2005 Project Abstract
For the Period Ending June 30, 2008

PROJECT TITLE: Biological Control of European Buckthorn and Garlic Mustard
PROJECT MANAGER: Luke Skinner
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FUNDING SOURCE: Minnesota Environment and Natural Resources Trust Fund
LEGAL CITATION:
[**ML 2005, First Special Session, [Chap. 1], Art. 2, Sec.[11], Subd. 5 (h).**]

APPROPRIATION AMOUNT: $200,000

Overall Project Outcome and Results
This project builds upon and continues work begun from a 2003 Trust Fund appropriation and has since received an additional 2007 Trust Fund appropriation to further continue and accelerate the work.

Buckthorn and garlic mustard are invasive species of highest priority for development of long-term management solutions, such as biological control (bio-control). This research aimed to help determine 1) if there are suitable insects that can be used to reduce impacts caused by buckthorn and 2) to implement introduction of insects to control garlic mustard and assess their establishment and success.

Buckthorn. Insects were collected and reared for carrying out host specificity testing. A total of 1,733 specimens (356 species) were collected from buckthorn infestations in this insect fauna survey. In total, 39 specialized arthopods were recorded from R. cathartica (common buckthorn) and F. alnus (glossy buckthorn) in Europe.

The reassessment of the potential for biological control of R. cathartica and F. alnus was conducted based on work done in Europe from 2002-2007 on potential biological control agents. A summary of 10 priority species for future research on biological control of R. cathartica is provided in Appendix A of the Work Program Final Report. This final suite of priority species are being tested for use as effective bio-control agents in future work.

Garlic mustard. Pre-release data is providing a greater understanding of normal year-to-year variation. To help differentiate normal fluctuation from changes due to the bio-control insect, data was collected over the course of this project. On average, less than 2% of the leaf area was damaged by herbivores. Garlic mustard plant populations do vary considerably from year to year. Two to three years of pre-release monitoring data have given us a good understanding of the year-to-year fluctuations in populations. At some sites, the population fluctuations are due to the changes in dominance between the seedling and adult stages.

After biological control insects are released we expect to see decreases in garlic mustard populations. With long-term data collection we can see long-term trends in garlic mustard populations (see Appendix B of Work Program Final Report).

<u>Project Results Use and Dissemination</u>
Information garnered from this study will be used to further our objective of developing an effective and efficient bio-control agent for buckthorn and garlic mustard. Effective bio-control agents will help reduce the damage and cost related to control of these invasive species. The information provided by this work helps to establish basic biological information pertaining to the types of species available for potential bio-control agents for buckthorn and narrow our efforts to a few priority species. The information gained on garlic mustard growth and impacts on native species will help us to assess the effectiveness of the current bio-control agents once they have been applied to the test sites. Without this type of baseline data a true understanding of the impacts the bio-control agent is having are impossible to attain. Information from these projects are being shared with multiple federal and state agencies to help the region better understand the potential control mechanisms for buckthorn and garlic mustard.

Information on this work has also been developed into peer reviewed scientific papers. The information has been presented at a variety of national and international conferences. Locally this information has been presented to a variety of interested practitioners and citizens at local conferences and meeting.

Project Completion and Summary Date: June 30, 2008
LCCMR 2005 Work Program Final Report

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

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

Total Biennial LCMR Project Budget:	**LCMR Appropriation:**	**$200,000**
	Minus Amount Spent:	**$200,000**
	Equal Balance:	**$0**

Legal Citation: ML 2005, First Special Session, [Chap. 1], Art. 2, Sec.[11], Subd. 5 (h).

Appropriation Language: 5(h) Biological Control of European Buckthorn and Garlic Mustard. $100,000 the first year and $100,000 the second year are from the trust fund to the commissioner of natural resources to research potential insects for biological control of invasive European buckthorn species for the second biennium and to introduce and evaluate insects for biological control of garlic mustard. This appropriation is available until June 30, 2008, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. AND III. FINAL PROJECT SUMMARY:
Buckthorn and garlic mustard are invasive species of highest priority for development of long-term management solutions, such as biological control. This research will help 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.

Buckthorn. Insects were collected and reared for carrying out host specificity testing. A total of 1733 specimens (356 species) were collected from buckthorn infestations in this insect fauna survey. In total, 39 specialized arthopods were recorded from R. cathartica and F. alnus in Europe.

The reassessment of the potential for biological control of R. cathartica and F. alnus was conducted based on work done in Europe from 2002-2007 on potential biological control agents. A summary of 10 priority species for future research on biological control of R. cathartica is given in Table 1, page 13 Appendix B. This final suite of priority species will be tested for use as effective biocontrol agents over the next three years.

Garlic mustard. Pre-lease data is providing a greater understanding of normal year-to-year variation, to help differentiate normal fluctuation from changes due to the biocontrol insect was collected over the course of this project. On average, less than 2% of the leaf area was damaged by herbivores. Garlic mustard plant populations do vary considerably from year to year. Two to three years of pre-release monitoring data have given us a good understanding of the year-to-year fluctuations in populations. At some sites, the population fluctuations are due the changes in dominance between the seedling and adult stages.

After biological control insects are released we expect to see decreases in garlic mustard populations. With long-term data collection we can see long-term trends in garlic mustard populations (see Appendix C).

IV. OUTLINE OF PROJECT RESULTS:

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

Description: Researchers from the Center for Applied Bioscience (CABI) in 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 preliminary information of 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 1:   **LCMR Budget**   **$90,000**
                                                     Balance   **$0**

Completion Date: 6/30/08

Final Report Summary: Over the course of this project researchers with CABI have surveyed, collected and tested a variety of insects for potential biocontrol of *R. cathartica* and *F. alnus*. A total of 1733 specimens (356 species) were collected from buckthorn infestations in this insect fauna survey. In total, 39 specialized arthropods were recorded from *R. cathartica* and *F. alnus* in Europe. Selceted species where tested for ability to oviposition on these plants and their choice

of oviposition plants. These species where also tested for their host specificity preference. These tests help to determine the effectiveness and efficiency of these species as biocontrol agents and any risk associated with other native related shrubs.

Once these surveys and tests were completed CABI researchers reassessed the data collected and prioritized the species for further testing. The reassessment of the potential for biological control of *R. cathartica* and *F. alnus* was conducted based on work done in Europe from 2002-2007 on potential biological control agents. A summary of 10 priority species for future research on biological control of *R. cathartica* is given in Table 1, page 13 Appendix B. This final suite of priority species will be tested for use as effective biocontrol agents over the next three years.

Three of the high priority species identified as priority species included *Philereme vetulata* (Lep., Geometridae), *Trichochermes walkeri* (Hom., Triozidae), and *Wachtiella krumbholzi* (Dipt.; Cecidomyiidae). These three species vary in the type of damage they do to *R. cathartica* ranging from the production of galls to attacking the fruits of the shrubs. One general finding is that there are few if any good biological control candidates for *F. alnus* (glossy buckthorn). All the candidates listed in the Table 1 are insects associated with *R. cathartica* (common buckthorn). The final report for Result 1 can be found in two CABI reports included in Appedix B. The first report describes in detail the research carried out on rearing and testing in 2006, and the second report covers work carried out in 2007 including the a reassement of the research completed to date and includes priorities for future work (appendix B.).

Further funding secured for the FY09/FY10 biennium will help to complete the work on these three potential biocontrol agents for *R. cathartica* control.

Result 2: Survey of insects on buckthorn in Minnesota

Description: Surveys will be continued out to determine what insect species currently utilize buckthorn in Minnesota. Such surveys are needed to determine if any native or non-native insect species are currently found on buckthorn or cause damage to buckthorn. Multiple sites will be surveyed periodically throughout the growing season to capture any insect species associated with buckthorn. Any immature insect collected will be allowed to complete development for identification purposes. A representative sample of each insect species collected will be mounted or preserved, and sent to the appropriate taxonomist for proper identification.

Summary Budget Information for Result 2:   **LCMR Budget $20,000**
                                                                        Balance $0
Completion Date: 02/28/07

Final Report Summary: The main effort in 2006 was to complete the sorting, pinning and identification of the large number of insects collected in 2004 and 2005. All insects have now been identified and included in data summaries and analysis. A total of 1733 specimens representing 356 species of insects were collected from buckthorn infestations in this insect fauna survey. Hemiptera was the most abundant order. It was followed by Hymenoptera, which consisted mostly of parasitoids simply using buckthorn as a resting spot or searching for their host. This data was analyzed to look at the relationship of habitat type to insect species richness and how this may affect the introduction of a biological control agent. Data indicates that ample feeding niches are available given that most herbivores collected can be classified as generalists. However, the abundance of parasitoids and predators may hinder establishment of potential

biological control agents. Data analysis are complete and we have attached the final report for result 2 to this document (Appendix B).

Result 3: Introduction and evaluation of Garlic Mustard biological control agents in MN

Description: Research activities will include selection of potential release sites, collection of pre-release plant community data, introduction of control agents and initial evaluation of establishment of agents. In anticipation of biological control agents becoming available for garlic mustard, up to 10 field sites will be select in different habitat types to implement a biological control program in Minnesota. At the chosen sites, we will 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. Once biological control insects are introduced, we will evaluate insect establishment and plant community response to the biological control.

Summary Budget Information for Result 3:   **LCMR Budget $90,000**
                                                                              Balance $0

Completion Date: 6/30/08

Final Report Summary:
        While evidence of insect feeding was widespread the actual amount of leaf damage was low. Across all sites, seasons and years the average amount of leaf area damaged due to insects was $1.8 \pm 0.03\%$. Leaf damage did not vary widely from site to site. The lowest mean leaf removal was 0.95% at Pine Bend in 2006, while the highest was 4.4% at Fort Snelling. When biological control weevils are released it is expected that insect damage, especially windowpane feeding, will increase.
        Garlic mustard's biennial life cycle drives some of the changes in garlic mustard cover and population density from year to year. At some sites, one life stage clearly dominates in each year. For example, a site may be dominated by adult flowering plants in spring 2005 and have few seedlings present. In the fall of 2005 there would be few rosettes. In the spring of 2006, the seedling stage would dominate and the site would have many seedling and very few adults. By fall 2006 there would be many rosettes. This pattern is demonstrated in Figure 2 with photos from Baker Park.
        Of the 12 sites, six showed a pattern of one life stage dominating each year (Fig. 1). Over three years of monitoring, the rosette population density cycled from low to high to low in some sites and from high to low to high at others (Fig. 1). It is important to take these population cycles into account when analyzing the impacts of biological control insects. A decrease in adult plants from one year to the next may simply be a result in this natural oscillation in life stage dominance. It will take several years of data to separate out natural population cycles from long-term decreases in population.
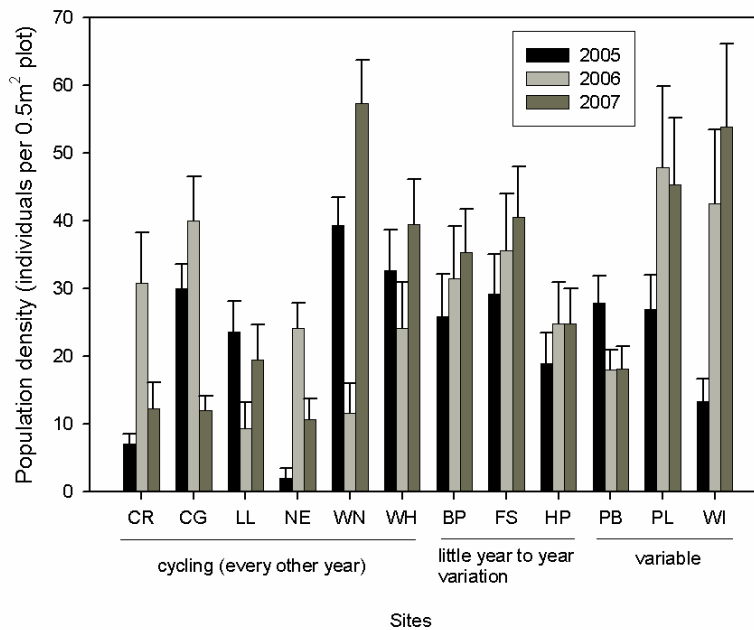
Figure 1. Population density of garlic mustard rosettes over time as measured in the fall at 12 monitoring sites in Minnesota, 2005-2007. Six sites show strong cycling (one life stage is dominant each year) with rosette densities peaking every other year. Three sites show little year to year variation in rosette population density (densities with standard error overlap from year to year). Three sites show variation over time with one site showing a decrease in rosette population density and two sites showing increases in rosette population density.

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

Data was collected on garlic mustard plant height and number of siliques as measures of vigor and reproductive output of the plants. It is anticipated that the introduction of biological control insects will stress the plants and result in smaller plants which produce fewer siliques. The year to year variation in garlic mustard average heights and numbers of siliques again underscores the importance of pre-release monitoring. Monitoring sites with and without biological control release will help us determine the impacts of biological control agents versus natural year to year variation. Large natural fluctuations in garlic mustard plant height and numbers of siliques were detected as height and siliques production decreased from 2006 to 2007.

One of the impacts of garlic mustard is that it forms dense populations which negatively impact native species. Sites with greater garlic mustard cover had lower native species richness and cover than those sites with less cover of garlic mustard. The negative correlations were consistent in both 2006 and 2007. Sites varied in the amounts of native and nonnative species present. Native species richness ranged from a low of 1.8 species/$0.5m^2$ quadrat at Baker Park in 2005 to a high of 6.7 species/$0.5m^2$ at Willmar in 2007. Native species cover ranged from a low of 9% cover at Baker Park in 2005 to a high of 50% cover at Nerstrand in 2007. Nerstrand also had the lowest nonnative species richness and cover (no nonnative species present in the spring 2005-2007). In addition to monitoring whether biological control insects will decrease garlic mustard populations, we can also monitor the response of the native vegetation. Ideally, native species cover and richness will increase as the populations of garlic mustard decrease. Monitoring data provides baseline information on native species cover and richness.

In addition to the proposed monitoring project we also looked at the allelopathic potential of garlic mustard. Garlic mustard roots exude allelochemicals which can negatively affect native

species by decreasing germination rates. The active allelopathic compound in garlic mustard is allyl glucosinolate (sinigrin). The seedbank of the study sites were also examined to provided a better understanding of the restoration potential of the sites infested by garlic mustard. These two portions of the study are discussed in Appendix C.

V.   TOTAL LCMR PROJECT BUDGET:

All Results: Other: $200,000 (for contracts)

TOTAL LCMR PROJECT BUDGET: $200,000

VI.   OTHER FUNDS & PARTNERS:

A. Project Partners:
<u>Anthony Cortilet and Monika Chandler</u>, MN Department of Agriculture - Mr. Cortilet and Ms. Chandler will work closely with DNR staff to develop and implement evaluations of garlic mustard biological control in the field. Mr. Cortilet will spend ~5% of his time (in-kind) and Ms. Chandler will spend 10% of her time on this project.
<u>Dr. Matthew Cock</u>, Director, Center for Applied Bioscience International (CABI), Delemont, Switzerland - Dr. Cock and his staff will be under contract to continue the ongoing buckthorn research. 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.
<u>Dr. Roger Becker</u>, University of Minnesota, will oversee garlic mustard biological control research under contract. Dr Becker will spend 5% of their time on this project. A post-doctoral researcher will spend 80% of their time on garlic mustard under the direction of Dr. Roger Becker.
<u>Dr. David Ragsdale</u>, University of Minnesota, will carryout surveys for insects on buckthorn in MN. Dr. Ragsdale will spend 5% of his time on this project while his graduate student will spend 50% of their time.
<u>Dr. Bernd Blossey</u>, Cornell University, will provide technical expertise garlic mustard research.

B. Other Funds being Spent during the Project Period:
<u>Buckthorn related spending</u>: The Department of Natural resources will contribute approximately $22,500 in additional funding towards this project.

C. Required Match (if applicable): Not applicable

D. Past Spending:
<u>Buckthorn related spending</u>: The DNR spent $20,000 in 2001 to initiate research on buckthorn bio-control. The DNR received $75000 in 2001 from the U.S. EPA to continue the buckthorn research. Currently, $109,000 of LCMR recommended funding along with an additional $50,000 grant from the U.S. EPA is being used to continue this research. If this research is successful in identifying potential control agents, future proposals will be forthcoming. We will continue to pursue other funding sources for this effort from other

states and federal agencies, which are likely to help pursue bio-control agent if some are identified.

<u>Garlic mustard</u> <u>related spending</u>: The DNR spent $25,000 in 1999 supporting garlic mustard biological control research. Between 2002 and 2003, the DNR received $105,000 from the U.S.D.A.-Forest Service to continue host specificity testing of garlic mustard agents. This research is taking place at the new quarantine facility at the University of MN, St. Paul Campus

E. Time:
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 2006 or 2007. 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 LCMR process.

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.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than January 2006, August 2006, February 2007, August 2007 and March 2008. A final work program report and associated products will be submitted by June 30, 2008.

IX. RESEARCH PROJECTS: See Appendix B and C.

Appendix A: Budget Detail for 2005 Projects

Proposal Title: Biological Control of European Buckthorn and Garlic Mustard-Continuation (H-02)

Project Manager Name: Luke Skinner

LCMR Requested Dollars: $ 200,000
    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

2005 LCMR Proposal Budget	Result 1, Activity 1 Budget:	Amount Spent 06/30/08	Balance 06/30/08	Result 1 Activity 2 Budget:	Amount Spent 06/30/08	Balance 06/30/08	Result 2 Budget:	Amount Spent 06/30/08	Balance 06/30/08	
	Buckthorn biological control- Europe			Buckthorn biological control- MN			Garlic Mustard biological control.			
BUDGET ITEM										**TOTAL FOR BUDGET ITEM**
Contracts	$ 90,000	$ 90,000	$ -	$ 20,000	$ 20,000	$ -	$ 90,000	$ 90,000	$ -	$ 200,000
Professional/technical	CABI-Bioscience Switzerand - research in Europe			Univ of MN- Research in MN			Univ of MN- Research in MN			
COLUMN TOTAL	$ 90,000	$ 90,000	$ -	$ 20,000	$ 20,000	$ -	$ 90,000	$ 90,000	$ -	$ 200,000

Appendix B

Result 1: Investigate potential insects as biological control of European Buckthorn
And
Result 2: Survey of insects on buckthorn in Minnesota

Result 1- Part 1: Investigate potential insects as biological control of European Buckthorn

Biological Control of Buckthorns
(*Rhamnus cathartica* and
Frangula alnus)

Annual Report 2006

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Summary

Preliminary screening tests with several buckthorn insects confirm host plant use observed in the field and the rejection of *Frangula* spp. by many insect species associated with *Rhamnus* in their native range. Work carried out 2002-2005 has lead to the rejection of several species because of their lack of specificity at the genus level: the stem boring cerambycid beetle *Oberea pedemontana*, the root-boring sesiid moth *Synanthedon stomoxiformis* and the leaf feeding tortricid moths *Ancylis apicella* and *A. derasana*. Other species have been discarded because of their likely oligophagy within genus *Rhamnus* and/or the difficulty in establishing a rearing colony in the future, i.e. the leaf feeding geometrid moths *Triphosa dubitata* and *Philereme transversata*.

Work carried out in 2006 confirmed that both *R. cathartica* and *F. alnus* are suitable hosts for the shoot-tip boring moth *Sorhagenia janiszewskae*. The selection of biological control agents which attack both *R. cathartica* and *Frangula alnus* in their native range will undoubtfully increase potential non-target impacts. Therefore it is suggested to give *S. janizewskae* a low priority for biological control of buckthorns.

Work in 2006 has also highlighted the difficulty to demonstrate with certainty the absence of consistence oviposition by *Trichochermes walkeri* on non target plants in no-choice conditions. However, the probability for high oviposition rate and gall and larval development on non-target *Rhamnus* species is extremely low. Oviposition choice tests should confirm the high specificity of *T. walkeri*.

Transfer of newly hatched larvae of *Philereme vetulata* on potted plants at the time of leaf bud expansion has produced good results. Species in genus *Rhamnus* appear to be suitable hosts for larval development of this moth although variability in food quality may result in lower pupal weight or longer time for development to pupal stage. The feasibility of oviposition choice tests will be studied in 2007.

Finally progress has been made in mass collection, and rearing to the adult stage of the seed feeding midge *W. krumbholzi*. Oviposition and larval development will be studied in 2007. Growing plants to fruiting stage and the synchronisation between adult emergence and plant phenology will be the main challenges in future tests.

1. Introduction

Rhamnus cathartica L. (common buckthorn) and *Frangula alnus* Miller (glossy buckthorn) (Rhamnaceae) are both shrubs and small trees of Eurasian origin which have become invasive in North America. *Rhamnus cathartica* was introduced to North America as an ornamental shrub in the late 1800s and was originally used for hedges, farm shelter belts, and wildlife habitats (Gourley, 1985; Randall and Marnelli, 1996; Gale, 2001). It has spread extensively and is currently found in most Canadian provinces (Nova Scotia to Saskatchewan) and 27 states predominantly in the north central and northeastern portion of the United States (Gale, 2001; USDA/NRCS, 2001).

Research to develop biological control for buckthorns was started in 1964 on behalf the Entomological Research Institute at Belleville, Ontario (former Agriculture Canada). Surveys for potential agents were carried out in 1964 and 1965 and preliminary screening tests in 1966-1967 (Malicky et al. 1970). The Minnesota Department of Natural Resources initiated a new programme in 2001 to reassess the potential of biological control of buckthorns in the light of the work carried out by Malicky et al. (1970) and the increasing importance of non-target impacts of biological control agents.

Preliminary screening tests with several buckthorn insects confirm host plant use observed in the field and the rejection of *Frangula spp.* by many insect species associated with *Rhamnus* in their native range. The likely geographically separate evolution of *Rhamnus* and *Frangula* has led to specialized diets in *Rhamnus* and *Frangula* species with only very few species specialized on *F. alnus* in its native range in Europe and relatively few species with no clear preference for either buckthorn species. Work carried out 2002-2005 has lead to the rejection of several species because of their lack of specificity at the genus level: the stem boring cerambycid beetle *Oberea pedemontana,* the root-boring sesiid moth *Synanthedon stomoxiformis* and the leaf feeding tortricid moths *Ancylis apicella* and *A. derasana.* Other species have been discarded because of their likely oligophagy within genus *Rhamnus* and/or the difficulty in establishing a rearing colony in the future, i.e. the leaf feeding geometrid moths *Triphosa dubitata* and *Philereme transversata.*

In our last report (Gassmann et al. 2006) we recommended to select the following species for further studies and host range testing in 2006: the shoot-boring moth *Sorhagenia janiszewskae*, the leaf feeding moth *Philereme vetulata*, the leaf margin gall psylloid *Trichochermes walkeri* and the seed feeding midge *Wachtiella krumbholzi*. There are few stem and root borers known on buckthorns and *S. janizewskae* was the only species which might be specific enough. Among the leaf chewing species, *P. vetulata* appeared to be the most specialised species for genus *Rhamnus*. Among the species which have been studied so far, *T. walkeri* is certainly the most specific and *W. krumbholzi* is one of the key species that could reduce the seed production of common buckthorn in North America.

