation of a CABI diagram depicting the centrifugal phylogenetic method of Wapsphere (1974).

Additionally, would there be a benefit to uninvaded communities if a biocontrol agent could avoid a boom and bust cycle of garlic mustard?

More broadly, the field of ecology is exploring fluctuations in population densities of invasive species (Simberloff and Gibbons, 2004, Ahern et al., 2010). In the experiences of an Extension State Weed Scientist at the University of Minnesota (Becker), it is well known that species shift, population densities ebb and flow, and weed patches move around on the landscape. Landscape-scale changes in problem species in agricultural systems are driven by what weed scientists call the 'big hammers'; typically system-wide shifts in tillage, fertility, periodicity of operations, or the periodic dominance of one herbicide mode of action in the marketplace. An example of dramatic population fluctuations that may inform invasions that dominate the landscape and then moderate, was the effort in the USA to domesticate the native common milkweed (*Asclepias syriaca* L.) during World War II to produce floss (pappi) to fill life jackets when imports of goose down were blocked. Common milkweed in North America can be found throughout a broad habitat range. As it naturally occurs, common milkweed remains at low population densities and scattered across the landscape. In attempts at domestic production, when planted in monocultures in fields, density-dependant diseases quickly became an impediment to successfully growing the crop in many locales, and in many cases resulted in abandonment of fields. Experienced weed scientists often recount such phenomena, but as is often the case with experiential knowledge, it is seldom documented in peer-reviewed journal articles. Similar to the disease limiting phenomena seen in milkweed, we have observed that Canada thistle populations approaching monotypic stands decline after six to seven years due to generalist pathogens *Fusarium* and *Pythium* resulting in reduced population densities that are relatively dispersed.

Herbaceous perennial or biennial weeds in the Upper Midwest USA are dynamic in population density, population size, and location in response to climate. Minnesota is at the intersection of the hardwood forest, boreal forest, and the tall grass prairie regions of the USA. Here, herbaceous invasive plants respond to temperature and moisture cycles. Historically, these occurred in 20-year cycles in records since the 1800s, but with climate change, the cycles are lengthening and becoming more local with drought and flood cycles occurring in the same season within the same county (Minnesota Climatology Working Group, 2011). These climate changes can be tracked by shifts in the species that become problematic for land managers. During wet cycles in Minnesota it is common to see Canada thistle (*Cirsium arvense* (L.) Scop.) and buttercup (*Ranunculus* spp.) thrive and expand geographically. Conversely, during dry cycles hoary alyssum (*Berteroa incana* (L.) DC.), wormwoods (*Artemisia absinthium* L.), and leafy spurge (*Euphorbia esula* L.*)* thrive and expand. This increase in localized variability due to climate change will accentuate changes in population dynamics of many of the invasive weeds with which we work.

Garlic mustard is a biennial species cycling in a perennial system. Sustaining a population is wholly dependent on constant regeneration of rosettes from seedlings. Seedling regeneration depends on disturbance and is subject to episodic widespread seedling mortality. At some of our garlic mustard monitoring sites in Minnesota, we see cycles where either the seedling/rosette or the flowering second-year growth stage dominate in a given year, while at other sites they occur simultaneously (Van Riper et al., 2010). By its biennial nature, garlic mustard populations will fluctuate dramatically, and in extreme climatic events, may even skip population cycles altogether, only to resurface in the future. Thus, multiple forces are at work resulting in garlic mustard populations that are very dynamic. Our challenge is to determine the long-term trends, and what that means within the construct of our original justification for biological control of garlic mustard.

Conclusions

Many on our team were also part of the biological control effort of purple loosestrife in North America, informing our approach to biological control of garlic mustard. Many of the same stakeholders and funding sources were used in both efforts, and the perceived success of purple loosestrife biocontrol resulted in built-in enthusiasm for the garlic mustard effort. For example, the network of pre-release garlic mustard monitoring sites included many managers who were

cooperators on the purple loosestrife effort. Blossey et al. (2001b) was in part a response to criticisms of the purple loosestrife biological control effort. Yet almost two decades after the release of *Galerucella* spp. for biological control of purple loosestrife, science has not settled the debate surrounding biological control of that invasive species (Lavoie, 2010). More studies are being proposed to answer the next set of garlic mustard research questions. We are on the cusp of gaining approval for release of *C. scrobicollis*, but are debating similar questions that are still debated for purple loosestrife. Experience indicates that scientific discourse will be unable to expeditiously address the complex interactions to manage higher-level drivers, nor quickly settle the more direct question of whether invasion by garlic mustard negatively impacts native ecosystems.

So, considering the debate over whether garlic mustard negatively impacts forest ecosystems and whether it is only present because of higher-level drivers, what can land managers do in response to public demands for action? As is the case for many pest problems, the default action is to treat the symptoms – in this case an invasive weed that has become abundant. This option is something we can do and can measure the success of in terms of cost and effectiveness, providing justification to those who fund such programs. Control of invasive, noxious weeds is often required via regulated weed laws in the USA. Managing higher-level drivers arguably might be the most efficacious and efficient approach; however, it would involve a higher degree of complexity, is more difficult to implement, and is an approach that takes a long time to provide results, thus, making it more challenging to garner and maintain support.