One current constraint in developing biological control of buckthorn is the difficulty to obtain seeds from a number of test plants or to grow plants from seeds. The difficulty is enhanced by the occurrence of the sudden oak death (*Phytophthora ramorum*) on *Frangula californica* and *F. purshiana* in North America and the need

to import in Switzerland cuttings or rootstocks "found free from non-European isolates of *Phytophthora ramorum*".

2. Studies on individual potential arthropod biological control agents

2.1. Lepidoptera

2.1.1 *Sorhagenia janiszewskae* (Lep., Cosmopterigidae)

Background
Sorhagenia janiszewskae, the larvae of which mine in the year's shoots of buckthorns is a difficult species to work with. As with many internal feeders or gall makers, larvae must be collected just before pupation, in this case just before they leave the shoots to pupate in the soil; cut shoot-tips decay or dry quickly and this may prevent completion of larval development. Thus, time for collection is critical and this can change from year to year depending on climatic conditions in late winter. Adult aestivation and hibernation was thought to be an insurmountable problem. Field observations and the absence of mines and larvae in plants exposed in the oviposition tests in 2004-05 suggest that there is no second generation and that *S. janiszewskae* overwinters in the egg stage. Oviposition most probably occurs within three weeks of adult emergence.

In 2004 we gave a low priority to *S. janiszewskae* because of unresolved problems in adult rearing and the likely lack of host specificity of the moth which occurs on both *R. cathartica* and *F. alnus* in its European range. However, the clear oviposition host preference of *S. janizewskae* reared from *F. alnus* for its field host plant in 2005 renewed interest in this species for biological control of *R. cathartica* and *F. alnus* in North America. It was hypothesized that *S. janiszewskae* from *F. alnus* and *R. cathartica* are either two different species or two different host races.

Collection of mature larvae and adult emergence
The collection of shoot-tips presumably attacked by larvae of *S. janiszewskae* is summarized in Table 1. The shoot tips were placed in an outdoor shelter in ventilated emergence cages / boxes filled with a mixture of sieved soil and vermiculite to allow pupation. Irrespective of the number of adults emerged, adult emergence for all collections sites started July 3-6 and was completed two weeks later (Figs 1, A-C). Emergence pattern in 2006 confirm that during normal years emergence of *S. janiszewskae* starts late June – early July and lasts for 2-3 weeks (see also Gassmann et al. 2006). Adult emergence in 2004-2006 was delayed by about two weeks as compared to 2003 which has been exceptionally warm in the whole Europe.

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Table 1 Collections of *Sorhagenia janiszewskae* in 2006

Site	Country	Collection date	Host plant	# shoot tips collected	# adults emerged
Neulengbach	Austria	22 May 2006	*F. alnus*	100	19
Unterwaltersdorf	Austria	22 May 2006	*R. cathartica*	180	73
Purgstall	Austria	22 May 2006	*R. cathartica*	15	7
Truman	Austria	23 May 2006	*R. cathartica*	85	64
Reisenberg	Austria	23 May 2006	*F. alnus*	85	32
Collet Bossy	Switzerland	29 May 2006	*F. alnus*	100	9
Satigny	Switzerland	29 May 2006	*F. alnus*	140	70

As shown in Table 1, one major difficulty with *S. janizewskae* remains to accurately predict adult emergence and hence best collection time. Low rate of adult emergence in a few sites is probably due to local conditions which slow down larval development and immature larvae will not complete development in cut shoots.
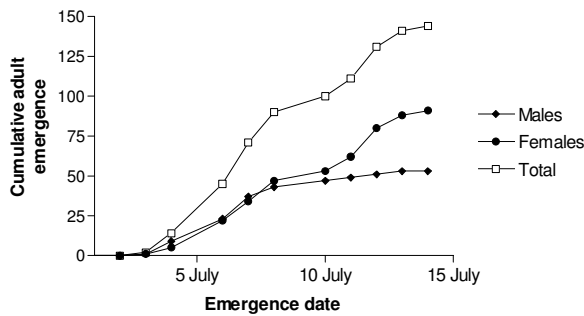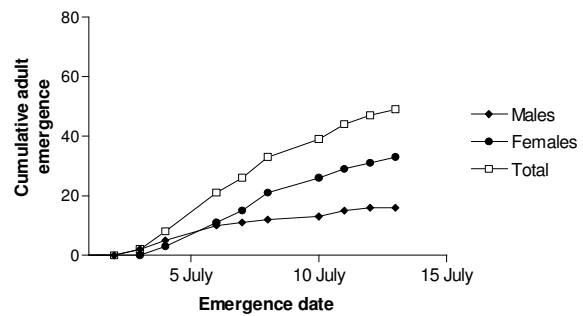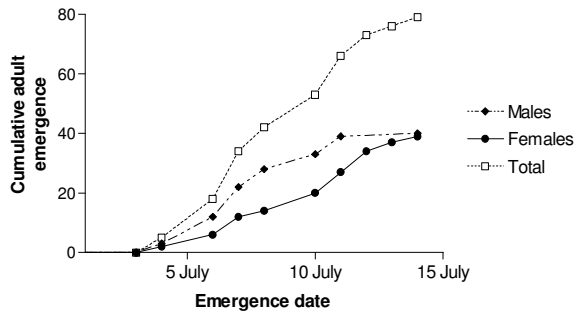

Fig 1A

Fig 1B

Fig 1C

Figs 1 A-C Emergence of *S. janizewskae* adults in 2006; A. from *R. cathartica* Austria; B. from *F. alnus* Austria; C. from *F. alnus* Switzerland

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Egg overwintering and egg hatch (2005-2006)

Sorhagenia janizewskae overwinters in the egg stage. Eggs presumably laid by *S. janizewskae* (reared from *F. alnus*) in single choice oviposition tests in 2005 (Gassmann et al 2006, table 5) were divided into batches of 10 in Petri dishes and kept under different conditions to study post diapause development. Batches of eggs were put at 10°C, 15°C and 20°C on 5 April 2006 after a cold treatment of 4.5 months at 3°C. Egg hatch at 10°C started on April 16 and was delayed by about one week compared to treatments at 15 and 20 °C (Fig 2). Egg hatch in an outdoor shelter started on April 12 and was completed on April 20. The percentage of egg hatch was comprised between 90-100% for all treatments.
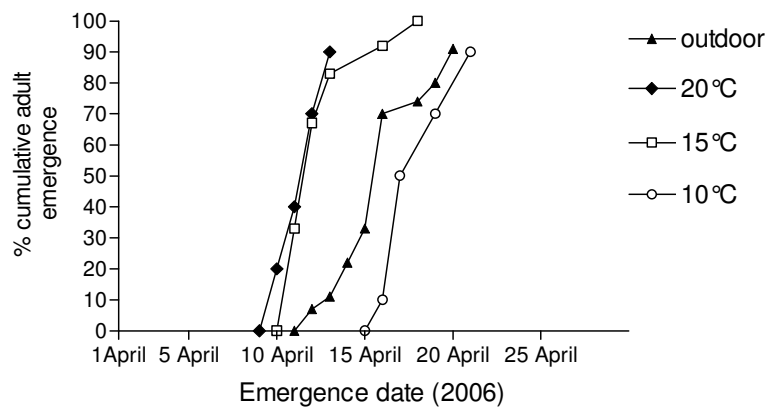

Fig 2 Egg hatch of *Sorhagenia janizewskae* under different temperatures after a cold treatment of 4.5 months at 3 °C (2005-2006)

Larval transfer and development

Larvae obtained from overwintering eggs were transferred into individual shoot buds of potted *R. catharctica* and *F. alnus* during April 12-28. Because the shoots were too thin to make a lateral hole near the top of the shoot, shoot tips were cut and a hole prepared at the cut end. All shoots were cut and dissected during June 9-12, i.e. before larvae left the shoots to pupate in the soil. Results are shown in Table 2.

Table 2 Larval development of *S. janizewskae* from *F. alnus*

Test plant	# of L1 transferred	# of larvae developed (%)
F. alnus	29	11 (37.9)
R. catharctica	37	11 (29.7)

Larval survival and development was quite similar on *F. alnus* and *R. catharctica*. The identification of adults emerged from this material is pending, but this is most certainly *S. janiszewskae*.

At the end of the 2005 field season, it was hypothesized that *S. janiszewskae* from *F. alnus* and *R. cathartica* are two different highly specific species or two different host races. The 2006 larval transfer tests infirm this hypothesis; the physiological host range of the larvae of *S. janiszewskae* from *F. alnus* includes both its field host plant and *R. cathartica*.

Single choice and no-choice field cage oviposition tests in 2006
Considering the results of preliminary single choice field cage oviposition tests in 2005, a large no-choice and single choice oviposition experiment was set-up with nine 2m x 2m x 2m field cages in the Centre's garden (Fig 3). Large 1m-1.5m tall potted *F. alnus* and *R. cathartica* were exposed singly or in combination to *S. janiszewskae* adults freshly emerged from *F. alnus* and *R. cathartica*. All cages were set-up July 4-10. All shoot buds of all plants were dissected in October. Very little oviposition occurred in the whole test (Table 3). The relatively good oviposition rate obtained in the 2005 oviposition test seems to have been exceptional and the best conditions for regular oviposition are still unknown. The field cage experiment carried out in 2006 does not show evidence of oviposition preference of *S. janiszewskae* from *F. alnus* and *R. cathartica*.

Fig 3 Field cage oviposition test with *S. janizewskae* in 2006

No choice oviposition tests in small cages in 2006
Three buckthorn species were individually tested each in two replicates in no-choice oviposition tests with three pairs of *S. janizewskae* from *R. cathartica* (Autrian population) in 80 x 40 x 40 cm cages. All cages were kept outside beneath a suspended tarpauline, protected from rain and sun. The tests were set up July 7-12. All shoot buds were dissected in late September. Results are summarized in Table 4. Again, very little oviposition occurred in the test and there is no evidence of oviposition preference of *S. janizewskae* for its field host plant.

Discussion

The absence of mines and larvae in plants exposed in the oviposition tests in 2005 and 2006 suggests that there is no second generation and confirm that *S. janiszewskae* overwinters in the egg stage. As indicated by the oviposition experiment carried out in 2004, oviposition most probably occurs within three weeks of adult emergence.

Sorhagenia janiszewskae eggs kept at 3°C hatched within one week when transferred to 15°C or 20°C. This suggests that, as for *Philereme vetulata* (This report), post diapause development requires a low number of degree-days and that temperature threshold for diapause development is < 3°C. Egg hatch in an outdoor shelter in 2006 occurred April 12-20. Egg hatch in many field situations is likely to occur slightly earlier when attacked shoot buds are exposed to local higher temperature or direct sunshine. In 2005, only newly hatched larvae were found in shoot buds of *F. alnus* collected 10-11 April at three sites in south-western Switzerland.

Following results obtained in 2005, we were expecting *S. janiszewskae* to oviposit readily in field cages. Preliminary data even suggested the possible occurrence of highly specific species or host races. Data obtained in 2006 are disappointing. First, *S. janiszewskae* females hardly oviposited in confinement. The higher number of eggs recorded in 2004 and 2005 was most probably due to normal oviposition by one or a very few females only, and this can't make oviposition tests reliable. Second, there is no further evidence of the occurrence of oviposition preference of the moth for its field host plant. Finally, larval transfer tests with newly hatched larvae showed that both *R. cathartica* and *F. alnus* are suitable hosts for larval development of *S. janiszewskae* from *F. alnus*. In our 2004-05 report (Gassmann et al. 2006), we were advocating a renewed interest for *S. janizewskae*. Results obtained this year and the difficulty to carry out reliable oviposition tests together with the need to give priority to potential biological control agents which are at least genus specific lead us to recommend discarding *S. janiszewskae* from the prime list of potential biological control agents for buckthorns in North America.

Table 3 Single choice and no-choice field cage oviposition tests in 2006

Cage design	*S. janiszewskae* from *F. alnus* (origin: Switzerland)	*S. janiszewskae* from *F. alnus* (origin: Austria)	*S. janiszewskae* from *R. cathartica* (origin: Austria)	# of shoot buds dissected		# of eggs in shoot buds	
				R. cathartica	*F. alnus*	*R. cathartica*	*F. alnus*
2 *F. alnus*	9 pairs	-	-	-	78	-	0
2 *R. cathartica*	9 pairs	-	-	256	-	0	-
2 *F. alnus*	-	5 pairs	-	-	183	-	1
3 *R. cathartica*	-	5 pairs	-	309	-	0	-
2 *F. alnus* + 2 *R. cathartica*	-	5 pairs	-	201	280	1	0
2 *F. alnus* + 2 *R. cathartica*	9 pairs	-	-	143	102	0	1
2 *F. alnus* + 2 *R. cathartica*	-	-	10 pairs	218	235	1	10
3 *R. cathartica*	-	-	10 pairs	371	-	1	-
2 *F. alnus*	-	-	10 pairs	-	240	7	-

Table 4 No-choice oviposition tests with *S. janiszewskae* from *R. cathartica* (Austrian population) in small cages in 2006

Test plant		# of shoot buds dissected	# of eggs in shoot buds
Rhamnus cathartica	Rep 1	70	3
Rhamnus cathartica	Rep 2	52	0
Rhamnus alnifolia	Rep 1	34	1
Rhamnus alnifolia	Rep 2	32	0
Frangula alnus	Rep 1	51	0
Frangula alnus	Rep 2	50	8

2.1.2 *Philereme vetulata* (Lep., Geometridae)

Philereme vetulata is exclusively associated with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965). Although the frequency of occurrence of this species on *R. cathartica* is only about 20%, populations are usually relatively common or abundant where they occur (Gassmann et al. 2006). Larvae of *P. vetulata* feed within folded leaves.

Collection and adult emergence
Following field collections, larvae were reared in ventilated plastic boxes stored in an outdoor shelter, where leaves were kept fresh with moist paper. Pupae were kept in ventilated plastic cups half filled with vermiculite and stored in an outdoor shelter for adult emergence. A total of 63 males and 118 females emerged from the collection of 318 larvae made in early spring in Germany and Switzerland (Fig 4). Another 98 males and 109 females emerged from larvae reared from eggs obtained in 2005. Batches of eggs kept at 3°C were regularly put at 20°C for this purpose. Newly hatched larvae were transferred onto young small folded leaves of potted plants before being transferred two weeks later in ventilated plastic boxes for completion of larval development. Last adult emerged on July 12, i.e. three weeks after the peak of adult emergence from field collected larvae.
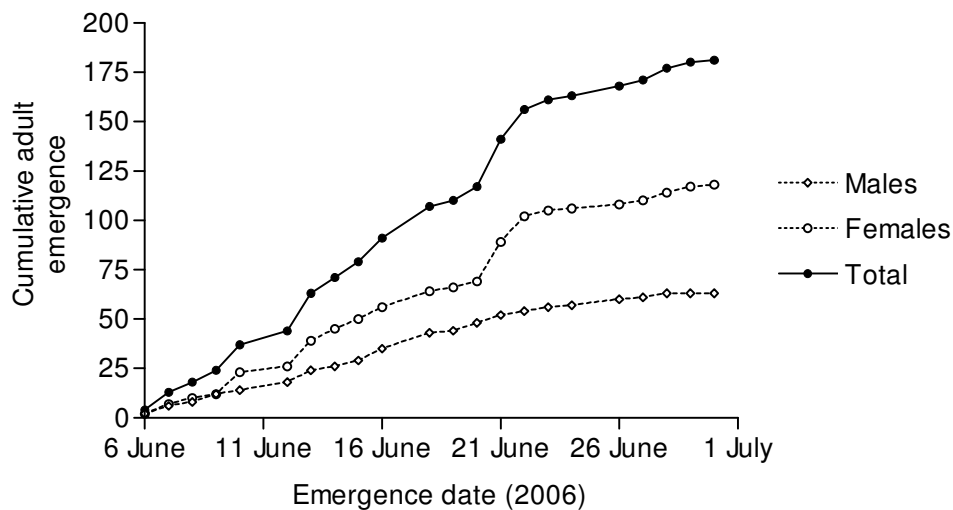

Fig 4 **Emergence of *Philereme vetulata* adults from three field sites in 2006**

Adult rearing and oviposition

A total of 120 females and 120 males were reared in 63 cardboard and plastic cylinders between 6 June and 24 July. Due to the high number of adults available, two

pairs were kept in each cylinder. A total number of 7475 eggs were obtained of which 75% (5603 eggs) were fertile. Average fecundity per female was 62 eggs, i.e. similar to that in 2004 when the females were reared in the same conditions, but lower than that of individual females in 2005 which was 88 eggs per female (Gassmann et al. 2005; 2006). Average fertile eggs per female were 47.

Diapause development and post diapause development of the egg of *Philereme vetulata* **(2005-06)**

Philereme vetulata is univoltine and overwinters in the egg stage. Observations made during larval transfer tests in previous years indicate that synchronisation between larval hatching and leaf bud development is important for the larvae to settle and start feeding. In order to best synchronize larval hatching and plant phenology for future larval feeding and development tests, diapause development and post diapause development of *P. vetulata* eggs were studied under different regimes of cold temperature and post diapause development temperatures. Studies in 2004-2005 indicated that a cold period at either 3°C or 10°C followed by a temperature of 20°C allowed over 80% of egg hatch within 20 days.

Material and methods: Studies were repeated in 2005-2006 under three different cold treatment (3°C, 10°C and 15°C) and five post diapause development temperature (20°C, 15°C, 12.5°C, 10°C and 7.5°C) in order to define more precisely the temperature thresholds for diapause and post diapause development. Eggs were divided into batches of 50 and kept relatively moist with short period of drier conditions during the whole experiment. Cold treatments were started on 11 November 2005, i.e. three weeks later than in the 2004-05 experiment.

Results: A cold treatment is necessary for diapause development. Fig 5A shows that a cold treatment of one month at 3°C followed by a temperature of 20°C allows some egg hatch, i.e. the diapause of a few eggs is less intense and requires a shorter exposure to cold treatment. In contrast to the 2004-05 experiment, a cold treatment of two months at 3 °C allows complete diapause and post diapause development. Time to egg hatch is slightly shortened when the cold treatment is prolonged to 3 and 4 months at 3°C.

Fig 5B shows egg hatch when the same cold treatments at 3°C were applied followed by a temperature of 15 °C. At 15°C, egg hatch is optimal after a cold treatment of 3 or 4 months at 3°C. Again, in contrast to the 2004-05 experiment, a cold treatment of 2 months at 3°C allows nearly complete diapause and post diapause development. Time to egg hatch is however longer than after a cold treatment of 3 or 4 months at 3 °C. 32% egg hatch occurred after one month only of cold treatment confirming that a 15°C temperature allows some diapause development.

Figs, 5C-E shows that egg development is optimal at any cold treatment of 3°C when the cold treatment is followed by temperatures of 12.5°C, 10°C and 7.5°C. Not surprisingly, time to egg hatch is slightly longer at 10°C than at 12.5°C and even longer at 7.5°C. As compared to the 2004-05 experiment, time to egg hatch at 10 °C was noticeably shorter in 2005-06 after a cold treatment of 2 months at 3 °C.
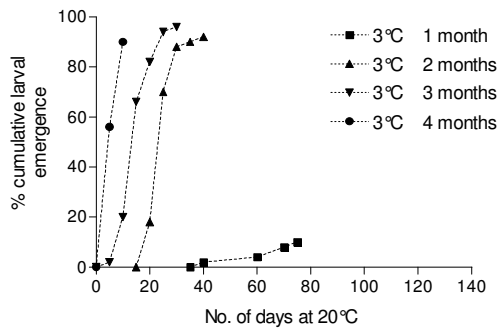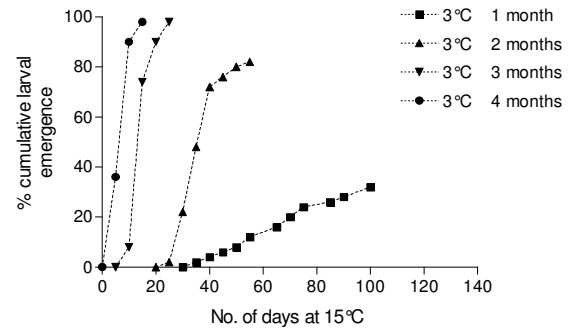
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Fig 5A
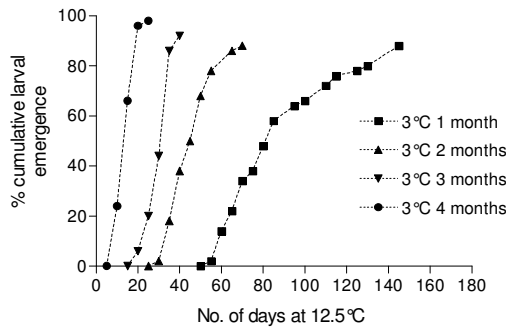
Fig 5B
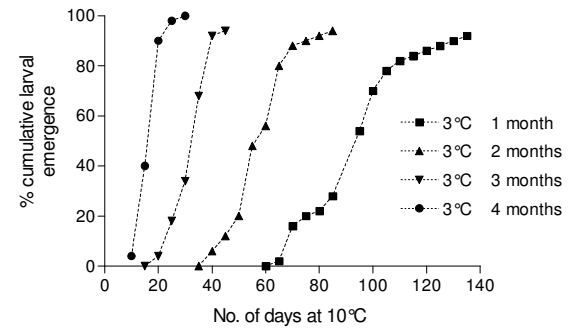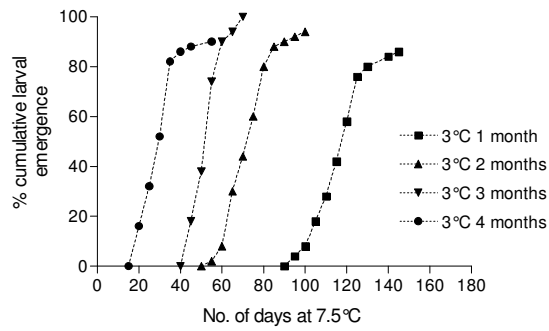
Fig 5C

Fig 5D

Fig 5E

Figs 5A-E Diapause development and post diapause development of *P. vetulata* eggs under different temperature regimes

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Fig 6A shows that a cold treatment of one month at 10°C followed by a temperature of 20°C allows only very little egg hatch. In contrast, a cold treatment of 2 months at 10°C followed by a temperature of 20°C allows a nearly complete diapause development and normal egg hatch. In the 2004-05 experiment, only 18% egg hatch occurred after a 2 months treatment at 10°C. Time to egg hatch was slightly longer with a cold treatment of 2 months at 10°C than with a cold treatment of 3 or 4 months at 10 °C. Larvae started to emerge at 10 °C at the end of the four months cold treatment

Fig 6B shows egg hatch when the same cold treatments at 10°C were applied followed by a temperature of 15 °C. 30% of egg hatch was recorded at 15 °C with a cold treatment of only one month at 10°C. In contrast to 2004-05, almost normal egg hatch was obtained at 15°C after two months cold treatment at 10°C. Time to hatch after 2 months cold treatment at 10°C was only slightly delay compared to longer cold treatment (3 and 4 months at 10°C). In this trial also, larvae started to emerge at 10 °C at the end of the four months cold treatment.

Fig 6C shows egg hatch at 20°C after a cold treatment of 1,2,3 and 4 months at 15 °C. Only few eggs hatched after 1, 2 and 3 months cold treatment at 15 °C.
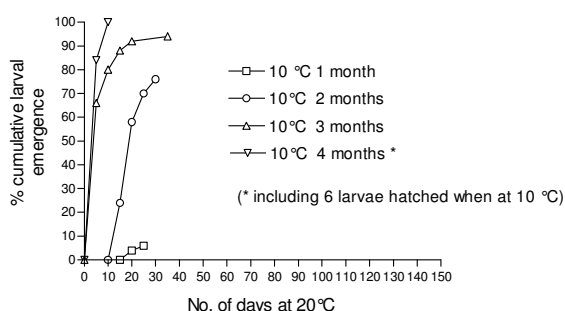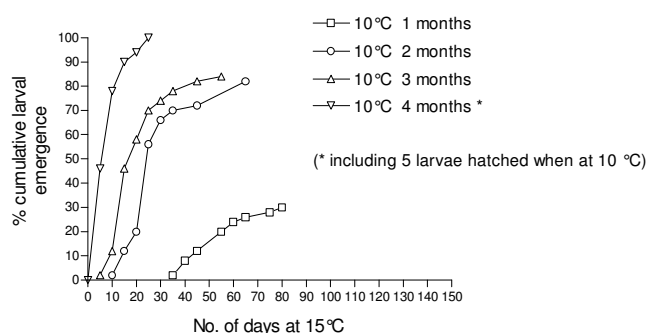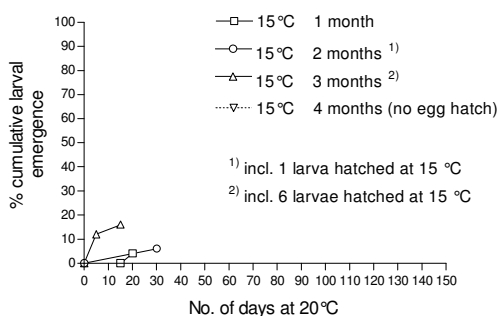

Fig 6A

Fig 6B

Fig 6C

Figs A-C   Diapause development and post diapause development of *P. vetulata* eggs under different temperature regimes

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Results are summarized in Fig 7. Normal egg hatch occurred after three months cold treatment at 3 °C and 10 °C. In contrast to the 2004-05 experiment, normal egg hatch occurs also after two months cold treatment at 3°C and nearly so after two months at 10°C. The difference of 20 days in the set-up of the experiments in 2004-2005 and 2005-2006 corresponds roughly to one month cold treatment at 10 °C: the mean daily temperature for the period 21 October – 10 November 2005 was 10.7 °C (meteorological data from FRI, Courtemelon). Thus, eggs have been exposed to additional cold treatment when kept in outdoor conditions in autumn 2005. Not surprisingly, mean time to egg hatch depends on the temperature following the cold treatment. The time to egg hatch and the difference in egg hatch between treatments is reduced with longer cold treatment at 3 °C or 10 °C, indicating that egg development occurs at 3°C and at 10°C.
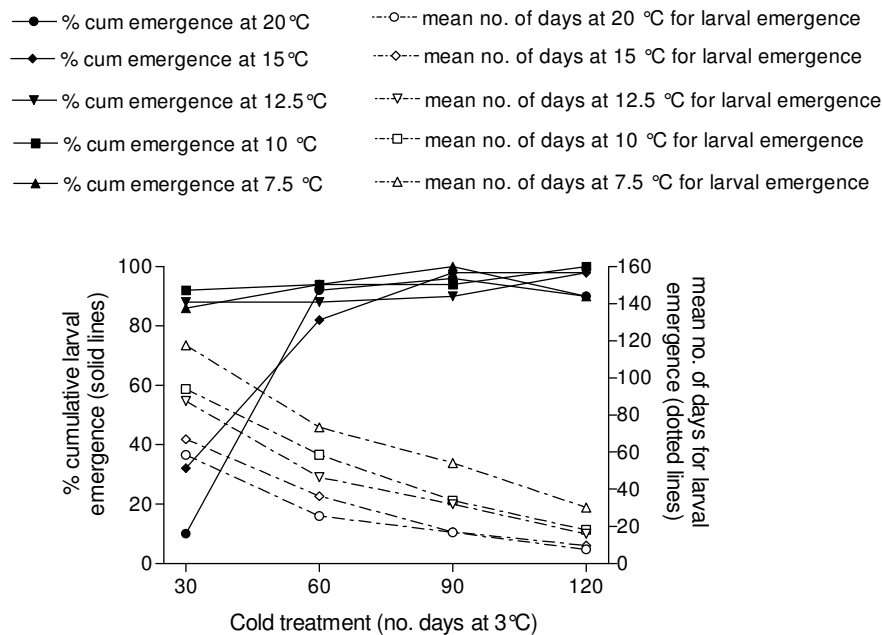

Legend:
— ● — % cum emergence at 20 °C   ‑‑‑o‑‑‑ mean no. of days at 20 °C for larval emergence
— ◆ — % cum emergence at 15 °C   ‑‑‑◇‑‑‑ mean no. of days at 15 °C for larval emergence
— ▼ — % cum emergence at 12.5 °C   ‑‑‑▽‑‑‑ mean no. of days at 12.5 °C for larval emergence
— ■ — % cum emergence at 10 °C   ‑‑‑□‑‑‑ mean no. of days at 10 °C for larval emergence
— ▲ — % cum emergence at 7.5 °C   ‑‑‑△‑‑‑ mean no. of days at 7.5 °C for larval emergence

Fig 7   Percent of cumulative larval emergence and mean time for larval emergence of *P. vetulata* under different temperature regimes

Fig 8 shows egg hatch at constant temperature of 3°C, 10°C, 15°C, and 20°C, and in outdoor conditions in 2004-2005 and 2005-2006. No egg hatches at 20 °C and only few at 15 °C, confirming that a temperature of 15°C allows both some diapause and post diapause development. Considering that diapause development may have occurred in part before the set-up of the experiment in autumn 2005, it is hypothesized that the upper limit for diapause development is slightly below 15°C. When kept continuously at 10 °C, egg hatch started in early March, i.e. less than one month later than in the 2004-2005 experiment, but the mean number of days to egg hatch since January 1 was identical in 2005 and 2006, i.e. 137 days. Eggs kept at a 3°C constant temperature started to hatch in mid-May, thus confirming that the

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temperature threshold for egg development of P. vetulata is below 3°C (Fig 8). 77% of egg hatch was recorded within 30 days only.

The calculation of the lower threshold for egg development is at least problematic since the 3°C cold treatment allows development. After a cold treatment of two months at 3 °C, the regression line (r^2 = 0.99) gives a temperature threshold of 1.6°C and a number of degree-days required for development of 480 (= slope $^{-1}$). After a cold treatment of three months at 3°C, the temperature threshold for development is 1.9°C and the number of degree-days required for development is 279 (= slope $^{-1}$) (r^2 = 0.85 for the regression line).

The period of egg hatch in outdoor conditions lasted only 12 days from late March until early-mid April and was similar in 2005 and 2006 (Fig 8). Assuming an hypothetical threshold for egg development of 1°C, the accumulation of temperatures above 1°C from January 1 for egg development in outdoor conditions was similar in 2005 and 2006, i.e. 275 (1 January - 7 April 2005) and 277 respectively (1 January – 20 April 2006).
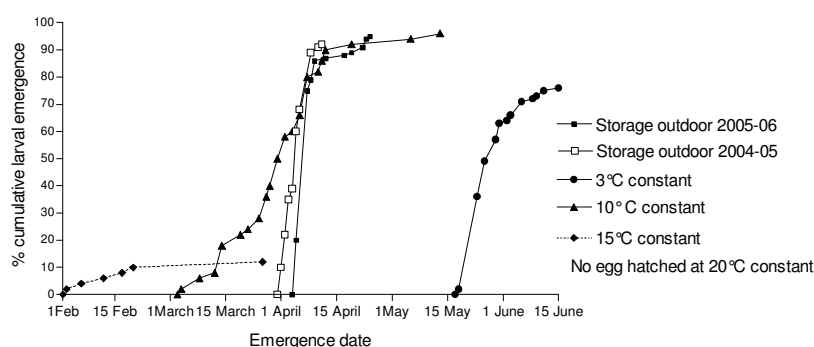

Fig 8   Percent of cumulative larval emergence of *P. vetulata* at constant temperatures and in an outdoor shelter (2005-2006)

Larval feeding and development tests
Introduction and methods: In 2005, it was observed that 43% of newly hatched larvae transferred onto potted plants with newly developed leaf buds successfully completed development. In contrast, a much higher mortality occurred with larvae transferred in individual Petri dishes with cut stem/leaves. Many larvae died because of an excess of humidity in the Petri dishes, or in contrast, young folded leaf material often dried very quickly when rearing conditions were kept too dry. As a result only few larvae established on cut unfolded leaves. Consequently, larval feeding and development tests in 2006 were carried out with potted plants only. Plants were regularly taken from the garden and put in a heated greenhouse to accelerate bud development when necessary. Batches of eggs kept at 3°C were transferred to 20 °C when leaf buds seemed to be at the right phonological stage. Plants with transferred larvae were covered with a gauze bag and kept in an unheated greenhouse for about one week before being transferred in the garden. Most tests were set-up 24 April – 10 May. The

number of larvae transferred onto each potted plant varied from 3 to 12 according to the number of leaf buds per plant and the number of plants available. All plants were dissected 2-3 weeks after set up and the larvae transferred in ventilated plastic boxes kept in an outdoor shelter for completion of larval development. Fresh food was provided to the larvae twice a week or more when necessary. Pupal weight was measured within five days after pupation.

Results and discussion: Results are summarized in Table 5. Survival to pupal stage was similar on *R. cathartica*, *R. alpina* and *R. alnifolia*. However, *R. alpina* and *R. alnifolia* seem to be providing a slightly less optimal food source for *P. vetulata*. The pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* and *R. alpina* (Dunnett's test, P<0.05), and the time to pupation was significantly shorter on *R. cathartica* than on *R. alnifolia* and *R. alpina* (Dunnett's test, P<0.05). No larval establishment and larval damage was observed on *F. alnus* and *F. caroliniana*.

Table 5 Larval survival and development of *P. vetulata* in no-choice conditions in 2006

Test Plant	# L1 transferred (# potted plants)	# larvae alive 2-3 weeks after set-up (%)	# pupae (%)	Pupal weight (mg) (SD)	# days to pupation ± SD	# adults emerged (% of pupae)
R. cathartica	119 (25)	92 (77.3)	86 (72.3)	0.055 ± 0.012 [a]	32.8 ± 3.7 [a]	73 (61.3)
R. alpina	80 (9)	63 (78.8)	48 (60.0)	0.051 ± 0.010 [a]	37.3 ± 3.9 [b]	46 (57.5)
R. alnifolia	58 (5)	41 (70.7)	40 (69.0)	0.046 ± 0.001 [b]	34.5 ± 2.4 [c]	38 (65.5)
F. alnus	80 (12)	-	-	-	-	-
F. caroliniana	75 (10)	-	-	-	-	-

The high rate of larval establishment on the field host plant confirmed the reliability of the method consisting in transferring newly hatched larvae on potted plants with newly developed leaf buds. Pupal weight and time to pupation on *R. alnifolia* are less than on *R. cathartica* but the former species is likely to be a suitable host for larval development. The fecundity of females reared on *R. alnifolia* will be studied in 2007. Species in genus *Frangula* are not suitable hosts for larval development of *P. vetulata*.

In partial larval feeding tests with medium-sized larvae, Malicky et al. (1970) found consistent feeding on *R. cathartica* and *R. alpina*, but inconsistent feeding on *R. saxatilis*, *R. alaternus* and *F. alnus* in a "short-term test" for the later species. No feeding was recorded on *F. purshiana*. These tests suggest that not all *Rhamnus* species are suitable hosts for *P. vetulata*, but this result will need to be confirmed in future tests with additional *Rhamnus* species. The tests carried out by Malicky et al. (1970) confirm that species in genus *Frangula* are not suitable for larval development of *P. vetulata*.

In the field in Europe, *P. vetulata* has been recorded almost exclusively on *R. cathartica*. This species has not been found on *F. alnus* (the single record from 2004 turned out to be a sampling mistake) and only once on *R. alpina* (Malicky et al. 1970). Likely specific requirements for larval establishment related to plant phenology, stage

of developing leaf bud, leaf shape and toughness, as well as habitat requirements will restrict host acceptance and host suitability to a few species in the genus Rhamnus. The realized host range will be evaluated by choice oviposition tests which are planned in large field cages in 2007. Eggs are laid on the bark of the host plant in natural conditions. The behaviour of ovipositing females in field cages has not been evaluated yet, so the reliability of such tests is uncertain for the time being.

2.2 *Homoptera*

2.2.1 *Trichochermes walkeri (Triozidae)*

Collections and adult emergence
430 adults of *T. walkeri* emerged from a total of 3000 leaf margin curl galls collected 25 July in the Jura Mountains and 9 August at another two collection sites in western Switzerland (Fig 9). Galls collected in the Jura Mountains mature earlier and gave a better adult emergence rate (28%) as compared to the galls collected in western Switzerland (8%). The sex ratio was nearly 1:1 at all collections sites.
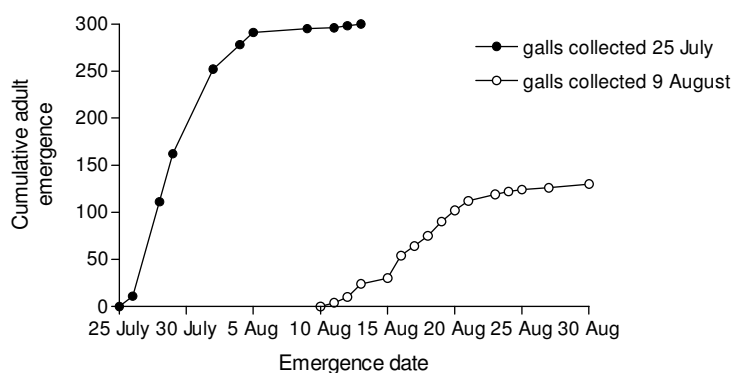

Fig 9 Emergence of adults of *T. walkeri* in 2006

Oviposition and gall development 2005-2006
80 branches of potted *R. cathartica* with 2527 eggs of *T. walkeri* marked with colour threads laid in August-October 2005 were protected from natural oviposition under a large gauze tent in the greenhouse until late November 2005 before being kept outdoors until early July 2006. The number of galled leaves, galls and larvae was counted during the second week of July, i.e. before the larvae leave the galls. A total of 325 galls and 347 mature larvae were obtained from the 2527 eggs laid in 2005 (Table 6). Thus 13.7% of the eggs developed successfully into mature larvae in well developed galls. The number of eggs (31.6 ± 21.9; N=80) and galls per branches (4.1 ± 4.4; N= 80) was much variable. 13 % of attacked leaves had more than one gall, usually no more than two. 59% of all galls contained one larva each but 17% were empty partly because of predatory mites. Two larvae were recorded in 19% of the galls and three larvae in 5%. No galls were found on 20 branches carrying a total of 422 eggs. No galls were recorded on *R. alnifolia.*

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Table 6 Oviposition and larval development of *T. walkeri* in 2005-2006

Test plant	No. eggs (2005)	No. of galled leaves (2006)	No. of galls (2006)	No. of larvae (2006)
R. cathartica	2527	285	325	347
R. alnifolia	20	0	0	0
R. alpina	1	0	0	0

Because eggs of *T. walkeri* are too fragile to be removed from their branches, cut branches with were placed in styrofoam boxes to study egg development and for larval transfer. Batches of 50 eggs were kept in a 3°C incubator September 2005 – March 2006 before being transferred to various temperatures (20°C, 15°C, 12.5°C, 10°C and 7.5°C). The material decayed quickly in all storage conditions and no egg hatch was observed. It is strongly suspected that manipulation and storage of eggs on cut plant material is not feasible.

Sequential no-choice oviposition tests
Background: Oviposition by *T. walkeri* started 3-4 weeks after set-up in all tests carried out during previous years. No eggs were laid in 2004 in no-choice oviposition tests carried out with *R. alnifolia*, *R. alpina*, *F. alnus* and *F. caroliniana*. However, because these preliminary results also indicated that none of the test plant was suitable for adult feeding and adult survival for a period extending until the oviposition had started, oviposition on non target plants could not be completely excluded. Therefore, in 2005, no-choice oviposition tests were carried out with females, which had been previously exposed to *R. cathartica* for three weeks. 71 eggs per female (N=19) on average were laid on *R. cathartica*. Twenty eggs only were laid on *R. alnifolia* by a total of 3 females. However, female longevity was much reduced on all test plants (after a three weeks feeding and preoviposition period on *R. cathartica*) as compared to the field host plant and additional oviposition could not be excluded, had the females survived for a longer period in no-choice conditions (Gassmann et al. 2006). Therefore, sequential no-choice oviposition tests were carried out in 2006.

Materiel and methods: All females and males were exposed to *R. cathartica* for four weeks in groups of 3 pairs each in 20 ventilated plastic cylinders (Ø 11.0 cm, height 15.0 cm) fixed on branches of potted *R. cathartica.* Four test plant species were then individually tested with one pair of *T. walkeri* in small ventilated plastic cups (Ø 7.0 cm, height 8.5 cm) fixed on branches of potted plants. The containers were kept outside beneath a suspended tarpauline, protected from rain and sun.
Because no-choice adult feeding and survival tests carried out in 2005 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 plant changed sequentially between the test plant and the target plant (*R. cathartica*). The assumption was that females would survive on the test plants before being exposed again to *R. cathartica*, thus allowing them to oviposit on perhaps less preferred but acceptable plant species. Sequential exposure to shorter periods was not feasible because of time constraints. For each test plant, 50% of the replicates started with the test plant and 50% with the target plant.

All sequential no-choice tests were set-up 27 July – 21 August. Branches with eggs were marked with colour threads. All plants used in tests been protected from natural infestation by *T. walkeri* and other herbivores in 2m x 2m x 2m field cages from mid-June until mid November. All plants are overwintering in the Centre's garden and will be put back in 2m x 2m x 2m field cages in April 2007. Gall development will be recorded from mid-May 2007 onwards.

Results and discussion: 60% of males and females survived the four weeks preoviposition period during which 390 eggs have been laid. Results of the sequential no-choice tests are summarized in Table 7. In the *R. cathartica – R. alnifolia* series, a total of 340 eggs and 16 eggs were laid on *R. cathartica* and *R. alnifolia* respectively. In the *R. cathartica – R. alpina* series, 163 and 20 eggs were laid on *R. cathartica* and *R. alpina* respectively. Only 10-12 eggs on average were laid per female on *R. cathartica* in both series. This is much lower than the 71 eggs recorded per female in the no-choice oviposition tests in 2005 (Gassmann et al 2006). However, the number of ovipositing females on *R. cathartica* was relatively similar to what had been observed on average during previous years, i.e. about 50% on average for the two series. The mean total female longevity in the two series was similar to that observed on *R. cathartica* in no-choice tests in 2005. Total longevity of ovipositing females was about 12 days longer than that of sterile females. Almost no eggs were laid in the *R. cathartica – F. alnus* series. All females but one died when first exposed to *F. alnus* for 3-4 days.

The tests carried out in 2006 confirm and refine that of previous tests. Very little oviposition occurred on the non target species in genus *Rhamnus* and no oviposition occurred on *Frangula*. 3-4 days periods of feeding on non target *Rhamnus* species (or the interruption of feeding on the target plant for 3-4 days) do not hamper significantly adult survival but this has a detrimental impact on the reproductive output of the females. Altogether, much fewer eggs were laid on *R. cathartica* but more oviposition attempts on non target hosts have been recorded. In the *R. cathartica – R. alnifolia* and *R. cathartica – R. alpina* series, 25% of all females laid a few eggs on the non target hosts (Table 7).

Perhaps surprisingly, in the *R. cathartica – F. alnus* series, all females except one died during the first exposure to *F. alnus* confirming that *Frangula* is not a suitable host for adult feeding and survival even in the alternate presence of the target host. Thus, if feeding probes occur, it is possible that *F. alnus* is lethal to the adults.
Finally, the possible detrimental impact of repeated manipulations - when transferring adults from one plant to another every 3-4 days - on adult fitness and fertility cannot be excluded.

Discussion: *Trichochermes walkeri* overwinters as eggs which are laid usually on the leaf axil. The manipulation and overwintering of eggs on cut material is not feasible. As shown in 2003, the transfer of first instar larvae or older larvae from young galls onto the leaves of potted plants does not provide conclusive results to assess the physiological host range of *T. walkeri*. Therefore, host specificity tests should rely on oviposition tests and subsequent larval and gall development.
No choice adult feeding tests carried out in 2005 indicate slightly prolonged longevity on non target hosts as compared to the no-food control, suggesting that feeding probes on the non target plants may have occurred. On *F. alnus*, slight feeding is perhaps

even lethal to the adults. Oviposition on non target hosts can be excluded in field situations where *R. cathartica* does not occur since *T. walkeri* females will die within ten days, i.e. much before the oviposition will start, usually 3-4 weeks after adult emergence. In mixed stands, evaluating oviposition behaviour of *T. walkeri* is not straightforward. No-choice tests carried out in 2005 with females, which had been previously exposed to *R. cathartica* for three weeks, resulted in very little oviposition on non target hosts. In these tests also, non target species were not suitable for sustained adult feeding and adult survival for a period extending much after the oviposition had started, thus consistent oviposition on non target plants could not be totally excluded. In 2006, sequential no-choice tests did not reduce adult longevity but resulted in a much lower oviposition rate on *R. cathartica*. This is maybe due to repeated manipulation inherent to the test, to the detrimental impact of slight feeding on insect fitness, or to the interruption of regular feeding on the target plant. Oviposition on non target hosts was low, consisting in 7% of the total number of eggs laid in the test. As in 2005, the number of eggs laid on non target hosts in 2006 will be most probably too low to get significant results as to whether any gall will develop in 2007. Single choice tests will be carried out in 2007 to further evaluate the suitability of *R. alnifolia* for oviposition by *T. walkeri*. New attempts of larval transfer are planned as well.

Table 7 Sequential no-choice oviposition tests with *T. walkeri* in 2006 (after a four weeks feeding and preoviposition period on *R. cathartica*)

	TEST SERIES					
	R. cathartica – R. alnifolia (N=29)		*R. cathartica – R. alpina* (N=16)		*R. cathartica – F. alnus* (N=11)	
	R. cathartica	*R. alnifolia*	*R. cathartica*	*R. alpina*	*R. cathartica*	*F. alnus*
Total no. of ♀ / days	290	302	161	143	40	59
Total no. of eggs	340	16	163	20	5	0
Mean no. eggs /♀ (SD)	11.7 (13.1)	0.6 (1.4)	10.2 (22.4)	1.3 (2.7)	0.5 (1.2)	0
Number of ovipositing females (% of total no. females))	17 (59)	7 (24)	6 (38)	4 (25)	2 (18)	0
Mean female longevity in the series (SD)	20.4 (12.7)		19.8 (13.5)		9.0 (4.4)	
Total female longevity (SD)	50.0 (12.1)		47.4 (13.0)		35.4 (7.1)	

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2.3 Flower and fruit feeding insects

2.3.1 *Wachtiella krumbholzi (Dipt.; Cecidomyiidae)*

In 2004, preliminary collections of flowers and fruits of *R. cathartica* were carried out in Austria, Germany, Switzerland and Serbia and an important population of the midge *Wachtiella krumbholzi* was discovered in the fruits of *R. cathartica* in northeastern Serbia. According to Skuhrava (pers. com. 2005), *W. krumbholzi* cannot be considered to be cecidogenous. Field observations in Serbia also suggest that *W. krumbholzi* is a seed feeder rather than a gall maker. The main characteristic of attacked fruits is similar to early fruit maturation with changes in colour. Attacked fruits become dark-red black while healthy fruits are still green. Gall swelling is not visible on damaged fruits. In the laboratory, the midge larvae leave the fruits and go into the soil to prepare a larval cocoon made of silk and soil debris.