One of our team members summed it up this way: We should address the symptoms, i.e., control garlic mustard if it: 1) provides additional time to address root causes, 2) prevents degradation in the meantime, 3) poses minimal risks, and 4) does not clearly jeopardize a long term solution. Doing so may spare uninvaded and minimally invaded habitat in the Midwest the upheaval of a garlic mustard invasion. This may not be true in parts of the northeast. Midwest ecosystems could benefit from delay or reduction of garlic mustard invasion considering our host specificity data suggest minimal risk.

We are left with a dilemma. On one hand, we must consider the implications of releasing an organism against a pest that may not be the root cause of detrimental changes. This would be an especially egregious error if the biological control agent caused unintended nontarget damage in the future. On the other hand, we must also consider the implications of not releasing a biological agent deemed safe for a target that many stakeholders feel has significant negative impacts. Managers may not be able to eliminate earthworms and deer, but biocontrol could give them a tool to reduce one stressor to the system: garlic mustard. Not releasing a biocontrol agent is particularly problematic if future work confirms significant impacts on forest ecosystems, and populations do not undergo a natural decline but rather persist across the landscape. Considering the ongoing controversies regarding biological control of weeds, we must also reflect on the implications these two scenarios may have for the future of biological control of weeds, both from a policy and funding viewpoint.

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Public Engagement with Biological Control of Invasive Plants: The State of the Question

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Abstract

The practice of biocontrol has been impacted by in the evolution of environmental values in societies, and difficulties in obtaining release permits. These challenge biocontrol stakeholders, researchers and regulators to foster more effective public engagement with invasive species management. To succeed, public engagement requires the disambiguation of research activities from public agency decision making. This requires greater up-front investment in public communication and consultation, and more transparency by agencies in the application of their decision making criteria. However, these additional costs can be offset if the result is attenuated surrounding controversies and amplified public support for invasive plant control. This article draws from a five year comparative study of biocontrol practice, policy & public engagement in the U.S., South Africa, New Zealand, and Australia. It presents key findings to guide public engagement with biocontrol of invasive plants.

Introduction

The social context of weed biocontrol has changed dramatically since the first International Symposium on Biological Control of Weeds (ISBCW). Formerly, biocontrol researchers labored in autonomy from society, but now they are increasingly expected to communicate their work to non-expert public officials and members of the public. With the rise of environmental values and legislation, environmental scientists and agencies funded with public monies were increasingly asked to justify their activities to the public (Speth, 2004). This gave rise to early efforts to cultivate public support for biocontrol of invasive plants, using the tools of public outreach and public consultation. These early efforts push information out to the public, or gather comments from the public. In the 21st century social context, unidirectional communication to or from the public regarding science is not sufficient to garner public monies, nor public support, for any type of scientific activity.

By studying cases where there is greater public support for the application of science and technology in addressing social needs, social scientists have articulated a new model for relating scientists and their institutions to society: public engagement (McCallie et al., 2009). Unlike the unidirectional communication implicit in public communication and comment, participatory public engagement with science and technology (shortened to "public engagement") facilitates mutual learning among publics, scientists, and others with respect to the development and application of science and technology in modern society (Rowe and Frewer, 2005; Mooney, 2010). Public engagement is more costly in terms of time and resources. However, members of the public are challenging publicly-funded researchers and regulatory agencies to be more transparent in their decision making, and the early models of public communication do not support effective responses. Public engagement has the potential to cultivate greater public support for biocontrol.

Invasive plant control in the future will require more than passive public support. It will require active and sustained engagement by citizens and stakeholders, who reasonably expect public agencies to demonstrate how this practice addresses economic and conservation goals. Public engagement fulfills democratic values, which is especially important for any public interest science (Warner et al., 2011), but also good practice to minimize social conflicts over biocontrol agent releases (Warner and Kinslow, *in press*). Since the public funds most invasive species control programs, it is reasonable to educate and engage them on a continuing basis. Controversies surrounding the introduction of biocontrol agents have dogged high profile and costly restoration projects, and the future of classical biocontrol as an invasive species management practice is threatened by persistent unresolved controversies (Strong and Pemberton, 2000; Warner, *in press*). To be effective, public engagement must:

1. Construct greater social understanding of the problems of invasive plants;

2. Create greater social consensus on the need to control invasive plants and the conditions under which biocontrol is a socially preferable approach; and

3. Incrementally increase the public's trust that government agencies are upholding the public's interest through appropriate regulatory review.