On 11 July 2005, over 3000 fruits of *R. catharica* apparently attacked by *W. krumbholzi* were collected in Serbia and sent to Switzerland. 280 larvae of presumably *W. krumbholzi* from *R. cathartica* were transferred into Petri dishes with a mixture of sieved soil and vermiculite. The soil was sieved in early September. Seven larvae alive and 123 larval cocoons were recovered (i.e. 44% of the larvae successfully built a larval cocoon for overwintwering). 60% of this material was kept in a 3°C incubator for overwintering. The remaining 40% was kept continuously in an outdoor shelter.

Emergence of gall midge adults kept in outdoor shelter started on 19 May 2006, i.e. at the same time as in 2005 (Gassmann et al. 2006), and was completed on May 28 (Fig 10). Percent of emergence was low (14%) as in the previous year. On 20 June 2006, gall midge cocoons kept in the 3°C incubator were moved into a 20 °C incubator. Adult emergence started two weeks later and was completed within six days. 10 males and 27 females were obtained (i.e. 46%) and were put in 80% alcohol for confirmation of their identification.

Eight parasitoids emerged from the same batches of larval cocoons. Keeping larval cocoons at 3°C and moving them to 20°C seem to be a reliable method to synchronize adult emergence with plant phenology. The high larval mortality in Petri dishes in the outdoor shelter seems to be due partly to drought. In 2006, larvae of *W. krumbholzi* are being stored in 9x9x9 cm ventilated plastic boxes half filled with a mixture of sterilized sieved soil and vermiculite.

In early July 2006, over 5000 fruits of *R. cathartica* apparently attacked by *W. krumbholzi* were collected in Serbia and sent to Switzerland on 14 July. Some 800 larvae have been reared from this material and transferred to Petri dishes filled with a mixture of sterilized sieved soil and vermiculite. In late August, the soil was sieved and 685 larval cocoons recovered (86%). Batches of 50 larval cocoons have been placed in 9x9x9 cm ventilated plastic boxes half filled with a mixture of sterilized sieved soil and vermiculite. 400 larval cocoons are being at in a 3°C incubator for overwintering and the remaining 285 ones in an outdoor shelter.
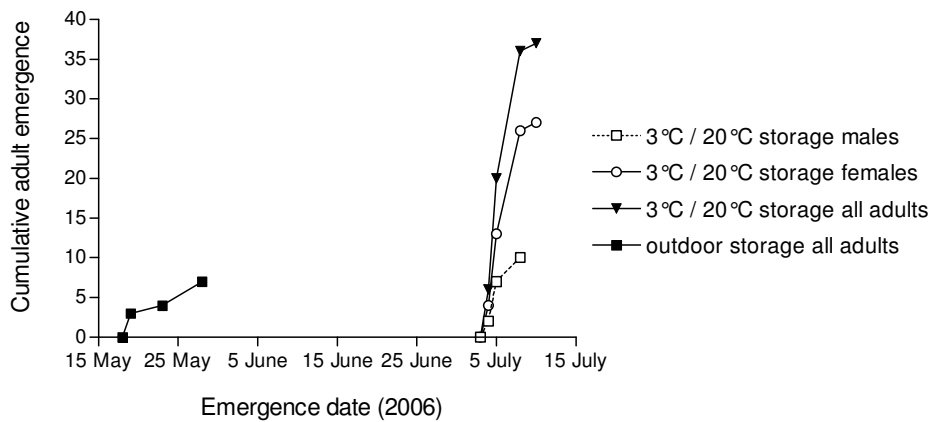
Fig 10 Emergence of *W. krumbholzi* adults under different conditions (2005-2006)

2.3.2 Others

Five larvae of an unknown lepidoptera species have been found in the fruits of *R. cathartica* in Switzerland in 2005. These larvae have been kept in an outdoor shelter for overwintering. No adult but one parasitoid emerged in 2006. Two adults of an unyet identified Lepidoptera species have emerged form the fruits collected in Serbia in early July 2006.

3   General discussion

Work carried out in 2006 has highlighted the difficulty to rear or to test some of the potential biological control agents for buckthorns. In spite of this, additional data with the shoot-tip boring moth *S. janiszewskae* confirm that both *R. cathartica* and *F. alnus* are suitable hosts for this species. The selection of biological control agents which attack both *R. cathartica* and *Frangula alnus* in their native range will undoubtfully increase potential non-target impacts. Therefore we suggest giving *S. janizewskae* a low priority.

Work in 2006 has also highlighted the difficulty to demonstrate with certainty the absence of consistence oviposition by *T. walkeri* on non target plants in no-choice conditions. However, the probability for high oviposition rate and gall and larval development on non target *Rhamnus* species is extremely low. Oviposition choice tests should confirm the high specificity of *T. walkeri*.

Work carried out in 2006 has also highlighted progress with the rearing and testing of another potential biological control agent. Transfer of newly hatched larvae of *P. vetulata* on potted plants at the time of leaf bud expansion has produced remarkable results. Species in genus *Rhamnus* appear to be suitable hosts for larval development of this moth although variability in food quality may result in lower pupal weight or longer time for development to pupal stage. *Philereme vetulata* is restricted to *R. cathartica* in its European native range. The feasibility of oviposition choice tests will be studied in 2007.

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Finally progress has been made in mass collection, and rearing to the adult stage of the seed feeding midge *W. krumbholzi*. Oviposition and larval development will be studied in 2007. Growing plants to fruiting stage and the synchronisation between adult emergence and plant phenology will be the main challenges in future tests.

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Result 1- Part 2: Investigate potential insects as biological control of European Buckthorn

Reassessment of the potential for Biological Control of Buckthorns (*Rhamnus cathartica* and *Frangula alnus*)

(2002-2007)

with

a summary of work done in 2007

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Summary

In total, 39 specialized arthopods were recorded from *R. cathartica* and *F. alnus* in Europe. The feeding guild on *R. cathartica* and *F. alnus* was dominated by leaf feeders (18 species), followed by sap-suckers/leaf gall formers (12 species), flower or fruit feeders (6 species), and shoot/root borers (3 species).

The reassessment of the potential for biological control of *R. cathartica* and *F. alnus* is presented by target species and by the arthropod feeding guilds. It is based on work done in Europe from 2002-2007 on 10 selected potential biological control agents.

This reassessment is based on the assumption that candidate biological control agents should be monospecific either to *R. cathartica* or to *F. alnus* or their host range should be restricted to either a few species in the genus *Rhamnus* or to a few species in the genus *Frangula*.

A summary of 10 priority species for future research on biological control of *R. cathartica* is given in Table 1, page 13.

The discovery of genus or species specific potential biological control agents for F. alnus will undoubtfully be difficult in Europe. Priority should be given to two fruit and seed feeding midges which are likely to be specific at the species or genus level according to field records in Europe.

The last part of this report gives a summary of work carried out in 2007.

PART I

Reassessment of the potential
for biological control of *R. cathartica* and *F. alnus*

Introduction

Rhamnus cathartica L. (common buckthorn) and *Frangula alnus* Miller (glossy buckthorn) (Rhamnaceae) are both shrubs and small trees of Eurasian origin which have become invasive in North America.

Rhamnus cathartica was introduced to North America as an ornamental shrub in the late 1800s and was originally used for hedges, farm shelter belts, and wildlife habitats (Gourley, 1985; Randall and Marnelli, 1996; Gale, 2001). It has spread extensively and is currently found in most Canadian provinces (Nova Scotia to Saskatchewan) and 27 U.S. states predominantly in the north central and northeastern portion of the United States (Gale, 2001; USDA/NRCS, 2001).

Rhamnus cathartica is found throughout Europe, but is absent from most parts of Scandinavia and the Iberian Peninsula, and from the extreme south. It prefers mesic to mesic-dry warm open or half-shaded habitats. It grows best in calcareous alkaline or neutral soils but it can also be found occasionally in humid or swampy areas (Rameau et al. 1989).

Frangula alnus was imported to North America prior to the 1900s as horticultural stock for landscape plantings and has become naturalized in the northeastern USA and southeastern Canada (Catling and Porebski 1994; Randall and Marnelli 1996; Haber 1997). Currently, *F. alnus* occurs from Nova Scotia to Manitoba, south to Minnesota, Illinois, New Jersey and Tennessee incorporating 23 states in the USA (Converse 2001; USDA/NRCS 2001).

Frangula alnus has a slightly wider distribution than *R. cathartica* extending from northern Scandinavia in the boreal zone up to the Iberian Peninsula and a southernmost enclave in western North Africa. In temperate Europe, *Frangula alnus* grows in various open to half shaded habitats. It can be found occasionally in dry calcareous stands but it is usually a widespread woody pioneer species on acid, moist soils (Rameau et al. 1989).

Research to develop biological control for buckthorns was initiated in 1964. Surveys for potential arthropod biological control agents were carried out mostly in Eastern Austria in summer 1964 and 1965 and preliminary screening tests in 1966-1967 (Malicky et al., 1970). A new programme was initiated in 2001 to reassess the potential of biological control of buckthorns with regard to the work carried out by Malicky et al. (1970). In recent years there have been ever-increasing concerns over potential non-target impacts of biological control agents and greater demands for high levels of specificity (e.g. Louda et al., 1997; Pemberton, 2000). The key question was whether *R. cathartica* and *F. alnus* are sufficiently distantly related that they would not share the same arthropod complex in Europe and, consequently which arthropod

species could be species or genus specific to be selected for further host range studies, and possibly later on be used for biological control of either *R. cathartica* or *F. alnus* without damaging native North American buckthorns.

The reassessment of the potential for biological control of *R. cathartica* and *F. alnus* is presented by target species and by the arthropod feeding guilds. It is based on work done in Europe from 2002-2007 on 10 selected potential biological control agents (Gassmann et al. 2004; 2006; 2007).

The last part of this report gives a summary of work carried out in 2007.

The arthropod fauna on *R. cathartica* and *F. alnus* in Europe

In total, 39 specialized arthopods were recorded from *R. cathartica* and *F. alnus* in Europe (Annex 1). The feeding guild on *R. cathartica* and *F. alnus* was dominated by leaf feeders (18 species), followed by sap-suckers/leaf gall formers (12 species), flower or fruit feeders (6 species), and shoot/root borers (3 species). A paper on the arthropod fauna on *R. cathartica* and *F. alnus* in Europe by A. Gassmann, I. Tosevski and L. Skinner entitled "Use of native range surveys to determine the potential host range of arthropod herbivores for biological control of two related weed species, *Rhamnus cathartica* and *Frangula alnus*" has been accepted for publication in Biological Control (2008).

Reassessment of the potential for biological control of *R. cathartica* (2002-2007)

This reassessment is based on the assumption that candidate biological control agents should be monospecific to *R. cathartica* or their host range should be restricted to a few species in the genus *Rhamnus*. In the United States, *Frangula* and *Rhamnus* include 5 and 7 native taxa respectively, but another two *Rhamnus* subspecies and ten Frangula subspecies have been recorded (USDA/NRCS, 2001). The following buckthorn species have been included in host range studies:

R. cathartica	(Europe)
R. alpina	(Europe)
R. alnifolia	(native in North America with a wide geographical distribution, largely overlapping the distribution of *R. cathartica*)
F. alnus	(Europe)
F. caroliniana	(native in North America, with a wide geographical distribution, partially overlapping the distribution of *R. cathartica*)

Internal borers

Oberea pedemontana (Col., Cerambycidae)

Status: *Oberea pedemontana* has a restricted geographical distribution, mainly around the Adriatic Sea. The beetle has been recorded in Northern Italy and former Yugoslavia, and less frequently in southern Austria and southern Hungary, western

Bulgaria and Rumania and perhaps in Turkey (Baronio et al. 1988). During our surveys, *O. pedemontana* was found on *R. cathartica* and *F. alnus* in Northern Italy and Serbia. The beetle has also been reported from *R. alpina* (Sama 1988) and from *Lonicera* species (Horion 1974; Demelt and Franz 1990). However, according to Frisch (1992), the record of *O. pedemontana* on *Lonicera* is probably due to the fact that *Lonicera* can occur in mixed stands with *F. alnus* and therefore adults of *O. pedemontana* have been collected as strays on *Lonicera*. Larvae make a gallery by boring along the centre axis of the branch. The species has a two or three year life cycle.

Two pairs that were reared from field collected *F. alnus* were subsequently successfully reared on *F. alnus*. Thus, rearing (including mating and oviposition) under confined conditions is possible. No oviposition and host suitability tests have been carried out so far.

Advantages: Field observations indicate that *O. pedemontana* is a very destructive species to the host trees (Lekic and Mihajlovic 1976; our observations). High level of parasitism in its native range suggests that populations of *O. pedemontana*, once established, could build up rapidly in the areas of introduction.

Disadvantages: According to literature records (see Annex 1), the beetle lacks specificity at the genus level unless the occurrence of host races can be demonstrated; it has a two- or three-year life cycle which could hamper its establishment in North America. The collection and rearing of *O. pedemontana* is an issue: larval sampling is very destructive for the host trees and adult rearing from field-collected larvae is problematic because of the multi-year life cycle of the species; in addition no adults have ever been collected on the host trees, perhaps due to a cryptic or nocturnal adult behaviour.

Prospects for biological control: Low

Potential future work: Molecular characterisation of *O. pedemontana* from *R. cathartica* and *F. alnus* to determine the possible occurrence of host races; in depth studies of the adult behaviour and the use of light traps to collect adult beetles; choice oviposition tests with native North American species.

Synanthedon stomoxiformis **(Lep., Sesiidae)**

Status: This root boring moth is widely distributed in the Palaearctic area (Doczkal and Rennwald 1992; Stadie, 1995; Bittermann, 1997). There are three subspecies which are all associated with *Rhamnus* and *Frangula* species in different geographical areas in Europe. *Synanthedon stomoxyformis* ssp. *stomoxiformis* is known from *R. cathartica* and *F. alnus* from central-southern Europe to Ural. According to literature records, *Sorbus aria* (Rosaceae) and more rarely *Corylus avelana* (Corylaceae) are alternative hosts of *S. stomoxyformis* ssp. *stomoxiformis* in upper Austria (Pühringer et al 1998; Spatenka et al. 1999).

Synanthedon stomoxiformis was found relatively commonly at several *R. cathartica* sites in the Deliblastiki Pesak and Pescara region in Serbia, where its presence has

been confirmed by the use of pheromone traps. According to literature, larvae have a biennial life cycle (Stadie, 1995; Spatenka et al., 1999). Eggs are laid on the trunk and branches. 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 down, boring into the roots. In autumn, the larva build a long and visible reddish exit tube above the ground made out of scraps, sawdust and silk out of which the adult emerges the following spring.

In no-choice larval feeding and development tests, best larval development was observed on *F. alnus* and *R. alpina*. Larval survival was lower on *R. cathartica* and similar to that recorded on the North American species *R. alnifolia* and *F. caroliniana*. These tests also revealed that *S. stomoxiformis* may complete development in one year. Larval development was much faster on *R. alpina* and an entire biennial life cycle was recorded on *R. cathartica* only. No larvae were found on any of the other 11 species tested outside the genera *Rhamnus* and *Frangula*. Mating was obtained only under open field conditions. No oviposition choice tests have been carried out so far.

Advantages: Although the impact of larval feeding to the trees is not obvious, *S. stomoxiformis* is an interesting species per se as it is the only root-boring species known on buckthorn.

Disadvantages: This species lacks specificity at the genus level. Given the larval biology, oviposition may not be a reliable indicator of host plant choice in mixed stands with desirable buckthorn species. The difficulty to get mating in confinement and a possible two year life cycle on *R. cathartica* could be a handicap for the establishment of this moth in North America.

Prospects for biological control: Low

Potential future work: larval choice tests and oviposition behavioural studies.

Sorhagenia janiszewskae **(Lep., Cosmopterigidae)**

Status: The larvae of *S. janiszewskae* mine in the current year's shoots of buckthorns. The species is found in most parts of Europe, except south of the Alps (Malicky and Sobhian 1971). The moth was found on *R. cathartica* and *F. alnus* in eastern Austria and on *F. alnus* in southwestern Switzerland. Our observations suggest that *S. janiszewskae* most probably does not overwinter in the adult stage as indicated in the literature, but in the egg stage. The species is univoltine.

Larval transfer tests gave inconsistent results but there are indications that the physiological host range of *S. janizewskae* from *F. alnus* includes both its field host and *R. cathartica*. However, the transfer of newly hatched larvae may likely produce false positive results. Therefore, host specificity tests should rely on oviposition tests in choice and no-choice conditions. The oviposition tests carried out so far gave poor results and did not show evidence of oviposition preference of *S. janiszewskae* from *F. alnus* and *R. cathartica* for its field host plant.

Advantages: The terminal leaves of the shoots attacked by *S. janiszewskae* wilt rapidly, later they may wither and fall off or they may recover. However, the attacked shoots show a reduced growth and are much smaller than unattacked ones.

Disadvantages: The species lacks specificity at the genus level. There is no consistent evidence of the occurrence of oviposition preference of the moth for its field host plant. It is difficult to accurately predict adult emergence in spring and hence best collection time. Cut shoot-tips decay or dry quickly and this may prevent the completion of larval development. The difficulty to carry out reliable oviposition tests is a serious handicap to determine the behavioural host range of this species.

Prospects for biological control: Low.

Potential future work: Additional surveys in western Europe to confirm the occurrence of the moth on *R. cathartica* and *R. alpina* (another host plant of *S. janiszewskae* according to Malicky et al. 1970); molecular characterisation of *S. janiszewskae* from *R. cathartica* and *F. alnus* and from different geographical areas to determine the possible occurrence of genetically distinct populations with distinct host preferences; in depth studies on adult oviposition behaviour to carry out reliable oviposition tests; study of the physiological host range of *S. janizewskae* from *R. cathartica*.

Other root/shoot boring species

There are no other internal root/shoot borers known on *R. cathartica* in Europe.

Defoliators

Ancylis apicella (Lep., Tortricidae)

Status: This species has a wide distribution in Europe. *Ancylis apicella* is bivoltine and overwinters as larva in a silk web in the soil. *Ancylis apicella* was collected more frequently on *F. alnus* than on *R. cathartica*. Malicky et al. (1970) found *A. apicella* on *R. cathartica*, *R. saxatilis*, *R. alaternus* and *F. alnus.* The species was also occasionally recorded on *R. alpina*.

Preliminary screening tests with neonate larvae indicate that *A. apicella* will develop on the North American *R. alnifolia* and *F. caroliniana* as well as on *R. alpina*.

Advantages: The impact is likely to be important at high population densities due to the bivoltine life cycle of the species.

Disadvantages: This species lacks specificity at the genus level. The oviposition behaviour is unknown.

Prospects for biological control: Low.

Potential future work: None.

Ancylis derasana (Lep., Tortricidae)

Status: This species has a wide distribution in Europe. *Ancylis derasana* is bivoltine and overwinters in the larval stage. The species prefers *R. cathartica* to *F. alnus*. No other field host is known in Europe. Malicky et al. (1970) recorded *A. derasana* exclusively from *R. cathartica*.

Preliminary no choice tests with neonate larvae indicate that *F. alnus*, *F. caroliniana* and *R. alpina* are less suitable hosts than *R. cathartica* and *R. alnifolia*.

Advantages: The impact is likely to be important at high population densities due to the bivoltine life cycle of the species.

Disadvantages: The species lacks specificity at the genus level. The oviposition behaviour is unknown.

Prospects for biological control: Low.

Potential future work: Study of the oviposition behaviour; oviposition choice tests with native North American buckthorn species to determine its likely ecological host range.

Triphosa dubitata (Lep., Geometridae)

Status: *Triphosa dubitata* was found in small numbers in nearly all surveyed areas in Austria, Germany, Switzerland and the Czech Republic. The species overwinters as an adult in natural caves (Cherix 1976; Jacobi and Menne 1991). According to Malicky *et al.* (1970), females mate prior to hibernation. Eggs and first instar larvae can be found in late April. The species is univoltine.

Triphosa dubitata was recorded exclusively on *R. cathartica* and *R. alpina* during our surveys. Malicky et al. (1970) also found *Triphosa dubitata* occasionally on *R. orbiculata* and on *F. alnus* at one location.

Preliminary no-choice larval feeding tests were carried out using field-collected eggs from *R. cathartica* and *R. alpina*. Larval survival to the pupal stage was higher on *R. alnifolia* than on *R. cathartica* and *R. alpina* in both populations. No larvae developed to the pupal stage on *F. caroliniana* and *F. alnus*.

Advantages: *Triphosa dubitata* is likely to be specific to the genus *Rhamnus*. High population densities could result in heavy defoliation of buckthorn in early spring.

Disadvantages: *Rhamnus alnifolia* is a more suitable host for *T. dubitata* from either field host (*R. cathartica* and *R. alnifolia*) in no-choice larval development tests. Oviposition preference tests would be needed to assess the potential behavioural host range of *T. dubitata*. However, this is hardly feasible considering the adult biology of the species.

Prospects for biological control: Low.

Potential future work: None.

Philereme transversata (Lep., Geometridae)

Status: Philereme transversata is reported to be common across Europe (Carter 1987). According to Malicky *et al.* (1965), the larvae of *P. transversata* feed on the leaves of buckthorn, but unlike *P. vetulata*, do not web the leaves together. Pupation takes place in the soil. The species is univoltine and hibernates in the egg stage.

The species was found exclusively on *R. cathartica* during our surveys. *Philereme transversata* was occasionally also found on *R. saxatilis*, *R. orbiculata* and on *F. alnus* at one location by Malicky *et al.* (1970).

No-choice larval development tests showed that Frangula alnus *and* F. caroliniana *are not suitable host plants for larval development of* P. transversata*;* R. alnifolia *seems to be a less preferred host than* R. cathartica *(no. of pupae and pupal weight are less on* R. alnifolia*), but this result will need to be confirmed.*

Advantages: *Philereme transversata* is likely to be specific to the genus *Rhamnus.* High population densities could result in heavy defoliation of buckthorn in early spring.

Disadvantages: The rearing of *P. transversata* is not straightforward. The oviposition behaviour is unknown.

Prospects for biological control: Medium.

Potential future work: Continue to study the physiological host range of *P. transversata* and the suitability of native North American *Rhamnus* spp.; study the oviposition behaviour and carry out oviposition choice tests.