Here is the state of the question: "could greater public engagement with biocontrol of invasive plants foster greater stakeholder support without hindering research?" Public engagement challenges scientists and their institutions to develop skills in public communication, and challenges public regulatory institutions to facilitate appropriate public review with biocontrol release decisions. Researchers and public agencies need forms of public engagement that do not:

1. Interfere with scientific research and practice;

2. Impose significant additional burdens on their own time;

3. Delay regulatory review.

To avoid these problems, public engagement should disambiguate scientific research activities from public agency decision making. This requires greater up-front investment in public communication and consultation, and more transparency by agencies in the application of their decision making criteria. However, these additional costs can be offset if the result is attenuated surrounding controversies and amplified public support for invasive plant control. The balance of this paper introduces the material and methods supporting this study; explains how public engagement differs from early forms of public communication; and summarizes conclusions from this study.

Methods and Materials

Social science field work was conducted in the U.S., South Africa, Australia and New Zealand. Between 2007 and 2009, 183 semi-structured interviews were conducted with 178 research scientists, laboratory directors, regulators, communication officers, critics, and clients of the practice of classical biocontrol in all four countries. Interviews addressed the following topics: the history of invasive species control, and biocontrol practice and their institutions; the impact of rising concern about nontarget effects of biocontrol agents; and how legislation and regulatory institutions have responded to risk concerns regarding biocontrol agent introductions. Several of these interviewees provided extensive documentation on the policymaking and regulatory processes in these countries.

Results

The introduction of a novel biocontrol agent is a socio-political decision as well as a biological and environmental action. At the time of the first ISBCW in 1969, virtually all biocontrol decisions could be made within one public agency. Target selection, agent selection, testing criteria, and release permitting were all internal to one institution, often a department of agriculture. Now these decisions are generally distributed between multiple public agencies and are at times more contested, reflecting broader social unease about environmental issues.

Public communication by public agencies has improved over the past 20 years, but the new media environment is remaking social context for mass communication faster than public agencies can respond (Press and Williams, 2010). The new media environment highlights trust-destroying events: facts and science are disputed, the public doubts the existence of the problem, and public skepticism of proposed remedies grows. This results in fraying of the relationship between scientists and society at large, and undermines the ability of scientists to address social needs. Public mistrust in scientists and government agencies (and what they do) is generally on the rise. Studies have demonstrated that the public does not evaluate novel risks on the basis of data, but on the basis of trust based on the trustworthiness of messengers. This finding has been repeatedly confirmed across scientific applications and novel technologies (Slovic, 2001). This mistrust—and the potential of public engagement to foster trust -- is an issue that has implications for all stakeholders in the biocontrol of invasive plants: researchers, regulators, conservationists, and beneficiaries.

Public engagement is a semi-structured transparent deliberative process that establishes consensus views on evidence, method, interpretation, and social values frameworks as the basis for making a scientifically-informed decision (Rowe and Frewer, 2005). Public engagement differs from public outreach or consultation in that it requires bidirectional communication between scientists, decision makers, and lay publics (McCallie et al., 2009). It is a deliberative "dialogue" in which publics and scientists both benefit from listening to and learning from one another, which can be described as mutual learning (McCallie et al., 2009). Public engagement includes members of the public doing more than merely asking questions of experts. It requires scientists to do more than merely present their knowledge and perspectives. Public engagement requires lay publics to learn about science and policy, and scientists to learn what members of the lay public know and don't know about science, but also about social values. Thus, "engagement" in this sense includes both political engagement and educational engagement. Participants from a variety of perspectives participate over a sustained period of time, guided by shared goals and a code of conduct. It has the ability to actually foster trust and consensus (McCallie et al., 2009).

The U.S. was a pioneer in early models of public participation; however, 1970s era legislation required only public communication and gathering public comments. This model is now unable to support social expectations of transparency and the need to cultivate active public participation these decisions. In the U.S., biocontrol agent review and permitting are functionally inaccessible to the public, and have remained so despite calls for greater transparency, peer review and public input (Strong and Pemberton, 2000). In contrast, New Zealand has created participatory public processes for identifying targets and cultivating support for biocontrol projects, and has created a new agency to review proposed introductions of all novel organisms, including biocontrol agents. New Zealand has a national extension system for the biological control of weeds (Hayes, 1999). Although described as a technology transfer program, in reality it is much more sophisticated, for it trains local land managers in the ecology of weeds, the management of released control agents, and public outreach. This has the potential to prompt public interest and demand for invasive species control. In 1996, New Zealand passed legislation to require transparency in decision-making processes regarding proposed novel organism introductions. It also requires the applicant to provide evidence of anticipated benefits exceeding risks (Campbell, 2010). This has created the world's most sophisticated decision-making process for evaluating novel organism introductions, with explicit reference to biocontrol agent introductions. It lays out clear decision-making criteria based on transparent and replicable ecologically-based risk-cost-benefit analysis, fixed time periods for

decisions, and participatory public engagement (Campbell, 2010). New Zealand has developed biocontrol decision making structures that best reflect the goals and methods of participatory public engagement. Australia and South Africa have also undertaken efforts to enhance public engagement activities surrounding biocontrol.

Discussion

This section summarizes key findings emerging from this study.

Most members of the public are not interested in invasive plants

There is little return on efforts to reach out to generic publics. Instead, public engagement strategies suggest public agencies should identify, reach out to, and convene all possible stakeholders, especially including potential critics. For public engagement to succeed, it is essential to begin by identifying stakeholders with strongly-held opinions, pro or con, and to convene them in a dialogical process. Stakeholders with strongly-held opinions -- but are unknown to those leading biocontrol projects -- are those most likely to contest and delay biocontrol projects. Identifying these stakeholders is a task proper to public agencies and the stakeholders themselves. For example, Australia has an on-line stakeholder registry, and New Zealand actively encourages public comments on proposed introductions. However, these need to be designed so as to not amplify risk concerns (Slovic, 2001).

A public process should enhance the capacity of stakeholders to understand science and agency decision making processes

For public engagement to succeed, it must convene a structured co-learning process in which everyone, from critics to supporters, participates over time in establishing the same scientific information about the invasive species and possible control methods. Public engagement fails if parties have divergent information about the problem and possible remedies. Most public concerns about biocontrol are founded, at least loosely, on conservation values, such as: is the invasive plant really a problem?; why introduce another organism?; what other organisms will the agent attack?; and what will the agent do when it consumes all its hosts? These have a scientific but a democratic dimension as well, because concerned citizens want to be heard and have their views respected. Few stakeholders are able to play any kind of constructive role with the knowledge that they bring to such a process, therefore, education of stakeholders is integral to any kind of engagement. For example, in South Africa, Rhodes University offers a two week short course which enhances the capacity of anyone to understand the basics of biocontrol, and a wide range of stakeholders are invited to attend it (Gillespie et al., 2003). In New Zealand, efforts to engage indigenous Maori communities have dealt with biocontrol issues chiefly from the perspective of cultural and ethical values, and not biology, however, they have been successful because everyone's opinion is dealt with respectfully (Hayes et al., 2008).

The beneficiaries (stakeholders, not researchers) are the most appropriate parties to explain why control of the invasive plant is in the public's interest

Creating greater consensus on the need to take action is a critical first step that is fundamental to success. For example, Australia has a national weeds strategy that justifies action (Natural Resource Management Ministerial Council of Australia, 2006). In New Zealand, regional councils serve as critical intermediaries between tax payers (or rate payers) as stakeholders with research institutions (Hayes, 1999). This insulates researchers from public suspicions of conflict of interest, in other words, that the researcher loses objectivity by promoting a project that advances their career.

The beneficiaries should present a risk/cost/benefit analysis that justifies a biocontrol strategy

In New Zealand, regional councils articulate an economic justification that makes clear the advantages of biocontrol over other forms of control to tax payers. In the New Zealand regulatory

system, these regional councils are generally those who petition for invasive plant biocontrol release permits, and they are better positioned to articulate these advantages, and to engage in discussions over conflicts of interest. These regional councils represent the public better than a scientist can, so the scientist serves as scientific expert advisor, and never the advocate for controlling a pest (Campbell, 2010). Legislation imposes the burden of public consultation and engagement on the petitioner for a permit. Although this appears costly, in practice it appears that this is more than offset by decreased costs and conflicts associated with the actual regulatory decision (Campbell, 2010). Other countries could benefit from this approach, although in the U.S., it would require going beyond what is required by law.

Public agencies should articulate their decision criteria clearly and gather stakeholder input of how their criteria apply to a specific permit application

The New Zealand permitting system is efficient because any decision to release a biocontrol agent is made on a very narrow basis. It presumes that there has been prior public engagement with the desirability of targeting the invasive plant and the suitability of the biocontrol agent. Then, the question upon which the decision is made is simple (as in straightforward): are the anticipated benefits greater than the costs and risks? In New Zealand, this has frontloaded costs and public engagement efforts, but has made release decisions less contested.

Conclusion

Biocontrol of invasive plants is a public interest science. It is chiefly funded by governments and is done on behalf of the public. Some form of public consent is necessary in a democratic society. To foster sustained public engagement over time, the problem definition of invasive plants should be disambiguated from the solution of biocontrol.

Public engagement can be structured so that it enhances public stakeholder support for biocontrol of invasive plants without imposing burdens upon researchers. However, lessons of prior public engagement suggest that scientific research activity should not be confounded with advocacy for invasive plant management using biocontrol. Fostering social consensus on the need to control the invasive plant is a pre-requisite. Public engagement requires careful attention to devising appropriate roles for stakeholders, and nodes for public input in decision making processes. Greater public engagement with biocontrol of invasive plants can be achieved by disambiguation of problem definition from solution options, and research activities from stakeholder advocacy.