Philereme vetulata (Lep., Geometridae)

Status: Larvae feed within young folded leaves. *Philereme vetulata* is univoltine and overwinters in the egg stage on the bark of its host plant.

Philereme vetulata is exclusively associated with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965).

Larval feeding and development tests on potted plants indicated that survival to pupal and adult stage was similar on *R. cathartica*, *R. alpina* and *R. alnifolia*. However, *R. alpina* and *R. alnifolia* seem to be a slightly less optimal food source for *P. vetulata*. The pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* and *R. alpina*, and the time to pupation was significantly shorter on *R. cathartica* than on *R. alnifolia* and *R. alpina*. No larval establishment or damage was observed on *F. alnus* and *F. caroliniana.* No oviposition on the field host plant was obtained in confinement.

Advantages: *Philereme vetulata* is specific to the genus *Rhamnus.* High population densities could result in heavy defoliation of buckthorn in early spring.

Disadvantages: The oviposition behaviour is unknown and no oviposition was recorded on *R. cathartica* in confinement, thus making oviposition tests in cages difficult, if not impossible.

Prospects for biological control: Medium

Potential future work: Further studies are needed on the oviposition behaviour of *P. vetulata*; carry out open field oviposition tests; study the progeny of females reared on *R. alnifolia*.

Other defoliators

Status: An additional 13 defoliators are known from buckthorns in Europe (Annex 1). Of these, *Gonopteryx rhamni*, *Ancylis obtusana* and *Triphosa sabaudiata* are known from both *R. cathartica* and *F. alnus*. They are therefore not further considered for biological control of *R. cathartica* in North America.

Sorhagenia lophyrella, *Odontognophos dumetata*, *Acrobasis romanella* and *Trachycera legatea* have only been recorded from *Rhamnus* spp. in Europe (Annex 1). Whether their host range is truly restricted to the genus *Rhamnus* would deserve further attention.

Five species (i.e. *Bucculatrix frangutella*, *B. rhamniella*, *Calybites quadrisignella*, *Stigmella catharticella* and *S. rhamnella*) mined in the leaves of buckthorn partially or during their entire life cycle. Among these, *B. rhamniella* and *S. catharticella* appear to be the most specific ones.

Advantages: High population densities of any of those species could result in heavy defoliation of buckthorn.

Disadvantages: Several of these species seem to be uncommon in the areas surveyed in Europe. Extensive additional surveys would therefore be necessary to collect workable populations. Studies carry out so far on several other defoliators suggest that monophagy on *R. cathartica* is unlikely.

Prospects for biological control: Low-medium.

Potential future work: Extensive surveys in Europe and preliminary host-specificity tests with those species known only from the genus *Rhamnus* in Europe.

Sap-suckers

Trichochermes walkeri (Hom., Triozidae)

Status: The leaf margin curl galler *Trichochermes walkeri* is known only from *R. cathartica* in Europe. It is also one of the most common insect species on *R. cathartica* and certainly one of the most conspicuous. The galls of *T. walkeri* seem to be aggregated on certain trees, while within a tree they appear to have a more normal distribution. The species overwinters at the egg stage. Eggs are laid on leaf buds axils.

Overwintering eggs do not survive on cut shoots. The transfer of first instar larvae or older larvae from young galls onto the leaves of potted plants gave very low survival rates and therefore does not provide conclusive results to assess the physiological host range of *T. walkeri*. Therefore, host specificity tests should rely on oviposition tests and subsequent larval and gall development.

No-choice adult feeding tests indicated slightly prolonged longevity (6-7 days) on non target hosts as compared to the no-food control (5 days), suggesting that feeding probes on the non target plants may have occurred. Females fed on *R. cathartica* lived up to 60 days.

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 since *T. walkeri* females will die long before oviposition starts. No-choice tests carried out with females, which had been previously exposed to *R. cathartica* for three weeks, resulted in very little oviposition on non target hosts (i.e. *R. alnifolia* and *R. alpina*). Oviposition on non target hosts was also very low in sequential no-choice tests with *R. cathartica* but also lower on the field host probably due to the fact that the adults could not feed continuously on their normal field host *R. cathartica*. Surprisingly, no eggs were obtained in single choice field cage oviposition tests with *R. cathartica* and *R. alnifolia*.

The number of eggs laid on non target hosts (*R. alnifolia* and *R. alpina*) was too low to obtain conclusive results as to whether any gall would develop the following year.

Advantages: *Trichochermes walkeri* is most likely monophagous on *R. cathartica*. High population densities could result in heavy leaf gall formation on buckthorn in spring thus hampering the photosynthetic process.

Disadvantages: Field observations indicate that populations of *T. walkeri* can be highly fluctuating. The probability of potential disease transmission has not yet been evaluated.

Prospects for biological control: High.

Future work needed: Repeat single choice oviposition tests and no-choice adult feeding tests with native North American *Rhamnus* spp. Potential disease transmission will need to be evaluated (see also below).

Other sap-suckers

Status: Another seven Hemiptera and four Eriophyidae are known from *R. cathartica* and *F. alnus* (Annex 1).

Based on their potential host range and availability, ***Cacopsylla rhamnicolla* (Hom., Psyllidae)** and ***Trioza rhamni* (Hom., Triozidae)** seem to be the most promising potential agents for a further phase of the project.

Advantages: There are several cases, in which Homopterans have successfully been used as biological control agents against invasive weeds. One example is the excellent control of *Mimosa invisa* by the psyllid *Heteropsylla spinulosa* released in 1993 in Papua New Guinea (Kuniata and Korowi 2004). Also, the broom psyllid *Arytainilla spartiophila* has been introduced with some success in New Zealand in 1992 and in Australia in 1994 for biological control of scotch broom *Cytisus scoparius* (http://www.landcareresearch.co.nz/research/biocons/weeds/broom/Broom_psyllid1.asp; http://www.csiro.au/resources/ps22n.html).
More recently the psyllid *Boreioglycaspis melaleucae* was successfully released in 2003 in Florida against *Melaleuca quinquenervia* (Gioeli and Neal 2004). In Europe, the psyllid *Aphalara itadori* has been studied for biological control of *Fallopia japonica* (Shaw et al. 2007). This species has been shown to be highly specific and potentially damaging, and a petition for release is in preparation. Psyllids can also be serious pests of trees such as in the case of *Leucaena leucocephala* in South-East Asia (Chazeau 1987).

Disadvantages: Homopteran can also transmit diseases as in the case of the Asian citrus psyllid, *Diaphorina citri*, and the citrus greening disease (Halbert and Manjunath 2004).

Prospects for biological control: High.

Future work needed: Collect and study the biology and host range of *C. rhamnicolla* and *T. rhamni*; carry out a literature review and gather expert advice on psyllid and triozid disease transmission from both pest and beneficial species; study the presence or absence of potential diseases;

Collect and identify eriophyid mites associated with *R. cathartica* in Europe.

Fruit and seed feeders

Wachtiella krumbholzi (Dipt.; Cecidomyiidae)

Status: Not much is known about this insect which was identified by Dr. M. Skuhrava. Interestingly, with the exception of a few specimens reared from *R. cathartica* in the Czech Republic, M. Skuhrava has not found this species during 50 years of investigations on Cecidomyiidae in 1800 European localities (Simova-Tosic et al. 2000, 2004; Skuhrava et al. 2005). According to Skuhrava (pers. com. 2005), *W. krumbholzi* cannot be considered to be cecidogenous. Field observations in Serbia also suggest that *W. krumbholzi* is a seed feeder rather than a gall maker. The main

characteristic of attacked fruits is similar to early fruit maturation with changes in colour. Attacked fruits become dark-red black while healthy fruits are still green. Casual observations revealed up to nine midge larvae per fruit and three larvae in one seed. The midge larvae leave the fruits and go into the soil to prepare a larval cocoon made of silk and debris.

Host range tests will rely entirely on oviposition tests. Preliminary work indicates that the midge mates and oviposits in confinement.

Advantages: Work done on midges in Europe for decades by Skuhrava and co-authors suggest that *W. krumbholzi* is specific to *R. cathartica*. The reduction of the seed production of *R. cathartica* will be a key element to reduce the spread of buckthorn in North America.

Disadvantages: Not much is known on *W. krumbholzi*, in particular its geographical distribution in Europe. The main difficulty will be to get the test plants at the right phenological stage at time of oviposition, i.e. most likely at the very early fruit development stage.

Prospects for biological control: High

Future work needed: Study the oviposition behaviour and phenological (?) requirements for successful oviposition and larval development; study the geographical range of the species in Europe and make extensive collections of fruit samples of other *Rhamnus* species to determine the field host range of *W. krumbholzi* in Europe.

Other fruit and seed feeders

Status: Another midge species known from the fruits of *R. cathartica*, ***Lasioptera kosarzewskella***, was found in the Ukraine in 1957 (M. Skuhrava pers. comm. 2004). This species should also be considered as a potential biological control agent of *R. cathartica*.

Neither ***Sorhagenia rhamniella (Lep., Cosmopterigidae)*** nor ***Hysterosia sodaliana (Lep., Tortricidae)***, which are known to feed in the fruits or the flowers of *R. cathartica* and *F. alnus* have been found during our surveys. However, none of them appears to be genus specific to be considered for biological control of *R. cathartica*.

Summary Table

A summary of 10 priority species for future work on biological control of *R. cathartica* and recommendations is provided in Table 1.

Table 1. Reassessment of the potential for biological control of *Rhamnus cathartica* based on work done in 2002-2007.

Species	Feeding guild	Field hosts	Experimental host range	Issues and comments
High priority species				
Trichochermes walkeri (Hom., Triozidae)	Sap-sucker/ leaf gall inducer	*R. cathartica*	*R. cathartica* / only a very few eggs were laid on *R. alnifolia* in no-choice and sequential-choice tests	1. Uncertainty regarding oviposition behaviour in large confined areas and feasibility of choice oviposition tests; 2. Potential risk of disease transmission needs to be evaluated; 3. Unstable population dynamics ?
Cacopsylla rhamnicola (Hom., Psyllidae	Sap-sucker	*R. cathartica*; occurrence on *F: alnus* needs to be confirmed	Not studied yet. This is a new species proposed for biological control of *R. cathartica*	1. *Psyllids* can be very damaging to their host plants; 2. Potential risk of disease transmission needs to be evaluated
Trioza rhamni (Hom., Triozidae)	Sap-sucker / pit-gall inducer	*R. cathartica*	Not studied yet. This is a new species proposed for biological control of *R. cathartica*	1. *Triozids* can be very damaging to their host plants; 2. Potential risk of disease transmission needs to be evaluated
Wachtiella krumbholzi (Dipt.; Cecidomyiidae)	Seed-feeder	*R. cathartica* /	Not studied yet	1. An important species to reduce the spread of R. cathartica; 2. Host range studies will rely on oviposition tests; 3. The main challenge will be to get test plants at the right phenological stage for oviposition tests
Lasioptera kosarzewskella (Dipt. ; Cecidomyiidae)	Seed-feeder	*R. cathartica*	Not studied yet	1. Additional field surveys to determine the distribution and field host range of the species
Medium priority species				
Philereme transversata (Lep., Geometridae)	Defoliator	*R. cathartica* / *Rhamnus* spp. / one old record on *F. alnus*	Larvae develop on *Rhamnus* spp. / *R. alnifolia* seems to be a much less suitable host than the *R. cathartica* /no oviposition tests have been carried out yet	1. Additional larval development tests need to be carried out to confirm the physiological host range; 2. Oviposition behaviour unknown; 3. Oviposition tests should be carried out; 4. Open field tests should be carried out
Philereme vetulata (Lep., Geometridae)	Defoliator	*Rhamnus cathartica* / *Rhamnus* spp.	Larvae develop on *Rhamnus* spp.; / *Frangula* spp. unsuitable for larval development	1. Oviposition behaviour unknown; 2. No oviposition occurred on the target host in confinement; 3. Additional oviposition trials should be attempted; 4. Open field tests should be carried out; 5. Study the progeny of females reared on non-target species

Bucculatrix rhamniella (*Lep., Bucculatricidae*) *Stigmella catharticella* (*Lep., Nepticulidae* *Sorhagenia lophyrella* (*Lep., Cosmopterigidae*)	Leaf miners and / or defoliators	*Rhamnus cathartica / Rhamnus* spp.	These are new species proposed for biological control of R. cathartica among several other potentially genus specific leaf feeding Lepidoptera species	1. Species probably uncommon in the areas already surveyed; 2. Additional contacts needed with lepidopteran experts in order to restrict the areas targeted for future surveys
Eryiphyidae	Sap-sucker	*R. cathartica*	Not studied yet	Determine the availability and the feasibility of working with mites associated with R. cathartica in Europe with the help of a mite expert in Serbia and Italy.

Reassessment of the potential for biological control of *Frangula alnus* (2002-2007)

This reassessment is based on the assumption that candidate biological control agents should be monospecific to *F. alnus* or their host range should be restricted to a few species in the genus *Frangula*.

Status and summary: The leaf-hopper ***Zygina suavis*** was the only species found on *F. alnus* but not on *R. cathartica*, although the literature lists *R. cathartica* as a host of *Z. suavis* (Ossiannilsson, 1981). No work has been done yet with Z. suavis.

In addition to *Z. suavis*, literature records indicated five additional arthropod species known from *F. alnus*, i.e. the gall midges ***Contarinia rhamni*** and ***Dasyneura frangulae***, the mirid bug ***Lygocoris rhamnicolla***, the Gelechiid moth ***Aristotelia pancaliella*** and the mite ***Eriophyes rhamni*** (Annex 1).

Extensive additional surveys would be needed to find and to assess the field host range of those species. The discovery of genus or species specific potential biological control agents for *F. alnus* will undoubtfully be difficult in Europe. Priority should be given to the **two fruit and seed feeding midges** which are likely to be specific at the species or genus level according to field records in Europe.

Other tasks

1. To prepare a test plant list and to submit it to TAG.

2. To start collecting and growing plant material. This task has proven to be difficult during the past years and additional considerable efforts will be needed to obtain plant material for host range testing. The difficulty is enhanced by the occurrence of the sudden oak death (*Phytophthora ramorum*) on *Frangula californica* and *F. purshiana* in North America and the need to import cuttings or rootstocks "found free from non-European isolates of *Phytophthora ramorum*" into Switzerland. It is likely that part of the host range screening will need to be done in the United States.

PART II

Summary of work done in 2007

1 *Philereme vetulata* (Lep., Geometridae)

The leaf-feeding moth *Philereme vetulata* is exclusively associated with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965). Although *P. vetulata* was found in about 20% of surveyed populations, it is usually relatively common or abundant where it occurs (Gassmann et al. 2006). Larvae of *P. vetulata* feed within folded leaves.

Collections and adult emergence

Following field collections, larvae were reared on leaves of *R. cathartica* in ventilated plastic boxes lined with moist paper to keep leaves fresh. Boxes were stored in an outdoor shelter. Pupae were kept in ventilated plastic cups half filled with vermiculite for adult emergence. A total of 112 males and 94 females emerged from the 380 larvae collected between 29 April and 3May 2007 in Switzerland (Fig 1).

In addition, 12 males and five females were reared from eggs obtained in 2006, which were transferred for larval development onto young folded leaves of potted *R. alnifolia* plants. Similarly, eight males and two females were reared from *R. alpina* from eggs obtained in 2006. These adults were used for rearing and oviposition.
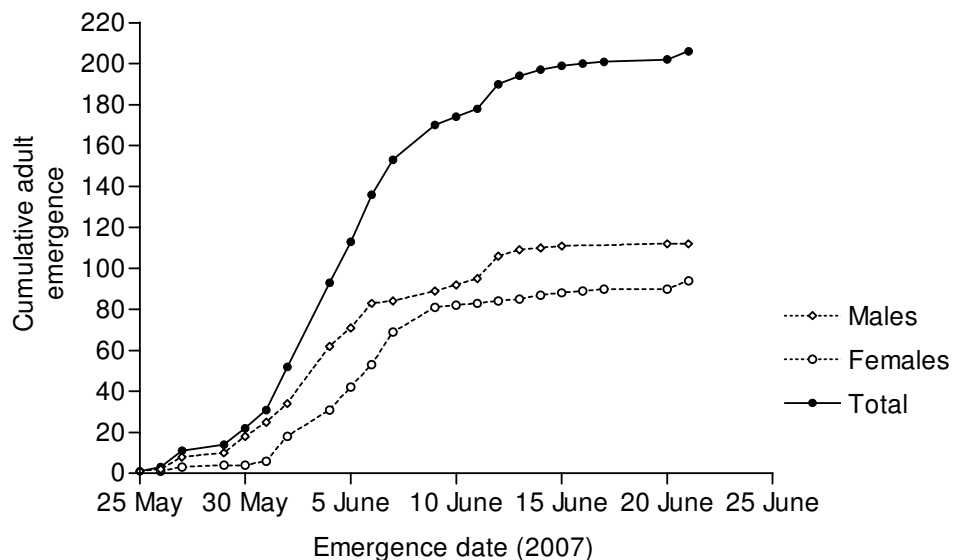

Fig 1. Emergence of *Philereme vetulata* adults reared from field collected larvae in 2007.

Adult rearing and oviposition

Between 25 May and 6 August, 50 pairs of *P. vetulata* reared from *R. cathartica* were kept individually in 50 cardboard and plastic cylinders (11cm x 15/25 cm) in an outdoor shelter. A total of 1,906 eggs were obtained of which 77% (1459 eggs) were fertile. Average fecundity per female was 38 eggs, i.e. in general lower than in previous years (Gassmann et al. 2006; 2007). Five pairs of *P. vetulata* reared from *R. alnifolia* and kept under similar conditions, laid a total of 220 eggs of which 68% (150 eggs) were fertile. Average fecundity per female was 44 eggs. No eggs were laid by two pairs of *P. vetulata* reared from *R. alpina*.

Eggs will be overwintered in an outdoor shelter and at 1°C for mass rearing on *R. cathartica* and to study the progeny by females reared on *R. alnifolia* in 2007.

Larval development

Rhamnus cathartica plants infested with newly hatched larvae were regularly dissected and larval head capsules measured. *Philereme vetulata* has four larval instars (Fig. 2). The second instar is reached after only 4-5 days at 20°C, and the third instar after 9-10 days. The last larval instar is reached during the third week.

Larval survival could not be assessed, since larvae were regularly killed to measure the head capsule size. Average pupal weight on *R. cathartica* within five days after pupation was 0.053 ± 0.01 mg (N=20). This was significantly higher than pupal weights of *P. vetulata* reared on *R. alnifolia* (0.042 ± 0.01mg; N=7; T-test; $P<0.05$). These data are similar to those obtained in 2006.
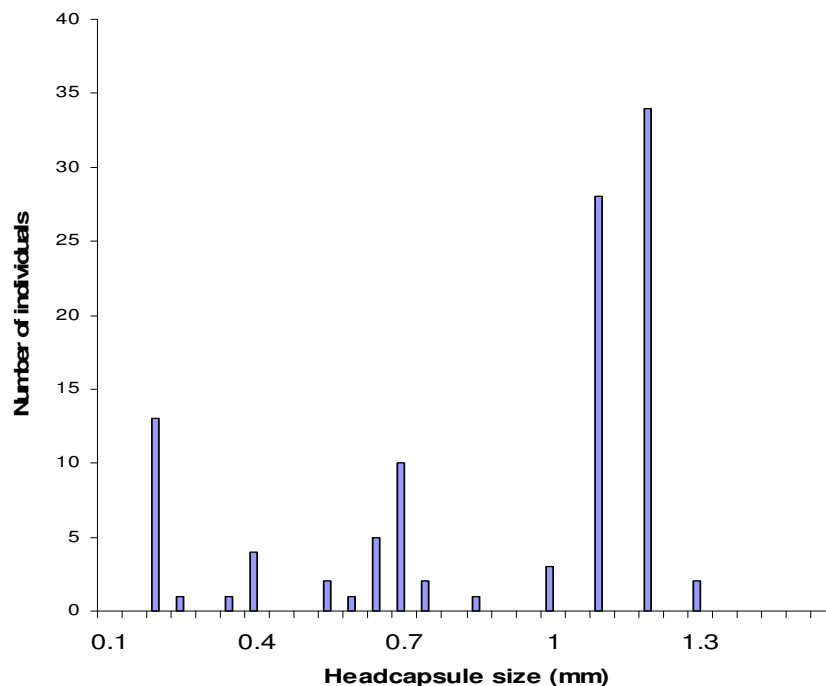

Fig 2. Headcapsule diameters of larvae of *P. vetulata* reared on *R. cathartica*

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No-choice and single-choice field cage oviposition tests

In the field in Europe, *P. vetulata* has been recorded almost exclusively on *R. cathartica*. It has never been found on *F. alnus* (the single record from 2004 turned out to be a sampling mistake) and only once on *R. alpina* (Malicky et al. 1970). No-choice larval feeding and development tests carried out in 2006 (Gassmann et al. 2007) and in 2007 (data not shown) indicate that *R. alnifolia* and *R. alpina* are suitable host plants for *P. vetulata*. However, both plants seem to be providing a slightly less optimal food source for *P. vetulata* than *R. cathartica*. The pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* and *R. alpina*, and the time to pupation was significantly shorter on *R. cathartica* than on *R. alnifolia* and *R. alpina*. No larval establishment and larval damage was observed on *F. alnus* and *F. caroliniana*.

The oviposition host range was evaluated in three 2m x 2m x 1.6m field cages. All cages were protected from excess of rain and sun by green gauze covers. Tests were established between 1 and 5 June. Two cages contained three potted *R. cathartica* (about 80-100 cm high) each to check the feasibility of cage oviposition tests. The third cage contained two *R. cathartica* and two *R. alnifolia* of about the same size. Twelve pairs of *P. vetulata* were released into each cage. *Philereme vetulata* hibernates in the egg stage on the bark of its host plant (Malicky et al 1970). Therefore, the bark of all branches and trunks as well as the leaves and leaf buds were carefully checked for eggs by the end of July. No eggs were found in any of the three cage oviposition tests.

When rearing *P. vetulata* in cardboard or plastic cylinders and in smaller cages (40x40x70 cm), eggs were always found on the bottom of the rearing containers or cages. No eggs have ever been found on the cylinder/cage walls, or on smaller potted plants, branches or hanging paper provided as a support for oviposition. The field cage oviposition tests confirm that *P. vetulata* apparently does not oviposit on its host plant in confinement. Open field tests should be carried out to tentatively determine the oviposition preference of *P. vetulata*.

Trichochermes walkeri (Hom., Triozidae)

The leaf margin gall psyllid *T. walkeri* has one generation per year. Females lay small orange eggs during late summer. The nymphs hatch in spring from overwintering eggs. First-instar nymphs migrate to the leaves, feed and induce rolling of the leaf margin.