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Outreach Challenges for Biological Control in Hawaii

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Abstract

Public understanding of Hawaii's use of biocontrol is limited. This can create problems when support for releases is sought. Release of a strawberry guava (*Psidium cattleianum* Sabine) enemy was delayed by public opposition. Raising awareness about invasive species in Hawaii is the purpose of the Hawaii Invasive Species Council Public Outreach Working Group (POWG). POWG organized statewide biocontrol educational activities. For Big (Hawaii) Island Invasive Species Committee (BIISC) outreach staff, biocontrol issues became particularly important with the strawberry guava proposal. One vocal Big Island activist raised public concern against biocontrol using a variety of tactics (described in Warner and Kinslow, 2011). BIISC outreach strategy focused on responding to issues that resonated with many members of the population. Key issues raised by the public to outreach staff revealed: the lack of agreement that strawberry guava is a problem that needs biocontrol (the tree has food value and natural area impacts are unseen); the public is primarily aware of examples of disastrous introductions and unaware of the extent and successes of biocontrol releases in Hawaii; the fear of rapid evolution of biocontrol agents to new hosts is pervasive; the lack of understanding of insect biology and genetics contributes to fear of rapid evolution; and, the public does not understand the selection process, research and testing protocols, and the regulatory process involved in classical biological control. A long-term education program with basic curricula plus materials on each species released would help agencies build public support for future releases.

Introduction

Biocontrol has a long history in Hawaii, with almost 800 species introduced, 300 established, complete control of approximately 40 insect species and substantial control of approximately 150 insect species, and successful control of approximately 10 weed species (Funasaki et al., 1988; Culliney and Nagamine, 2000; Culliney et al., 2003).

However, many people are familiar only with the famous mistakes (mongoose, cane toad) and not at all familiar with the extent or successes of other biocontrol releases. Biocontrol history in Hawaii commenced under the leadership of King Kalakaua. This last king, revered for his leadership in preserving Hawaiian culture, also passed laws (1890) to prevent immigrant insect pests from entering Hawaii. The first biocontrol release (1890) was the vedalia beetle (*Rodolia cardinalis* Mulsant), which successfully controlled the cottony cushion scale (*Icerya purchasi* Maskell). After the reign of Queen Liluokalani, Albert Koebele was hired as entomologist and biological control expert for the Republic of Hawaii. In the early period, attention was focused on agricultural pests and the general public had little knowledge of biocontrol.

One might characterize the 20th century in "biocontrol eras", beginning with a long period of introductions to address agricultural pests with little review, then an era euphoric about pesticide efficacy,

and next an era impressed with biocontrol. With the greater ecological consciousness of the 1970's and entomological research, awareness of non-target impacts began to increase. However, there was also developing interest in the idea of using natural enemy introductions to slow the spread of weeds in conservation areas. Concurrently, the regulatory review process became increasingly strict, with committees of specialists reviewing proposals, and requirements for NEPA documents.

Still, most biocontrol proposals were not widely noticed by the public until an activist became concerned about proposals to introduce a scale insect to control strawberry guava (*Psidium cattleianum* Sabine). This vocal Big Island resident activist raised public concern against biocontrol using a variety of tactics. His tactics have been described in Warner and Kinslow (2011) and were familiar to BIISC, as he has opposed numerous other projects to control coqui, mangrove, and invasive species work in general.

Methods

Raising awareness about invasive species in Hawaii is the primary purpose of the Hawaii Invasive Species Council Public Outreach Working Group (POWG). Core members of the group include the outreach staff of the invasive species committees (ISC) on each island. In 2009 four focal topics were identified as outreach priorities, one of which was biocontrol. POWG organized several biocontrol educational activities, including a documentary video, a biocontrol communications conference held March 2010, and a general brochure (produced collaboratively with the Hawaii Department of Agriculture) for public and legislator education (distributed at Ag Day at the Capital). Several video segments about biocontrol were shown on Outside Hawaii (an audience of 20,000 every week on TV alone, plus viewers at the website). The video focused on the recovery of the native wiliwili tree after a successful biocontrol effort. There were also some interviews about the impacts of strawberry guava and the need for biocontrol as a separate segment (http://www.oc16.tv/shows/32) A website was posted about strawberry guava biocontrol specifically to assist with the EIS public review process (http://www.hear.org/strawberryguavabiocontrol/).

The biocontrol communications workshop brought agency staff, researchers, land managers and outreach specialists together to talk about challenges and approaches to communicating about biocontrol. Since then, the biocontrol working group was convened for one meeting. The Maui Invasive Species Committee (MISC) worked with their county council to pass a resolution supporting the use of biocontrol. The Big Island County Council, in response to the strawberry guava controversy, passed a resolution against biocontrol. A site visit to a public forest infested by dense strawberry guava convinced the participating council members of the need for biocontrol, but not all council members chose to or were able to attend.

BIISC outreach strategy, particularly with regards to the strawberry guava proposal, focused on responding to biocontrol issues that resonated with many members of the public. The BIISC program participates in an average of one public outreach event per week, often in the form of information booths at varied festivals, plant sales, farmers markets, or spoken presentations to public or school groups. The BIISC outreach specialist presented an oral presentation on the history and successes of biocontrol in Hawaii at the 2009 Hawaii Conservation Conference. Presentations were also developed to educate and intrigue the public on the biology and importance of insects. Better understanding of insects will help the public to assess risk.

Results

Key issues raised by members of the public to outreach staff revealed that: the public generally is aware of one or two examples of disastrous failed introductions and is totally unaware of the extent and successes of biocontrol in Hawaii; fear of rapid evolution of the host to new targets is pervasive; a lack of understanding of insect biology and genetics contributes to the fear of rapid evolution; and the public does not understand the quarantine testing, regulatory process and limits on biocontrol releases. Through discussions and exhibits, many individuals expressed relief that biocontrol introductions were not as haphazard and uncontrolled as they had thought them to be. Most significantly, other

biocontrol releases have not been met with much opposition, before, during, or since the strawberry guava biocontrol issue came to a head.

The public's view of the invasive species considered for biocontrol affects whether or not a project receives support. For example, in the case of the wiliwili tree decimated by an accidently introduced wasp, people saw the trees die and understood the gall wasp was a problem. Biocontrol of the gall wasp was not opposed. People do not see the watershed, do not see the full extent of the strawberry guava invasion, and therefore, they do not understand the impact (on groundwater, on cultural values, and on native species). Strawberry guava is not a recently introduced species and so has social familiarity and is perceived as a useful tree. Because it has some food value, attempts to control strawberry guava were portrayed as attempts by government to control the food supply, which is linked to fears of genetically modified foods. The relationship of strawberry guava fruit in promoting damaging fruit flies is not well understood by the public.

Discussion

Agencies may give undue weight to public opposition to biocontrol projects if that opposition is based on misinformation which can be corrected. Public opinions can change rapidly when a broader context of history, methods, successes, and regulation is described. Biocontrol is an important management tool for the threats facing Hawai'i. For biocontrol to be successful, agencies must be committed to and have the resources necessary for the research, development and education necessary before a release. This strong agency support and education will help the public in supporting this tool. Limited support runs the risk of achieving neither conservation goals nor reducing public concern with risk.

It is recommended that agencies and resource managers in Hawaii devote significant resources to produce educational materials to publicize biocontrol methodology and successes in Hawaii. Basic curricula should educate and intrigue the public on the biology and importance of insects. A discussion of genetics and reasons for host specialization is also important. Good guy and bad guy cards, identification cards, and the current fascination with forensic anthropology may be useful lures. Another interesting possibility would be to engage citizen groups in rearing of approved biocontrol agents, as has been done elsewhere in the world. Future biocontrol projects should evaluate public attitudes towards the particular species, and plan outreach accordingly, while building general awareness and support.

Other current limitations for the state are the shortage of adequate quarantine facilities for testing. Public support for biocontrol proposals would help convince policy makers that these facilities should be funded.

Acknowledgements

Interviews with biocontrol staff from the Hawaii Department of Agriculture, USFS, and biocontrol researchers present at the second Miconia conference (Hana, Maui, 2009). Members of the Public Outreach Working Group.

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The Role of Implementation in Weed Biological Control in South Africa

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Abstract

Biological control of weeds was initiated in South Africa in 1913 with the introduction of the cochineal insect *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae) on the invasive cactus *Opuntia monacantha* Haw. (Cactaceae) (Moran et al., 2011). Since that time some 113 agent species have been released against 48 weed species with varying levels of success (Klein 2011). The implementation of weed biological control agents has historically been neglected and there is very little research on this topic (Grevstad 1999, Memmott et al., 1998). In South Africa, initially agents were mass-reared and released by the researchers and a few landowners. In 1996 with the advent of the Working for Water Programme biological control implementation officers were appointed in each province of the country to serve as a conduit between the research scientists and the landowners (Gillespie et al., 2004). The role of the implementation officers was to mass-rear, release and monitor for establishment of the agents and redistribute, where necessary. Key to the success of this programme was record-keeping and the ensuring that information regarding releases and establishment of agents was provided to the researchers. This was achieved through the establishment of biannual technical liaison committee meetings and annual weed biological control workshops. More recently the task of mass-rearing has been outsourced to a commercial facility, which has greatly improved the quantity and more importantly the quality of agents being released. In the last five years weed biological control implementation has been rolled out to a number of schools and this has facilitated the incorporation of weed biological control into the National School Curriculum. Further, a programme that trains physically challenged individuals to mass-rear and distribute weed biological control agents around South Africa has been highly successful.