Collections and adult emergence

A total of 90 adults of *T. walkeri* emerged from 1,500 leaf margin curl galls collected between 25 July and 3 August in the Jura Mountains and in western Switzerland (Fig 3). As in previous years, galls collected in the Jura Mountains matured earlier and gave a better adult emergence rate (12%) as compared to the galls collected in western Switzerland (4%). In general, the number of adults emerged was much lower than in

2006 and 2005. The sex ratio of emerged adults was nearly 1:1. The adults were used in single-choice oviposition field cage tests.
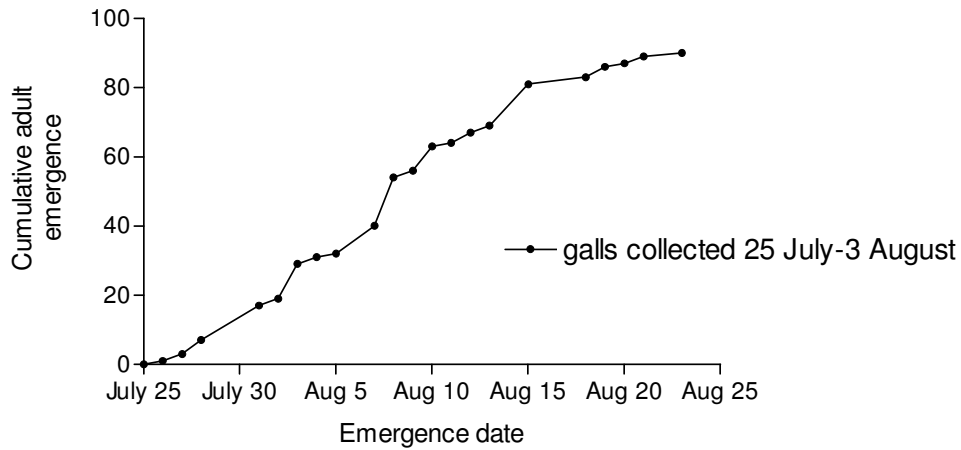

Fig 3. Emergence of *T. walkeri* adults in 2007.

Oviposition and gall development 2006-2007

Branches (n=41) of 26 potted *R. cathartica*, onto which 716 eggs of *T. walkeri*, had been laid in autumn 2006 (Gassmann et al. 2007) were protected from natural oviposition under a large gauze tent in a greenhouse until the end of November 2006, and then kept outdoors until early July 2007. The number of galls and larvae was counted during the second week of July, i.e. before the larvae leave the galls to pupate nearby on the leaves. A total of 63 galls and 51 larvae were obtained from the 716 eggs laid in 2006. A quarter of the galls (15) did not contain any larvae. Thus, only 7.1% of the eggs developed successfully into mature larvae in well developed galls. On almost half of the branches (n=19) no galls developed although 253 eggs had been laid. Gall and larval development were less successful than in 2005-2006 (Gassmann et al. 2007). Only 16 eggs and 20 eggs had been laid in 2006 on R. alnifolia and R. alpina respectively and no galls developed on these plants in 2007.

Single choice field cage oviposition tests

In 2005 and 2006, little oviposition occurred on R. alnifolia in no-choice tests, without gall and larval development recorded the following year. Single-choice oviposition tests were therefore evaluated in three 2m x 2m x 1.6m field cages All cages were protected from excess of rain and sun by green gauze covers. The tests were set-up between 30 July and 10 August. Two cages contained two potted *R. cathartica* and two potted *R. alnifolia* each, and one cage only one potted plant each of *R. cathartica* and *R. alnifolia*. Care was taken to expose plants of similar size (60-80 cm). Twelve newly emerged *T. walkeri* were released into each cage. The first cage was checked for eggs on 19 September, i.e. seven weeks after set-up. Oviposition in the other two cages was checked on 5 November. Unfortunately, no eggs were found in any of the three oviposition tests.

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At this stage, it is difficult to explain why no oviposition was recorded in this trial. Oviposition tests in previous years were designed to allow close contact between the insect and its host plant in either small cages (about 40x40x70 cm in size), ventilated plastic cylinders (Ø 11.0cm, height 15cm) or even smaller ventilated plastic cups (Ø 7.0 cm, height 8.5 cm). Therefore, the absence of oviposition might result from a typical "cage effect" negatively influencing insect behaviour and oviposition. Another reason could be that too few pairs of *T. walkeri* were released. In all previous tests, about 50% adult mortality was recorded prior to oviposition, which probably resulted in only few adults surviving, to mate and to oviposit in particular under adverse weather conditions. Due to low adult emergence in 2007, we were not able to release more adults.

Conclusions and outlook: *Trichochermes walkeri* overwinters as eggs which are laid usually on the leaf axil. The manipulation and overwintering of eggs on cut material and the transfer of first instar larvae or older larvae from young galls onto the leaves of potted plants are not feasible to assess the physiological host range of *T. walkeri*. Therefore, host specificity tests should rely on oviposition tests and subsequent larval and gall development. Additional single-choice oviposition tests should be carried out in 2008 using more adults and/or smaller cages.

Wachtiella krumbholzi (Dipt.; Cecidomyiidae)

In 2004, preliminary collections of flowers and fruits of *R. cathartica* were carried out in Austria, Germany, Switzerland and Serbia and an important population of the midge *Wachtiella krumbholzi* was discovered in the fruits of *R. cathartica* in northeastern Serbia. According to Skuhrava (pers. com. 2005), *W. krumbholzi* cannot be considered to be cecidogenous. Field observations in Serbia also suggest that *W. krumbholzi* is a seed feeder rather than a gall maker. The main characteristic of attacked fruits is similar to early fruit maturation with changes in colour. Attacked fruits become dark-red black while healthy fruits are still green. Gall swelling is not visible on damaged fruits. In the laboratory, the midge larvae leave the fruits and go into the soil to prepare a larval cocoon made of silk and soil debris in August.

Adult emergence from larvae collected in 2006: In early July 2006, over 5,000 fruits of *R. cathartica* apparently attacked by *W. krumbholzi* were collected in Serbia and sent to Switzerland on 14 July. Some 800 larvae have been reared from this material and transferred to Petri dishes filled with a mixture of sterilized sifted soil and vermiculite. In late August, the soil was checked and 685 larval cocoons recovered (86%). Batches of 50 larval cocoons were placed in 9x9x9 cm ventilated plastic boxes half filled with a mixture of sterilized sifted soil and vermiculite. Cocoons were either being kept in an incubator at 3°C (N=400) for overwintering or in an outdoor shelter (N=285). All fruits were kept in an outdoor shelter for emergence of additional midges or other fruit feeding insects.

Emergence of gall midge adults kept in an outdoor shelter started on 10 May 2007, i.e. at the same time as in 2006 (Gassmann et al. 2007), and was completed on May 22. Percent of emergence was low (10%), as in previous years. Adults (N=66) of an as yet undetermined braconid species emerged from the same batches of larval cocoons.

Another 150 midge adults (43♂ and 107♀) emerged from the decaying fruits overwintered in the outdoor shelter.

No-choice oviposition tests: On 18 May 2007, 20 females and 8 males of *W. krumbholzi* were released on one large R. cathartica plant with developing fruits covered with a gauze bag. On 11 July, the one fruit that was produced was dissected and one gall midge larva was found. This result is encouraging, as it indicates that *W. krumbholzi* mates and oviposits in confinement. Unfortunately, no additional oviposition tests could be carried out, because the majority of our mature *R. cathartica* trees were male trees (although they were sold as females trees), and because of the difficulty to have the female flowers pollinated. Consequently, we have bought a set of mature female *R. cathartica* trees to be used in the following years. It is also planned to keep mature trees of both sexes in a greenhouse or in large cages and to use honey bees for pollination. Hand pollination will also be considered.

Collection of gall midge larvae in 2007: Another unexpected difficulty was encountered this year as no attacked fruits could be found in Serbia in 2007. This is probably due to the high parasitism rate of the 2006 midge generation and to the extreme warm spring conditions which have occurred in the Balkans this year and which might have provoked a much earlier emergence of the adult midges. However, midge larvae have been found in the fruits of R. cathartica in southern Germany and in western Switzerland. No sign of attack was found in the fruits of *F. alnus* in the Swiss site where *R. cathartica* and *F. alnus* co-occur. A few midge larvae are being kept in an outdoor shelter for adult identification in 2008.

Shipment of plant material

In 2007 we started collaborating with Dr. Ryan Stewart, University of Illinois, and Dr. Theresa Culley, University of Cincinnati, on a preliminary project to determine the genetic structure of *R. cathartica* and *F. alnus* in the U.S. and in Europe. *Rhamnus cathartica* has been sampled at two sites in Switzerland (Geneva and Neuchâtel) and at one site in southern Germany (Zienken). *Frangula alnus* has been sampled at Neuchâtel in Switzerland where *F. alnus* and *R. cathartica* grow together. *Rhamnus cathartica* was also sampled at four sites in North, Central and East Serbia.

At each site, a few newly expanded leaves per tree were sampled and dried in silica gel. Ten trees were sampled per population/site, and one herbarium voucher specimen kept per site. GPS coordinates were recorded and digital images of the trees sampled were taken. The samples from Switzerland and Germany were sent on 18 June 2007, and the material from Serbia on 25 July 2007.

Acknowledgements

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Annex 1   Specialized arthropods associated with *Rhamnus cathartica* and *Frangula alnus* in Europe (* field records from our surveys; ** Malicky et al. 1970; remaining records from literature as indicated) (from Gassmann et al. 2008).

Species	Host plants	Specificity[1]	Food niche	References
Coleoptera				
Cerambycidae				
Oberea pedemontana Chevrolat	*R. cathartica*, *F. alnus*, *R. alpina*, *Lonicera* ?	O?	stem, woodboring	(Horion, 1974; Lekic and Mihajlovic, 1976; Contarini and Garagnani, 1980; Baronio et al., 1988; Demelt and Franz, 1990; Frisch, 1992)
Diptera				
Cecidomyiidae				
Contarinia rhamni Ruebs.	*F. alnus*	M	gall forming (flowers)	(Houard, 1909; Barnes, 1951; Buhr, 1965; Zerova et al., 1991)
Dasyneura frangulae Ruebs.	*F. alnus*	M	gall forming (flowers)	(Barnes, 1951; Buhr, 1965)
Lasioptera kozarzewskella Mar.	*R. cathartica*	M	gall forming (fruits)	(Stelter, 1975; Zerova et al., 1991)
Wachtliella krumbholzi Stelter	R. cathartica*	M	gall forming (fruits)	(Stelter, 1975)
Heteroptera				
Miridae				
Heterocordylus erythrophtalmus *Hb*	*R. cathartica***, *F. alnus***	O	sap sucking	(Gollner-Scheiding, 1972)
Lygocoris rhamnicola *Reuter*	*F. alnus*	M	sap sucking	(Coulianos, 1998)
Homoptera				
Aphididae				
Aphis commensalis Stroyan	*R. cathartica*	M	gall forming ? (leaves)	(Buhr, 1965; Heie, 1986)
Aphis mammulata Gimingh. & HRL	***R. cathartica***	M	sap sucking, free living	(Heie, 1986; Blackman and Eastop, 1994)
Cicadellidae				
Zygina suavis Rey	*F. alnus* */ R. cathartica*	O	sap-sucking, free living	(Ossiannilsson, 1981)
Psyllidae				

Cacopsylla rhamnicola (Scott)	**R. cathartica*[/]**/ F. alnus****	O	sap-sucking, free living	(Ossiannilsson, 1992)

Triozidae

Trichochermes walkeri Foerster	*R. cathartica*[/]**	M	gall forming (leaves)	(Buhr, 1965; Okopnyi and Poddubnyi, 1983; Meyer, 1987; Zerova et al., 1991; Ossiannilsson, 1992; McLean, 1993)
Trioza rhamni Schrank	*R. cathartica*[/]**/ F. alnus **	O	gall forming (leaves)	(Buhr, 1965; Ossiannilsson, 1992)

Lepidoptera
Bucculatricidae

Bucculatrix frangutella Goeze	*R. cathartica*[/]**/ F. alnus*[/]**/ R. alpina*[/]**	O	leaf miner/ leaf chewer	(Hering, 1957; Heath and Emmet, 1985)
Bucculatrix rhamniella H.-S.	*R. cathartica*	M	leaf miner/ leaf chewer	(Hering, 1957; Buszko, 1992)

Cosmopterigidae

Sorhagenia lophyrella Douglas	*R. cathartica**/ R. saxatilis**	O	leaf roller	(Baran, 1997 ; Malicky et al., 1970)
Sorhagenia janiszewskae Riedl	*R. cathartica*[/]**/ R. alpina**/ F. alnus*[/]**/	O	shoot miner	(Malicky et al., 1970)
Sorhagenia rhamniella Zeller	*R. cathartica**/ F. alnus*	M?	flower feeder	(Malicky et al., 1970; Emmet, 1969)

Gelechiidae

Aristotelia pancaliella Stgr.	*F. alnus*	M	leaf chewer	(Ivinskis et al., 1982)

Geometridae

Odontognophos dumetata Treitschke	*R. cathartica*	M	leaf chewer	(Forster and Wohlfahrt, 1981)
Philereme transversata Hufnagel	*R. cathartica*[/]**/ R. saxatilis**/ R. orbiculata**/ F. alnus**	O		(Skinner, 1984)
Philereme vetulata Den. & Schiff.	*R. cathartica,* *[/]**/ R. alpina**	O	leaf chewer	(Forster and Wohlfahrt, 1981; Skinner, 1984)
Triphosa dubitata L.	*R. cathartica*[/]**/R. alpina*[/]**/ F. alnus *[/]**/ Prunus ?/ Fraxinus ?/ Crataegus ?*	O?	leaf chewer	(Blaschke, 1914; Forster and Wohlfahrt, 1981; Skinner, 1984; Jacobi and Menne, 1991)
Triphosa sabaudiata Dup.	*R. cathartica**/ R. saxatilis**/ R. orbiculata**/ F. alnus/ R. alpina*	O	leaf chewer	(Blaschke, 1914; Forster and Wohlfahrt, 1981)

Gracillariidae

Calybites quadrisignella Zeller	*R. cathartica*[/]**/ F. alnus*	M?	leaf miner/ leaf chewer	(Hering, 1957)

Nepticulidae

Stigmella catharticella Stainton	*R. cathartica*[/]**/ R. alaternus*	M?	leaf miner	(Hering, 1957; Heath, 1976; Speight and Cogan, 1979; Puplyasis, 1984; Puplesis, 1994; Michalska, 1996)

Stigmella rhamnella H.-S.	***R. cathartica**/**/ *R. alpina**/ *F. alnus***	O	leaf miner	(Hering, 1957; Puplesis, 1994; Michalska, 1996)
Pieridae				
Gonopteryx rhamni L.	*R. cathartica**/**/ *R. orbiculata**/ *F. alnus**/**	O	leaf chewer	(Frohawk, 1940; Bergmann, 1952; Pollard and Hall, 1980; de Freina, 1983; Bibby, 1983; Rippey, 1984; Heath and Emmet, 1989; McKay, 1991; Gutierrez and Thomas, 2000)
Pyralidae				
Acrobasis romanella Mill.	*R. cathartica**/ *R. alaternus***	O	leaf chewer	(Malicky et al., 1970)
Trachycera legatea Haw.	*R. cathartica***/ *R. saxatilis***	O	leaf chewer	(Mihajlovic, 1978)
Sesiidae				
Synanthedon stomoxiformis Hb.	*R. cathartica**/ *F. alnus**/ *Sorbus aria*?/ *Coryllus avelana* ?	O?	root miner	(Doczkal and Rennwald, 1992; Stadie, 1995; Bittermann, 1997; de Freina, 1997; Spatenka et al., 1999)
Tortricidae				
Ancylis apicella Den. & Schiff.	*R. cathartica**/**/ *F. alnus**/**/ *R. alpina***/ *Ligustrum* ?/ *Cornus* ?/ *Prunus* ?	O?	leaf chewer	(Razowski, 2003)
Ancylis derasana Hb. (= *A. unculana* Haw.)	*R. cathartica**/**/ *F. alnus**/ *Corylus* ?/ *Rubus* ?/ *Populus* ?	O?	leaf chewer	(Razowski, 2003)
Ancylis obtusana Haw.	*R. cathartica*/ *F. alnus*	O	leaf chewer	(Razowski, 2003)
Hysterosia sodaliana Haw.	*R. cathartica***/ *F. alnus*	O	fruit feeder	(Hannemann, 1964; Razowski, 1970)
Acari				
Eriophyidae				
Aceria rhamni Roiv.	*R. cathartica*	M	sap sucker, free living	(Amrine and Stasny, 1994)
Eriophyes rhamni (Pgst)	*F. alnus*	M	leaf erineum ?	(Amrine and Stasny, 1994)
Phyllocoptes annulatus (Nal.)	*R. cathartica**	M	leaf erineum	(Amrine and Stasny, 1994)
Tetra rhamni Roiv.	*R. cathartica*	M	sap sucker, free living	(Amrine and Stasny, 1994; Petanovic, pers. com. 2005)

[1] M = monophagous, restricted to *R. cathartica* or *F. alnus*, O = oligophagous, restricted to species in the genus *Rhamnus* and/or *Frangula*

Result 2: Survey of insects on buckthorn in Minnesota

Common buckthorn, *Rhamnus cathartica* L.: Available feeding niches and the importance of controlling this invasive woody perennial in North America

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Summary

Common buckthorn, *Rhamnus cathartica* L., an invasive woody perennial of northern hardwood forests in North America, has been targeted for classical biological control and research has been underway since 2001. In support of biological control research, a survey was conducted for insects associated with common buckthorn in a portion of its introduced range in the state of Minnesota. This survey provides baseline information on available feeding niches for potential control agents of common buckthorn and identifies the natural enemy community that could potentially interfere with agent establishment. In two years of sampling, 356 species representing 111 families and 13 orders were collected from common buckthorn in Minnesota. There was no significant defoliation observed at any of the study sites. We surmise that ample feeding niches are available given that most herbivores collected can be classified as generalists. However, the abundance of parasitoids and predators may hinder establishment of potential biological control agents. Further research is needed to determine if biotic resistance could play a significant role in preventing establishment of herbivores in a classical biological control programme for common buckthorn in North America.

Keywords: Rhamnaceae, arthropod herbivores, natural enemies, biological control

Introduction

Common buckthorn, *Rhamnus cathartica* L., is an invasive woody perennial that has become established in northern hardwood forests of North America. It was introduced as a landscape plant and used as a shelterbelt tree because of its winter hardiness and its ability to grow in multiple soil types and habitats (Archibold et al., 1997). In North America, common buckthorn is one of the most invasive woody perennials in natural ecosystems (Archibold et al., 1997; Catling, 1997). Common buckthorn retains its leaves longer than native tree species, creating a competitive advantage (Harrington et al., 1989). In addition, Archibold et al. (1997) suggested that common buckthorn might be allelopathic, allowing its seedlings to grow below mature female trees while inhibiting native tree species. Common buckthorn produces a dense branching structure that attracts nesting songbirds; however, the American robin, *Turdus*

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migratorius L., experiences higher levels of predation when nesting in common buckthorn compared to native species (Schmidt and Whelan, 1999). Others have documented that common buckthorn causes changes in soil properties, leaf litter composition, and micro-arthropod communities (Heneghan et al., 2002; 2004).

Common buckthorn has negative impacts on agriculture. It is the spring host for oat crown rust, *Puccinia coronata* Corda, which can cause severe yield losses in oats (Harder and Chong 1983). Common buckthorn was identified as a suitable overwintering host for soybean aphid, *Aphis glycines* Matsumura, which was first discovered in North America in 2000 and by 2007 has spread to 24 states and 3 Canadian provinces (Ragsdale et al., 2004; Voegtlin et al, 2005).

Common buckthorn is currently on the noxious weed list in six states and two Canadian provinces (University of Montana-Missoula, 2007; USDA, 2007). Multiple methods of control have been employed against common buckthorn including cut stump treatments, foliar herbicide applications, and burning (Archibold et al., 1997). Such control efforts are expensive and for the most part are only effective on a small scale because seedlings tend to re-grow after a burn or a chemical treatment (Archibold et al., 1997). In the early 1960s, several potential biological control agents were identified by Malicky et al. (1970), but their study was not continued. In 2001, biological control research was resumed by the Minnesota Department of Natural Resources in collaboration with CABI Europe Switzerland (CABI) to identify and screen potential control agents.

Our first objective was therefore to conduct a survey of herbivorous insect species associated with common buckthorn, while our second objective was to identify which predators and parasitoids are found on common buckthorn in Minnesota. These data will provide key information in understanding the availability of feeding niches for potential biological control agents and provide insights on what biotic resistance might be present to interfere with agent establishment in Minnesota.

Methods and Materials

Field sites

In 2004 and 2005, eight common buckthorn sites were sampled in three different habitat types, i.e. urban (three sites), rural (two sites), and agricultural (three sites), in seven (2004) or six (2005) southern Minnesota counties (see Yoder 2007 for specific locations). Sites were characterized for their plant communities by randomly sampling ten, $1m^2$ plots. Data collected in each plot included: percent cover of common buckthorn, percent cover of other plant species, common buckthorn stem density, other plant species stem density, number of different plant species, and percent canopy cover. Canopy cover was estimated using a densiometer. To characterize mature trees in the forest, which were not captured by the $1m^2$ plots, we counted the number of trees for each species that were at least 1.5m in height, in a 2m radius around each $1m^2$ plot.

Insect sampling

In 2004 and 2005, 12 common buckthorn plants: four small (<1m in height), four medium (1-3m), and four large (>3m), were marked for repeated insect sampling at each site. Sites were visited every two weeks throughout the growing season (15 June - 15 September 2004; 15 May - 15 September in 2005). All reachable branches were visually surveyed and any insect present was collected, and immediately returned to the laboratory for either identification

if adults were captured or reared to adult stage if immature insects were collected. In addition to the 12 plants sampled biweekly, two transects were established at either six (2004) or five (2005) of the largest sites. The first transect consisted of 25 consecutive common buckthorn trees growing along a path, roadway, or other opening where trees had full exposure to the sun resulting in common buckthorn trees that were larger. The second transect was perpendicular to the first transect and consisted of another 25 consecutive common buckthorn trees and included trees growing in the under-story in shade or filtered sunlight. All trees selected were visually surveyed for up to 2 minutes and all insects observed were collected and returned to the laboratory.

Adults reared and collected in the field were preserved and pinned for later identification. Soft-bodied insects and immature insects that failed to reach the adult stage were preserved in vials containing 70% ethanol. Voucher specimens were deposited in the Entomology Museum at the University of Minnesota.

All adult specimens were categorized as herbivores, predators, parasitoids, or scavengers. For a species to be included in the statistical analysis, a minimum of five specimens per species was required. A qualitative Sorenson index (Magurran, 1988) was used to characterize differences in insect assemblages between habitat types. The equation for the qualitative Sorenson index (C_S) is $C_S=2j/(a+b)$ where j is the number of species found in both groups, a is the number of species in group x, and b is the number of species in group y. We used a quantitative Sorenson index (Magurran, 1988) to characterize differences in insect assemblages in relation to abiotic factors such as the amount of sunlight (forest edge vs. interior) and biotic factors such as tree size (small, medium, large). The equation for the quantitative Sorenson index (C_N) is $C_N=2j_N/(aN+bN)$ where j_N is the sum of the lower of the two abundances recorded for a given species found in both groups, aN is the total number of specimens in group x, and bN is the total number of specimens in group y. The closer C_S or C_N are to 1, the more similar the groups are, and the closer to 0, the more dissimilar.