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"Of Miconia and Men": The Story of a Scientifically and Socially Successful Biological Control Program in Tahiti, French Polynesia

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Abstract

Many biological control programs against invasive plants have failed or have been abandoned because of negative human perceptions or strong conflicts of interests, *e.g.*, the fear of introducing alien predators or pathogens (the so-called "pathophobia", Warner, in press), the potential threats for related species of economic or conservation value, and the uncertainty of successful control (see e.g. Louda & Stiling, 2004). In this regard, biological control scientists often appear as sorcerer's apprentices. This talk describes how a biological control program against the invasive tree *Miconia calvescens* (Melastomataceae), a formerly popular ornamental plant species, was successfully conducted (1997-2010) on the island of Tahiti (French Polynesia, South Pacific) using a fungal pathogen (Meyer et al., 2008; Meyer et al., in press), despite the very bad reputation of past "biological control experiments" in the region (carnivorous snails introduced to control the Giant African snail, myna birds for wasps, raptors for rats, *etc.*). This case-study tries to demonstrate that rigorous scientific (pre- and post-release) studies are necessary but not sufficient for the acceptance of biological control by human society. Information and education at all levels (from public to politicians), consultation process including all stakeholders, and communication involving different media are equally important to avoid that "*The best laid schemes of mice and men go often askew*" (inspired by Robert Burns' famous poem written in 1785). Paradoxically, biological control projects provide excellent opportunities to explain basic ecological processes and the methodology of science to the general public and schoolchildren in particular.

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Russian Olive – a Suitable Target for Classical Biological Control in North America?

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Abstract

Projects to develop biological control solutions against invasive plants are mid- to long-term endeavors that require considerable financial support over several years. Discussions of concerns and potential conflicts of interests often occur when biological control agents are first being proposed for release into the environment. Such late discussion, which in some cases results in delays or in the halt of ongoing biological control programs, has led to uncertainty, confusion and frustration among the various stakeholder groups, including the biological control practitioners. Russian olive (*Elaeagnus angustifolia* L.), a small tree or multi-stemmed shrub native to south-eastern Europe and Asia, was introduced to North America in the late 19th century as a horticultural plant. It has since spread into the environment, particularly along river courses where it now occupies similar habitats as tamarisk. To date, Russian olive has become a declared noxious weed in four US states. Because of the perceived benefits of planting Russian olive in some regions, developing a classical biological control program against Russian olive could give rise to a conflict of interests. To address and discuss potential conflicts of interests right at the onset of this new biological control initiative, we recently created a platform to collect, analyze and disseminate science-based information on Russian olive. Particular emphasis is being put on the following questions: 1) what are the economic, environmental or social impacts caused by Russian olive in North America or in other parts of the invaded range, 2) what are the goals of Russian olive management, and 3) is classical biological control a useful and feasible way to achieve these management goals? We will present first results of our data analysis and propose a way forward to reach common ground among key stakeholders regarding under which conditions Russian olive is a suitable target for biological control.

The Economics of Classical Biological Control: A Meta-Analysis of Historic Literature and Suggested Framework for Future Studies

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Abstract

Classical biological control (CBC) programs are initiated to protect natural resources, agricultural and other human interests. CBC programs typically involve an investment of public funds and their success is often determined by welfare measures such as benefit/cost analyses. An initial review of the literature shows previous efforts at measuring program benefits in monetary terms have often been incomplete and/or misguided. This review reveals that the basic analytical challenge can be broadly traced to two areas; project benefits lacking marketable measures and confusing or under reporting of project costs and benefits. The economics of CBC projects should be analyzed within the neo-classical economic view of supply and demand. On the supply side, costs are expenses directly related to project development and implementation. These include all direct expenditures necessary to locate and test the control agent and affect its release. These costs are typically covered by public funds and justified by the public nature of the anticipated project benefits. However, cost should also include any value lost to agents as a result of the project's success. On the demand side, agents with marketable goods and services that benefit from the project will provide a direct measure of the economic gain. Furthermore, their gains will lead to an indirect benefit or ripple effect through the economy. However, there are also benefits that lack market value and include items such as improved ecological services and other non-market activities such as improved fishing, hunting, *etc*. CBC projects would benefit from a strategic approach to assessing their economic efficiency. A meta-analysis of the use of economics in historic CBC literature is conducted and an analytical framework introduced to guide future benefit/cost studies for CBC projects. The framework will help generate support for CBC programs by providing a clear guideline for their effective economic evaluation.

Biological Control of Strawberry Guava in Hawaiian Forests

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Abstract

Over the last two decades, scientists in Hawaii, Florida and Brazil have researched biological control as a new tool for managing strawberry guava, an invasive tree in Hawaiian forests. A leaf galling scale insect from Brazil, *Tectococcus ovatus* (Hemiptera: Eriococcidae), was found to be highly target-specific and has been proposed for release in Hawaii. This natural enemy is expected to slow the spread of strawberry guava into native forests by reducing growth rates and seed and fruit production over time. A State of Hawaii environmental assessment of the proposed biocontrol release included detailed data from researchers as well as inputs from stakeholders and the public in recent years. Although this project has been strongly supported by partner agencies and conservation workers in Hawaii, it has encountered substantial opposition from some quarters of the public who value strawberry guava for a variety of reasons. As a prominent and provocative target for biocontrol, the case of strawberry guava offers some important lessons on the challenges and opportunities facing biocontrol as a management tool for conservation and restoration of Hawaiian forests.