Results

Site characteristics

Overall, urban sites had the highest density of common buckthorn and the lowest plant species diversity (Table 1). Those sites characterized as agricultural sites had the opposite, with the lowest density of common buckthorn and the greatest plant diversity (Table 1). Those sites characterized as rural had an intermediate percent cover of common buckthorn, but on a stem density per m^2 had common buckthorn densities equal to those of the urban landscapes. Plant species diversity was low in the rural sites, but the percent cover of other plant species and stem density of other plant species was intermediate (Table 1). Interestingly, if common buckthorn was excluded from this analysis, the most common plant species found in either urban or rural sites was garlic mustard, *Alliaria petriolata* L., another invasive plant of hardwood forests. When surveying the mature tree composition, four sites had common buckthorn as the dominant mature tree. Urban sites had a significantly higher density of mature common buckthorn trees when compared to rural or agricultural sites with one urban location having a maximum of 11 mature buckthorn trees per $1m^2$. Agricultural sites had the lowest number of mature buckthorn trees and the highest number of other mature tree species (Table 1). For all sites, the most dominate mature tree species, other than common buckthorn, was American elm, *Ulmus americana* L., followed by box elder, *Acer negundo* L.

Insect fauna

Over the two-year study, a total of 1,733 arthropods representing 13 orders, 111 families and 356 species were collected from common buckthorn. Hemiptera was the most abundant order, followed by Hymenoptera, which consisted mostly of parasitoids (Tables 2 & 3). Several species were abundant, each with over 75 specimens collected: *Metcalfa pruinosa* (Say) (Flatidae), *Lasius alienus* (Förster) (Formicidae), *Harmonia axyridis* (Pallas) (Coccinellidae), *Graphocephala coccinea* (Forster) (Cicadellidae), and *Trissolcus* sp. a. (Scelionidae).

For the analysis we used 606 herbivores representing 32 different species, 154 predators representing 5 different species, and 140 parasitoids representing 4 different species (Table 2 & 3). An additional 314 species were excluded from analysis because fewer than five specimens were collected over the 2 year sampling effort or because species were known to be saprophagous, mycetophagous, scavengers, or non-feeding as adults. The Sorenson index (C_S) showed that all three habitat types, agriculture, rural, and urban landscapes, were very similar in insect species diversity (range 0.71-0.73). The majority of predators were captured at sites in agriculture habitats (56%) and the majority of parasitoids were captured in rural habitats (61%). The quantitative Sorenson index for insect diversity for forest edge and interior using data collected from the two perpendicular transects was (C_N=0.54). It is not surprising that transects are different since more insects were collected on common buckthorn along the transects where plants were along a forest edge (62% of captures) compared to the interior (38% of captures). When comparing tree sizes, large and small trees had the least similar insect composition (C_N=0.44); whereas medium and small trees had the most similar insect composition (C_N=0.59). Medium trees tended to have higher diversity and abundance compared to large and small trees.

In general, there was very little evidence of feeding damage on common buckthorn. The most common type of damage was leaf miner tunnels, followed by damage caused by lepidopteran larvae. Nine species were reared in the laboratory after collecting immature insects from common buckthorn indicating these nine species are able to complete their development solely on buckthorn. These included three hemipteran species, *Acanalonia conica* (Say), *M. pruinosa*, and *Gyponana quebecensis* (Provancher), three orthopterans, *Neoxabea bipunctata* (De Geer), *Oecanthus fultoni* Walker, and *Oecanthus niveus* (De Geer), and three lepidopterans collected as eggs and reared to adult, which included *Choristoneura rosaceana* (Harris), *Machimia tentoriferella* Clemens, and *Spilosoma virginica* (Fabricius). The two tortricids, *C. rosaceana* and *M. tentoriferalla* experienced high mortality during rearing and adult specimens that did emerge often had abnormal wing development. However, a literature search revealed that these nine species listed above can be categorized as generalist herbivores and are not considered specialist herbivores that only feed on common buckthorn.

Discussion

Urban sites, which had the densest common buckthorn infestation and lowest plant species diversity, also had the lowest insect abundance when compared to other habitat types. All urban sites sampled were located in highly populated areas where human activities could easily disturb the natural habitat. In contrast, agricultural sites had the highest plant diversity with more insects collected at those sites. Predators were collected at higher rates in agricultural sites than the other sites possibly drawn there by agricultural pests that would be found in the adjacent crop fields.

The main objective of this study was to identify major herbivores present on common buckthorn in Minnesota. Overall, there were many herbivores collected, however, most insects collected were represented by fewer than five specimens suggesting that they were transient

feeders or generalist herbivores that do not utilize common buckthorn. In reports of herbivores collected from *R. cathartica* in Europe, the most common insect species found were Lepidopterans (Malicky et al. 1970). Here we show that in Minnesota, defoliators were common, but unlike Europe more Hemipterans were encountered in Minnesota than Lepidopterans. During our 2-year study we did not find any insect feeding internally on buckthorn, and thus one potential niche that could be exploited successfully would be an internal feeder such as the stem-boring beetle, *Oberea pedemontana* Chevrolat (Coleoptera: Cerambycidae) which has been identified in Europe as a possible biological control agent of *R. cathartica* (Gassmann, 2005). Even though we found many generalist herbivores feeding on leaves at no time did defoliation exceed 5% on any one tree, thus a specialist herbivore would have an abundant resource to utilize in Minnesota.

The second objective of this study was to identify possible sources of biotic resistance if non-native herbivores were introduced as classical biological control for common buckthorn. There were numerous parasitoids and predators, all considered generalists, collected from common buckthorn. The abundance of parasitoids and predators may indeed hinder establishment of potential biological control agents. Generalist predators have been known to interfere with biological control agents released for purple loosestrife control (Sebolt and Landis 2004). Currently, there have been a few species proposed as potential biological control agents for common buckthorn in North America (Gassmann 2005). As agent selection continues for common buckthorn, the species diversity and abundance of natural enemies collected from buckthorn documented here needs to be considered. In particular *H. axyridis,* could play a significant role in preventing establishment of herbivores since it was the most abundant generalist predator collected and this coccinellid is known to prefer arboreal habitats. This recently introduced coccinellid was more common in spring and fall (Figure 1). *Harmonia axyridis* could exert strong biotic resistance on biological control agents especially if a vulnerable life stage was present when *H. axyridis* densities were high. For example, one group of candidate biological control agents for common buckthorn are the psyllids, *Cacopsylla rhamnicolla* and *Trichohermes walkeri* (Gassmann 2005) and it is possible that *Harmonia axyridis* would pose a particular threat to psyllids. This potential negative interaction could be studied as part of the host testing procedure.

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Table 1. Site characteristics for three habitat types (urban, rural, and agricultural) surveyed for insect fauna on *Rhamnus cathartica,* common buckthorn.

All vegetation (1 m^2)	Urban sites	Rural sites	Agricultural sites
% Cover of common buckthorn	61.0 ± 0.1	48.0 ± 0.1	31.0 ± 0.1
% Cover of other plant species	39.0 ± 0.1	52.0 ± 0.1	69.0 ± 0.1
Common buckthorn stem density m^2	6.1 ± 1.1	6.1 ± 1.1	4.3 ± 0.9
Other plant species stem density m^2	11.5 ± 2.4	28.4 ± 3.4	26.4 ± 3.6
Number of other plant species	3.5 ± 0.4	3.8 ± 0.3	6.8 ± 0.5
% Canopy cover	82.0 ± 1.1	83.0 ± 0.8	80.0 ± 0.8

Mature Tree Survey (12.56 m^2)[a]

Number of common buckthorn trees	6.1 ± 1.5	2.8 ± 0.7	1.1 ± 0.3
Number of other trees	1.9 ± 0.4	1.4 ± 0.2	2.6 ± 0.3
Number of other tree species	1.5 ± 0.3	1.1 ± 0.1	1.4 ± 0.1

[a], trees at least 1.5 m tall in a 2 m radius from center of plot (12.56 m^2)

Table 2. Herbivores collected on *Rhamnus cathartica,* common buckthorn in Minnesota. Only species for which a minimum of five specimens were collected were included, except for *Oecanthus* spp., because of the high abundance of immature specimens collected.

			No. of specimens		
Order	Family	Genus species	2004	2005	Total
Orthoptera	Gryllidae	*Neoxabea bipunctata* (De Geer)	10	5	15
		Oecanthus fultoni Walker	2	1	3
		Oecanthus niveus (De Geer)	1	2	3
Subtotal			**13**	**8**	**21**
Hemiptera	Acanaloniidae	*Acanalonia conica* (Say)	9	11	20
	Aphididae	*Aphis glycines / nasturtii*	0	39	39
		Aphis glycines Matsumura	7	17	24
		Aphis nasturtii Kaltenbach	0	26	26
	Cercopidae	*Clastoptera obtusa* (Say)	0	7	7
		Philaenus spumarius (L.)	2	8	10
	Cicadellidae	*Empoasca sp.* b	0	5	5
		Graphocephala coccinea (Forster)	21	64	85
		Gyponana quebecensis (Provancher)	4	9	13
		Jikradia olitorius (Say)	9	6	15
	Derbidae	*Cedusa incisa* (Metcalf)	8	2	10
	Flatidae	*Metcalfa pruinosa* (Say)	11	164	175
	Miridae	*Hyaliodes harti* Knight	7	6	13
		Hyaliodes vitripennis (Say)	3	2	5
		Paraproba capitata (Van Duzee)	6	8	14
		Phytocoris spicatus Knight	2	4	6
	Pentatomidae	*Euschistus tristigmus* (Say)	8	9	17
	Tingidae	*Corythucha pergandei* Heidemann	0	5	5
Subtotal			**97**	**392**	**489**
Coleoptera	Chrysomelidae	*Diabrotica longicornis* (Say)	1	5	6

	Curculionidae	*Polydrusus sericeus* (Schaller)	2	5	7
	Pyrochroidae	*Pedilus impressus* (Say)	1	4	5
Subtotal			**4**	**14**	**18**
Lepidoptera	Arctiidae	*Spilosoma virginica* (Fabricius)	0	10	10
	Gracillariidae	*Phyllonorycter caryaealbella* (Chambers)	2	6	8
	Psychidae	*Thyridopteryx ephemeraeformis* (Haworth)	0	5	5
	Tortricidae	*Choristoneura rosaceana* (Harris)	4	5	9
		Machimia tentoriferella Clemens	2	3	5
Subtotal			**8**	**29**	**37**
Diptera	Cecidomyiidae	*Parwinnertzia notmani* Felt	2	5	7
Hymenoptera	Cynipidae	*Diplopepsis* sp. a	0	9	9
		Liodora sp.	0	6	6
	Tenthredinidae	*Fenusa* sp.	0	19	19
Subtotal			**0**	**34**	**34**
Totals			**122**	**477**	**599**

Table 3. Predators and parasitoids collected on *Rhamnus cathartica,* common buckthorn in Minnesota. Only species for which a minimum of five specimens were collected were included.

			No. of specimens		
Order	Family	Genus species	2004	2005	Total
Hemiptera	Nabidae	*Lasiomerus annulatus* (Reuter)	10	15	25
Coleoptera	Cantharidae	*Podabrus rugulosus* LeConte	5	0	5
	Coccinellidae	*Coleomegilla maculata* DeGeer	3	2	5
		Harmonia axyridis (Pallas)	44	68	112
			52	70	122
Diptera	Empididae	*Tachypeza* sp. a	4	3	7
Hymenoptera	Platygasteridae	*Leptacis* sp. c	0	8	8
	Scelionidae	*Idris* sp.	18	0	18
		Trissolcus sp. a	37	39	76
		Trissolcus sp. b	0	38	38
			55	85	140

Figure 1. Seasonal abundance of *Harmonia axyridis* observed on *Rhamnus cathartica,* common buckthorn in Minnesota. Data pooled for 2004 and 2005.

Appendix C

Result 3: Introduction and evaluation of Garlic Mustard biological control agents in Minnesota

LCCMR Final Report

Title: Monitoring garlic mustard (*Alliaria petiolata*) in anticipation of future biocontrol release

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EXECUTIVE SUMMARY

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. Three *Ceutorhynchus* weevil species are being studied to determine their suitability as biological control agents for garlic mustard. In 2008, a proposal to approve the release of *Ceutorhynchus scrobicollis* was submitted to the USDA Technical Advisory Group. In anticipation of the future release of garlic mustard biological control agents, a garlic mustard population monitoring program was initiated in Minnesota in 2005.

Garlic mustard is a biennial and its population can vary widely from year to year. Several years of monitoring are necessary to provide an accurate assessment of the pre-release population and to understand normal levels of year to year population fluctuation. 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 2007.

Three 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. Overall, garlic mustard plants had an average of 1.8% of their leaf area removed by herbivores. An introduced biological control agent could greatly increase the amount of damage garlic mustard is currently experiencing. At about half of the 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 1st or 2nd year plants in any given year. For example, at Warner Nature Center, the density of adult garlic mustard cycled from 1 plant/m^2 in 2005 up to 85 in 2006 and down to 15 in 2007. The other sites had more stable or increasing garlic mustard populations. 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.

We were also able to characterize the plant community in which garlic mustard is growing and estimate the potential for native species recovery should garlic mustard populations decrease. Sites with greater garlic mustard cover had lower native species richness and cover then sites with lower garlic mustard cover. After biological control agent release, we will be able to determine if garlic mustard is reduced, and if so, how native species populations respond. To determine which species are likely to germinate if garlic mustard populations are reduced, we collected soil samples to describe the composition of the seed bank at seven sites. We found that seeds of native species were more common than nonnative species. There are several sites that may need additional

restoration help because of a low proportion of native species in the seed bank and in the existing vegetation. However, other sites are likely to have better recovery as they have larger covers of existing native vegetation and a variety of native species present in the seed bank.

To further examine the impacts of garlic mustard on native species and the potential for native species recovery in the absence of garlic mustard, several greenhouse experiments were designed to explore the effects of garlic mustard's allelopathic root exudates. Activated carbon was used to apply treatments that negated the impact of the allelochemicals. We found that plants growing with garlic mustard were negatively impacted due to competition with garlic mustard; however, removing the allelopathic effect did not significantly improve plant performance. A second experiment determined that native plants did not have less above-ground biomass in soils in which garlic mustard had grown previously, indicating that there was no legacy effect of garlic mustard allelochemicals. While allelopathy did not have a direct impact on plants growing with garlic mustard or in garlic mustard soils, this does not exclude the potential for indirect effects due to negative impacts on the native mycorrhizal soil fungi upon which many native species are dependent. If native plants have difficulty reestablishing following a reduction in garlic mustard, it likely would be due to indirect impacts, not due to direct residual allelopathic impacts of garlic mustard.

Chapter 1

Garlic mustard (*Alliaria petiolata*) monitoring in anticipation of future biological control release

INTRODUCTION

Nonnative invasive species are one of the main threats to native species diversity (Williamson 1996, Schmitz et al. 1997, Wilcove et al. 1998). Nonnative species may displace native species as well as alter entire ecosystem processes (Mack et al. 2000, Mack and D'Antonio 1998). Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] is an invasive, nonnative species that is invading forested regions throughout the United States (Cavers et al. 1979, Meekins et al. 2001). Garlic mustard is able to invade high quality forests in addition to disturbed areas (Nuzzo 1999). Garlic mustard is a concern because of its ability to invade high quality forests, form dense populations, and decrease abundance of native species (Blossey et al. 2001).

Due to the number of negative impacts of garlic mustard, a program was initiated to develop biological control agents (Blossey et al. 2001). Ideal candidate insects to be a biological control will only feed and complete its life cycle on the target organism. In this way, the biological control agent can reduce the population of the invasive plant and allow native species populations to return to non-impact levels. Currently, three weevil species are being tested at the University of Minnesota quarantine facility to determine their host specificity and their suitability as biological control agents. The three species are the root-crown feeding weevil *Ceutorhnychus scrobicollis*, and the stem-mining weevils *Ceutorhnychus alliariae* and *Ceutorhnychus robertii* (Blossey et al. 2001, Katovich et al. 2005). A proposal to approve the release of *Ceutorhnychus scrobicollis* was submitted to the USDA-APHIS Technical Advisory Group committee in the first quarter of 2008.

To determine if the biological control agents are effective at reducing garlic mustard, it is necessary to monitor the plant communities into which the insects are released. The impact of the control agents and the response of the plant community can be assessed by comparing data gathered before and after the insects are released (Blossey 1999). Garlic mustard is a biennial species which can cause populations to fluctuate significantly from year to year (Meekins and McCarthy 2002). Due to the variability of garlic mustard populations it is preferable to have several years of plant monitoring data before the insects are released. A grant for implementing and monitoring biological control releases was proposed to the Legislative-Citizen Commission on Minnesota Resources (LCCMR) and was accepted (Skinner 2005).

Garlic mustard is part of a complex community and its role in that community is not clear. Nonnative earthworms have invaded many forests and denuded the litter layer, altered soil processes, and decreased native species abundance (Bohlen et al. 2004, Hale et al 2005). Bartuszevige et al. (2007) found that garlic mustard seedlings had the greatest establishment in plots with litter removed versus control or litter added plots. Garlic mustard is often found in areas with little to no litter layer, implying that garlic mustard may succeed in sites that have been invaded by earthworms (Blossey et al. 2005). The overpopulation of deer in many areas has put additional pressure on native

plants. Garlic mustard seems to be grazed less than natives and so may do better than natives in sites with high deer population density (Blossey et al. 2005). Garlic mustard itself may change soil properties. Garlic mustard has allelopathic root exudates which can inhibit germination in some species (Prati and Bossdorf 2004). The root exudates have also been found to have a negative impact on mycorrhizal fungi (Stinson et al. 2006). Many late-successional native species are dependent on mycorrhizae, so the loss of mycorrhizae can negatively affect native species abundance.

By monitoring garlic mustard populations and the associated plant community and environmental conditions we can begin to answer questions about garlic mustard impacts and the effectiveness of biological control agents. We can assess the level of herbivory garlic mustard is experiencing in Minnesota in the absence of biological control insects. Low herbivory levels indicate that existing herbivores are not having a large impact on garlic mustard populations and that the biological control agents could have a large impact. Monitoring data also allows a characterization of the year to year fluctuations in garlic mustard cover, population density, heights, and siliques (seed pod) production so normal population variation can be separated from long-term impacts of biological control agents. By monitoring the plant population growing with garlic mustard we can gain a better understanding of the relationship and impacts of garlic mustard on native and nonnative plant species.

METHODS

The main goal of the garlic mustard monitoring project was to establish long-term monitoring sites to characterize garlic mustard populations in anticipation of biological control insect release. Twelve monitoring sites were selected based on the presence of a garlic mustard population of sufficient size and population density to accommodate the plots and a willingness of the owners to refrain from any management to reduce the garlic mustard during the course of the study. At each site, 20 permanent 1m x 0.5 m plots (0.5m^2 quadrats) were established. To monitor the plant communities, we used the protocol developed by the Ecology and Management of Invasive Plants Program in 2003 (available at http://www.invasiveplants.net). Monitoring data is collected in June and October. Following the protocol, data is collected on 1st year garlic mustard plants (cover and number of individuals), 2nd year garlic mustard plants (cover, number of individuals, height, and number of siliques), cover of other species present, amount and type of insect damage to garlic mustard, ground cover, and litter depth. This information will allow us to determine if the biological control agents have their desired effects of reducing cover of garlic mustard, decreasing seed set of garlic mustard, and increasing native species cover.

In June 2005, garlic mustard monitoring plots were established and data was collected at five sites. All 12 sites were established by the fall monitoring data collection period in October 2005 (Table 1). In 2006 and 2007, data was collected from all 12 sites in June and October. City and county information is provided for each site in Table 2. All sites are upland deciduous forests, except for Coon Rapids and Fort Snelling which are floodplain forests (although flooding is currently rare due to management of adjacent rivers). There is also variation from site to site in terms of dominant tree and herbaceous layer species composition.

Data is summarized by season and site. In all graphs, the error bars are standard errors. Garlic mustard is a biennial, so there are three terms used to describe garlic mustard life stages. In the first year of life, garlic mustard germinates from a seed and enters the "seedling" stage in the spring. By the fall of that year, the seedling will have grown into a basal "rosette". The rosette over-winters and then in the spring, the second year of life, a stem bolts from the rosette and forms the "adult" flowering stalk. Adult plants flower in the spring, set seed in the summer, and die by the fall. The spring data includes both the seedling and adult stages, while only the rosette stage is present in the fall. The adult plants can be further divided into those with siliques present and those with no siliques present.

In addition to collecting data on garlic mustard, data was also collected for the other plants growing within the garlic mustard plots. Other species within a plot were identified to species, when possible, and their percent cover in the plot recorded. The species were then categorized as native, nonnative, or unknown (species that cannot be identified to the taxonomic level where native or nonnative status can be determined). All native or nonnative determinations were based on the Minnesota Department of Natural Resources species list for Minnesota (http://files.dnr.state.mn.us/ecological_services/plant_list9-25-02.pdf). By collecting data on the other species present, we can track the changes of the plant community over time.

RESULTS: 2005 through 2007 monitoring period
1. Garlic mustard herbivory levels

While evidence of insect feeding was widespread (Table 2), the actual amount of leaf damage was low (Fig. 1). Across all sites, seasons and years the average amount of leaf area damaged due to insects was $1.8 \pm 0.03\%$. Leaf damage did not vary widely from site to site. The lowest mean leaf removal was 0.95% at Pine Bend in 2006, while the highest was 4.4% at Fort Snelling (Fig. 1). Most of the damage was in the form of edge or internal hole feeding (Table 2). Leaf mining and windowpane feeding also occurred. While edge and hole feeding remained common in the fall, leaf mining was much less common, dropping from occurring in 31% of the plots in the spring to only 1% in the fall, indicating that most leaf mining was on adult plants. When biological control weevils are released it is expected that insect damage, especially windowpane feeding, will increase.

2. Fluctuations in garlic mustard populations over time

Garlic mustard's biennial life cycle drives some of the changes in garlic mustard cover and population density from year to year. At some sites, one life stage clearly dominates in each year. For example, a site may be dominated by adult flowering plants in spring 2005 and have few seedlings present. In the fall of 2005 there would be few rosettes. In the spring of 2006, the seedling stage would dominate and the site would have many seedling and very few adults. By fall 2006 there would be many rosettes. This pattern is demonstrated in Figure 2 with photos from Baker Park.

Of the 12 sites, six showed a pattern of one life stage dominating each year (Fig. 3). Over three years of monitoring, the rosette population density cycled from low to high to low in some sites and from high to low to high at others (Fig. 3). It is important

to take these population cycles into account when analyzing the impacts of biological control insects. A decrease in adult plants from one year to the next may simply be a result in this natural oscillation in life stage dominance. It will take several years of data to separate out natural population cycles from long-term decreases in population.

Not all sites were dominated by one life stage in a given year. At many of the sites there were similar amounts of first and second year plants coexisting each year. Three sites showed little variation in rosette population density from year to year (Fig. 3). The final three sites showed either increasing or decreasing rosette population density over time. Of the six sites that didn't show life stage cycling, five showed a trend of an increase in rosettes over time. At these sites, it is likely that garlic mustard populations are still growing and expanding, causing an increase in rosettes over time. In addition to changes in rosette population density due to the cycling of life stages or a growing population, population density can also change in response to abiotic and biotic factors. For example, in years of low precipitation, we would expect to see lower cover of garlic mustard.