The Economic Benefits of TSA Biological Control

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Abstract

Tropical Soda Apple (*Solanum viarum* Dunal) (TSA) is an invasive exotic plant from South America that has become a weedy pest, choking pastures and afflicting Florida's beef producers. In 2007, state-wide economic losses were documented to range from $6.5 million to $16 million annually. In 2008, efforts to control TSA resulted in the release of the green tortoise beetle (*Gratiana boliviana* Spaeth) (GTB) across central and southern portions of the state. Also a native of South America, the GTB is particularly fond of TSA foliage with no alternative native hosts. Initial results indicate the beetle is spreading rapidly and significantly reducing TSA density in many areas of the state. During the summer of 2010, a survey of Florida's cattle producers was conducted to evaluate the impact of the recent TSA biological control efforts (*Gratiana boliviana* Spaeth) in central and southern Florida. A survey was mailed statewide to 3,500 members of the Florida Cattleman's Association. The survey asked participants to identify their type of cattle operation, the distribution of TSA in their pastures and their assessment of TSA density and the effort required to control this plant. Slightly more than 30% of those surveyed responded. When compared to 2007, preliminary results indicate significant declines in both TSA density and control efforts across central and southern Florida. On the other hand, northern Florida has experienced an increase in TSA density and control effort. These preliminary results support the hypothesis that the GTB has reduced TSA density and lowered control costs to cattle producers.

Is Post Hoc Development of Risk Management in Weed Biological Control Too Late? Lessons Learned from *Cactoblastis cactorum*

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Abstract

The Argentine cactus moth, *Cactoblastis cactorum* (Berg), is renowned for its success as a biological control agent against exotic *Opuntia* spp. in many locations including Australia, South Africa and Hawaii. However, in 1957, its introduction into the Caribbean to control native *Opuntia* spp. ultimately resulted in its arrival to southern Florida where it became an invasive pest of native and rare *Opuntia* species and a threat to the *Opuntia*-rich areas of the western U.S. and Mexico. To mitigate this risk, survey and control tactics were developed in the U.S. and an awareness campaign was initiated in Mexico. A Bi-National Cactus Moth Control Program was established to facilitate risk management, which involved identifying, evaluating, selecting and implementing actions to prevent, reduce or control adverse effects of *C. cactorum*. The risk management process included comparing the risks of taking no action with the risks associated with each remedial alternative, while taking into account social, cultural, ethical, economic, political, and legal considerations. Although these risk management activities were undertaken after the initial release of *C. cactorum*, management tactics were available and used successfully to eradicate this pest when there was an incursion in Mexico. Efforts remain ongoing in the U.S. where the westward expansion of *C. cactorum* has been mitigated through regulatory and control actions. The lessons learned from *C. cactorum* in North America underscore the need to have regional involvement in the risk analysis process and in the development of risk management prior to the release of a weed biocontrol agent.

Biological Control as a Tool to Mitigate Economic Impacts of Facilitative Ecological Interactions between the Giant Reed and Cattle Fever Ticks

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Abstract

Annual domestic impacts associated with introduced weeds are conservatively estimated at $27 billion, which incorporates costs of weed management, crop losses and displacement of productive rangeland, and displacement of some environmental services. Estimating the total economic damage of invasive weeds can be difficult, especially when they impact non-market services, or when impacts are indirect. The giant reed, *Arundo donax* L., is an invasive grass infesting riparian corridors and waterways in the southwestern U.S. and northern Mexico. In addition to the economic implications of water loss in this arid agricultural area, deleterious non-market effects ascribed to giant reed invasion include riparian habitat fragmentation, biodiversity loss, stream-bank erosion, and physical and logistical obstruction for border security and enforcement. These thick swaths of giant reed are also a highly suitable habitat for the cattle fever tick, *Rhipicephalus microplus* (Say), an important vector of the protozoa causing bovine babesiosis. Survival rates, fecundity, and fertility of engorged adult female cattle fever ticks were tested in tick cohorts placed in pastures, mixed brush, and arundo stands. Ticks were more likely to lay eggs and larger egg masses in giant reed and mixed brush when compared to ticks in mixed-grass pastures where microclimatic conditions are less favorable. Animals such as cattle, horse, and white-tailed-deer traversing through nearly-impenetrable stands of giant reed create common-use corridors that in effect facilitates parasitism of suitable hosts by cattle fever ticks thriving in that habitat. Our findings document the economically significant indirect impact by giant reed as a complicating factor to keep the U.S. free of cattle fever ticks and bovine babesiosis. Such considerations should be incorporated when modeling the total economic costs associated with an invasive plant. The use of biological control agents against giant reed stands represents a sustainable strategy to mitigate the indirect economic impacts of giant reed and disrupt facilitative ecological interactions between invasive species like cattle fever ticks and giant reed in south Texas.