The springtime population densities of garlic mustard adults and seedlings varied from year to year (Fig. 4). Since only five sites were established in 2005, there are only five sites with three years of spring data. The patterns that were clear in the fall data are less visible in the spring as most sites have only two years of data. In most cases, the sites that showed strong year to year population cycling in the fall reflect strong year to year variation in the spring population densities of seedlings and adults (Fig. 4). For example, the population density of adult plants at Warner Nature Center fluctuated widely, going from 0.5 plants per $0.5m^2$ plot in 2005 to 42.6 plants/plot in 2006 and then down to 7.7 plants/plot in 2007 (Fig. 4). The low of 0.5 plants/plot (1 plant/m^2) and the high of 42.6 plants/plot (85.2 plants/m^2) at Warner Nature Center were the lowest and highest densities found across all sites and years. Seedling densities also ranged widely, from a low of 14.1 plants/plot (18.2 plants/m^2) to a high of 114.7 plants/plot (229.4 plants/m^2) (Fig. 4). Comparing the population density of seedlings in the spring with rosettes in the fall, it is clear that many seedlings die before reaching the rosette stage (Figs. 3 and 4).

In addition to monitoring garlic mustard population densities, we also monitored the percent cover of garlic mustard. At some sites, small numbers of plants were large and covered a large percentage of the plot, while at other sites, large numbers of very small plants covered only a small area. The percent cover of garlic mustard is another way to visualize the dominance of garlic mustard at a site and to track impacts of biological control agents. The biological control agents may stunt plants, causing them to be smaller in stature then before. The total cover of garlic mustard in the spring (adults plus seedlings) ranged from 20 to 70% (Fig. 5). Garlic mustard cover decreased in the fall because the adult plants have set seed and senesced, so only first year rosettes are present. Total garlic mustard cover did vary from year to year, although the range of garlic mustard cover was similar from year to year.

3. Fluctuations in garlic mustard plant height and reproductive output

Data was collected on garlic mustard plant height and number of siliques as measures of vigor and reproductive output of the plants. It is anticipated that the introduction of biological control insects will stress the plants and result in smaller plants

which produce fewer siliques (Gerber et al. 2007). The year to year variation in garlic mustard average heights and numbers of siliques again underscores the importance of pre-release monitoring. Monitoring sites with and without biological control release will help us determine the impacts of biological control agents versus natural year to year variation.

Large natural fluctuations in garlic mustard plant height and numbers of siliques were detected as height and siliques production decreased from 2006 to 2007 (Fig. 6 and 7). The mean height of garlic mustard plants decreased at all twelve sites from 2006 to 2007 (Fig. 6). Mean heights ranged from 48 to 82 cm in 2006, but only 21 to 56 cm in 2007. Environmental factors, such as below normal precipitation in 2007, were the likely cause of decreased growth at all the sites. Smaller garlic mustard plants then produced fewer siliques (Fig. 7). In 2006, silique production was high with 67 to 444 siliques present in a $0.5m^2$ quadrat, but by 2007 there were only 43 to 240 siliques/$0.5m^2$ (Fig. 7A). The number of siliques per quadrat gives an estimate of seed output at a site. After biological control agent release, silique production on the site level should decrease. The number of siliques produced per quadrat will also vary from year to year because in some years the silique-producing adult plants will dominate, but the next year the non-reproductive seedling stage will dominate. The numbers of siliques per plant are likely to remain relatively constant from year to year in the absence of biological control or strong environmental stress (Fig. 7B). The mean number of siliques per stem is a measure of the fecundity of individual plants at a site. Since plants were generally smaller in 2007 than 2006, they correspondingly produced fewer siliques per plant with 5-14 siliques/stem in 2006 versus 2-11 siliques/stem in 2007 (Fig. 7B). When biological control is released, we expect that individual plants will produce fewer siliques.

To further characterize the population, adult stems were categorized as to stems with siliques or with no siliques. Almost all adult plants produced siliques; stems without siliques were rare (Fig. 8). At most sites, fewer than 5% of the adult stems did not produce siliques. With one exception, the other sites had fewer than 10% barren stems (Fig. 8). The high percentage of barren stems (26%) observed at Hilloway Park in 2006 was due to early season buckthorn (*Rhamnus cathartica*) control which resulted in herbicide drift onto garlic mustard plants. This caused reduced and delayed silique development in many plants. The low percentages of barren stems across sites indicate that most adult plants have the resources to complete their life cycle and produce seed. It is anticipated that the number of stems without siliques will increase with the introduction of biological control insects as the insects stress the plants.

4. Relationship between garlic mustard and native species

One of the impacts of garlic mustard is that it forms dense populations which negatively impact native species (Nuzzo 1999, Blossey et al. 2001). Sites with greater garlic mustard cover had lower native species richness and cover than those sites with less cover of garlic mustard (Fig. 9). The negative correlations were consistent in both 2006 and 2007 (Fig. 9). Sites varied in the amounts of native and nonnative species present. Native species richness ranged from a low of 1.8 species/$0.5m^2$ quadrat at Baker Park in 2005 to a high of 6.7 species/$0.5m^2$ at Willmar in 2007. Native species cover ranged from a low of 9% cover at Baker Park in 2005 to a high of 50% cover at Nerstrand in 2007. Nerstrand also had the lowest nonnative species richness and cover

(no nonnative species present in the spring 2005-2007). The highest nonnative species richness was found at Baker Park in 2007 (1.6 nonnative species/0.5m^2) and the highest nonnative cover was found at Coon Rapids in 2006 (26.3%).

In addition to monitoring whether biological control insects will decrease garlic mustard populations, we can also monitor the response of the native vegetation. Ideally, native species cover and richness will increase as the populations of garlic mustard decrease. Monitoring data provides baseline information on native species cover and richness. By continuing to monitor after biological control release, we will be able to determine if native species remain stable or increase or if other nonnative species are increasing. This data will provide information on the response of the plant community to the release of biological control agents and indicate whether additional restoration work may be necessary.

5. Garlic mustard and leaf litter

When nonnative earthworms invade a forested site, they cause a dramatic decrease in the litter layer (Bohlen 2004, Hale et al. 2005). In Minnesota, litter layer depth decreased from 10 cm to 0 cm with the presence of earthworms (Hale et al. 2005). Blossey et al. (2005) suggested that garlic mustard invasion follows earthworm disturbance. Data on the percent cover of bare soil and the depth of the litter layer were collected to assess the impact of earthworms at the sites. We used low litter layer depth and high cover of bare soil as indicators of earthworm disturbance. All sites had very low litter depth with average spring litter depths ranging from 0.09 cm to 2.4 cm. There was no accumulated litter from previous years; the litter that was measured was recent leaf fall. The low variation in litter depths across sites made it difficult to detect any correlation between increased garlic mustard densities in sites with low litter depth (Fig. 10A). The percent cover of bare ground did vary widely across the sites (ranging from 0 to 84% of the ground cover in the spring). Even with a range of bare ground cover, there was no indication of increasing garlic mustard population density with increasing amounts of bare ground (Fig. 10B). Our data showed little evidence for increased garlic mustard with increasing impacts of earthworms. However, all sites were found to have significantly impacted litter layers, so there was no comparison with undamaged sites.

DISCUSSION

Garlic mustard in Minnesota is currently experiencing very little herbivory. On average, less than 2% of the leaf area was damaged by herbivores. The 1.8% leaf damage levels in Minnesota are similar to the 3.3% leaf damage levels reported in Michigan (Evans et al. 2007). This low level of damage may be one reason why garlic mustard has been such a successful invader. An introduced biological control insect has the potential to greatly increase insect damage from its present level.

Garlic mustard plant populations do vary considerably from year to year. Two to three years of pre-release monitoring data have given us a good understanding of the year to year fluctuations in populations. At some sites, they population fluctuations are due the changes in dominance between the seedling and adult stages. After biological control insects are released we expect to see decreases in garlic mustard populations (Davis et al.

2006, Gerber et al. 2007). With long-term data collection we can see long-term trends in garlic mustard populations. When biological control insects are available they will only be initially released at half of the monitoring sites. This will help in separating population changes due to biological control insects from changes due to environmental factors, such as years of low precipitation.

Through the monitoring data we will be able to see both the impact of the biological control insects on garlic mustard and the impact of changes in garlic mustard abundance on other plant species in the community. Individual species and functional groups have been found to vary in their responses to experimental removal of garlic mustard (McCarthy 1997, Stinson et al. 2007). Tree seedlings and native grasses especially susceptible to the presence of garlic mustard and are some of the first species to increase after garlic mustard removal (McCarthy 1997, Stinson et al. 2007). Through monitoring, we will find out if garlic mustard populations decline due to biological control and whether or not those declines allow native species to increase. We will be able to characterize the sites and determine if native species cover and species richness improve when garlic mustard populations are reduced. If other nonnative species increase as garlic mustard populations decrease then additional restoration work may be necessary. It will likely take several years of reduced garlic mustard populations before impacts on the forest understory can be observed (Hochstedler et al. 2007).

After biological control release, there is the potential for large differences in native plant community recovery among the different sites. Some sites have high levels of disturbance (low litter levels, high nonnative species cover) while others have a more robust native plant community. For example, Nerstrand had no nonnative species cover measured in the spring in all three years. If garlic mustard decreases, there is a large, diverse native species population ready to expand. In contrast, Baker Park had the lowest cover of native species and the highest diversity of nonnative species. It is not clear how the native plant community will respond to the reduction in garlic mustard. The twelve sites encompass a range of disturbance levels and their responses to biological control will help clarify the impacts of the biological control agents and whether those impacts are consistent across sites. Finally, with the monitoring program in place and with key pre-release release baseline data, we will be able to determine the benefits, or vegetative outcomes, of the garlic mustard biological control efforts.

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TABLES

Table 1. Garlic mustard monitoring sites in Minnesota. The ID column gives the abbreviation for the site found in the data summaries.

Site #	ID	Site Name	City	County
1	BP	Baker Park Preserve*	Maple Plain	Hennepin
2	CR	Coon Rapids Dam Regional Park	Coon Rapids	Anoka
3	CG	Cottage Grove Ravine Regional Park	Cottage Grove	Washington
4	FS	Fort Snelling State Park*	Saint Paul	Ramsey
5	HP	Hilloway Park	Minnetonka	Hennepin
6	LL	Luce Line	Long Lake	Hennepin
7	NE	Nerstrand State Park, Prairie Creek SNA*	Nerstrand	Rice
8	PB	Pine Bend Bluffs SNA*	Inver Grove Heights	Dakota
9	PL	Plainview – private land	Plainview	Winona
10	WN	Warner Nature Center*	Marine on St. Croix	Washington
11	WH	Westwood Hills Nature Center	St. Louis Park	Hennepin
12	WI	Willmar - private land	Willmar/New London	Kandiyohi

*=site was established in time for spring 2005 data collection

Table 2. Garlic mustard presence and types of insect feeding present. The percent of plots with garlic mustard present out of the 20 plots at each of 12 study sites in Minnesota over 3 years. Of the plots with garlic mustard present, the percentages of those plots with various types of leaf damage are listed.

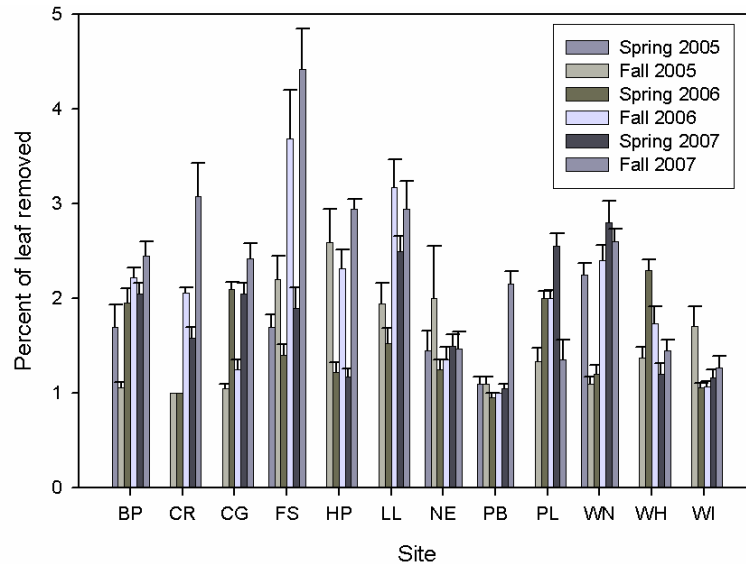| Time	Garlic mustard present (% of all plots)	Edge feeding	Holes	Leaf miner	Windowpane feeding
		(% of plots with garlic mustard present that showed this type of damage)			
Spring 2005	100	96	98	31	4
Fall 2005	87	99	98	1	1
Spring 2006	98	96	97	31	9
Fall 2006	84	97	98	0.5	0.5
Spring 2007	99	100	100	33	0
Fall 2007	88	97	96	1	0

Figure 1. Levels of insect damage to garlic mustard leaves in the absence of biological control agents by site in Minnesota. Damage was quantified as visual mean percent of leaf removed. The percent of leaf removed per site is the average of 20 plots per site. Data was collected during the June and October of 2005 through 2007. Data was collected in the spring at 5 monitoring sites in 2005 (NE, WN, BP, FS, PB) and at all 12 monitoring sites in fall 2005 and spring and fall in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, 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. Photos of a single plot over time, showing the dominance of different garlic mustard life stages at Baker Park, MN 2005-2006. The adult flowering plants were dominant in the spring 2005 with few seedlings present. By fall 2005, the adults senesced and there was little other vegetation present. In spring 2006 there was a carpet of garlic mustard seedlings. By fall 2006 the surviving seedlings had grown into rosettes.
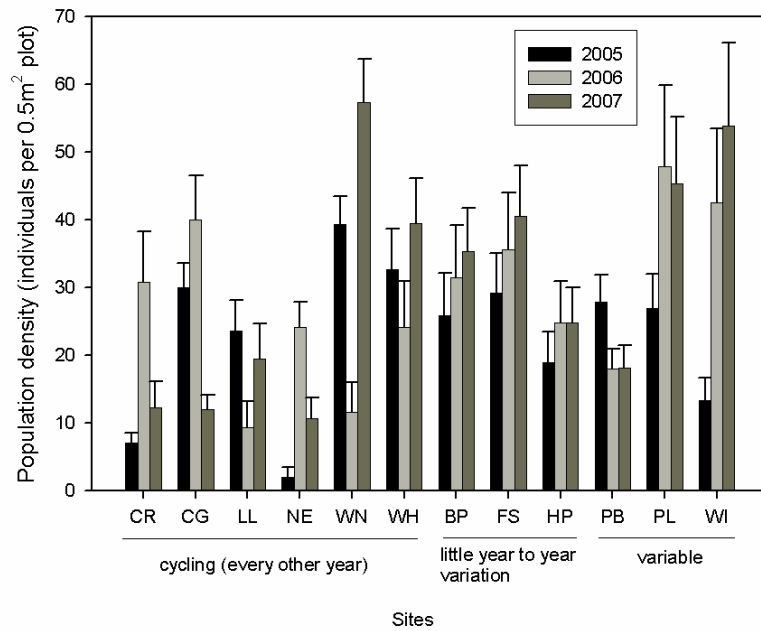
Figure 3. Population density of garlic mustard rosettes over time as measured in the fall at 12 monitoring sites in Minnesota, 2005-2007. Six sites show strong cycling (one life stage is dominant each year) with rosette densities peaking every other year. Three sites show little year to year variation in rosette population density (densities with standard error overlap from year to year). Three sites show variation over time with one site showing a decrease in rosette population density and two sites showing increases in rosette population density.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar
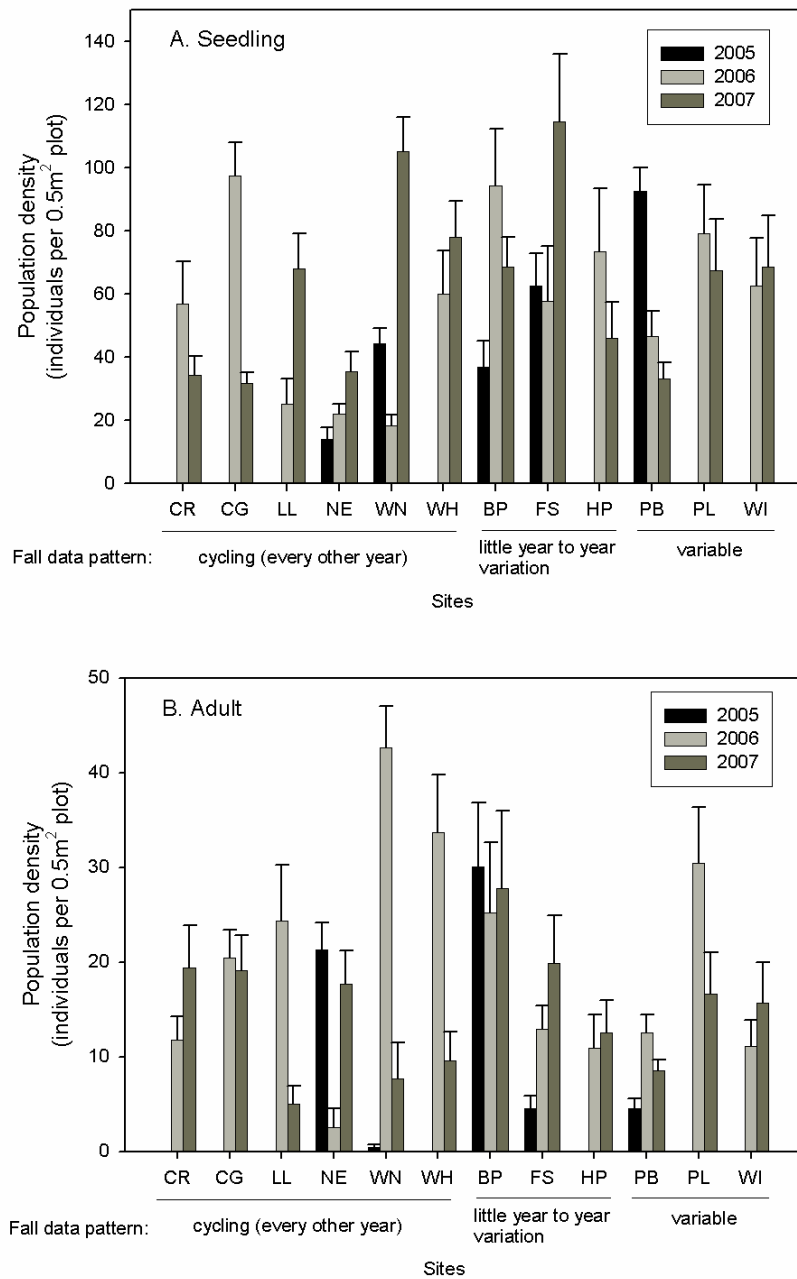
Figure 4. Population density of garlic mustard seedlings (A) and adults (B) over time. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (NE, WN, BP, FS, PB) and at all 12 monitoring sites in 2006 and 2007. Sites are organized according the density patterns seen in the fall data. With only 2 years of data at many of the sites, it is difficult to see the same cycling patterns present in the fall data, although WN clearly shows an alteration between the seedling and adult stages over the three years with seedlings dominant in 2005 and 2007 and adults dominant in 2006.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar
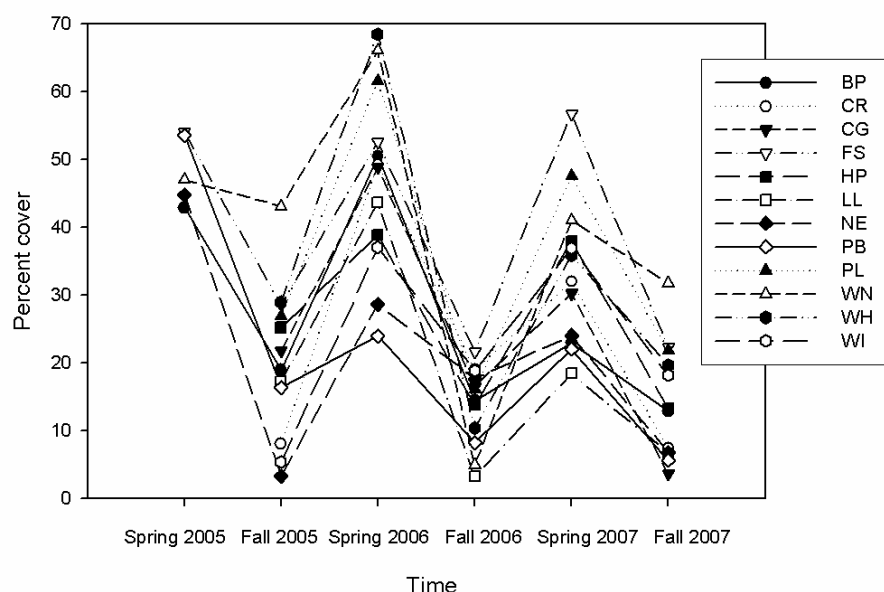
Figure 5. Visual total of the percent cover of garlic mustard over time. Spring garlic mustard cover is the total cover of adults and seedlings. Fall cover is cover of the rosettes. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar
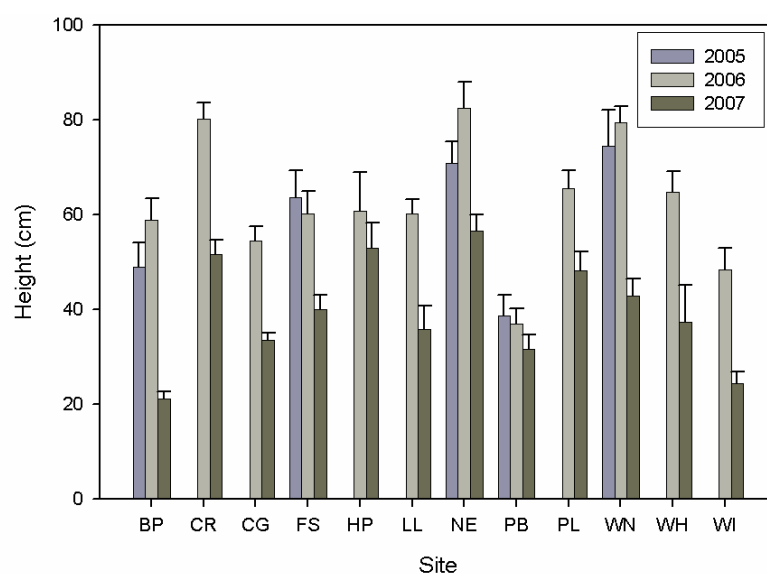

Figure 6. Mean garlic mustard plant height per quadrat averaged by site. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.

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BP=Baker Park, CR=Coon Rapids, 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. A: Mean number of siliques per quadrat (an estimate of seed output at a site). B: Mean number of siliques per stem (a measure of the fecundity of individual plants at a site). Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, 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. Percent of adult stems without siliques present. Note that the 2006 Hilloway
Park (HP) percent barren plants is high because many garlic mustard plants were
impacted by herbicide directed toward buckthorn. Data was collected in Minnesota in the
spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites
in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce
Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature,
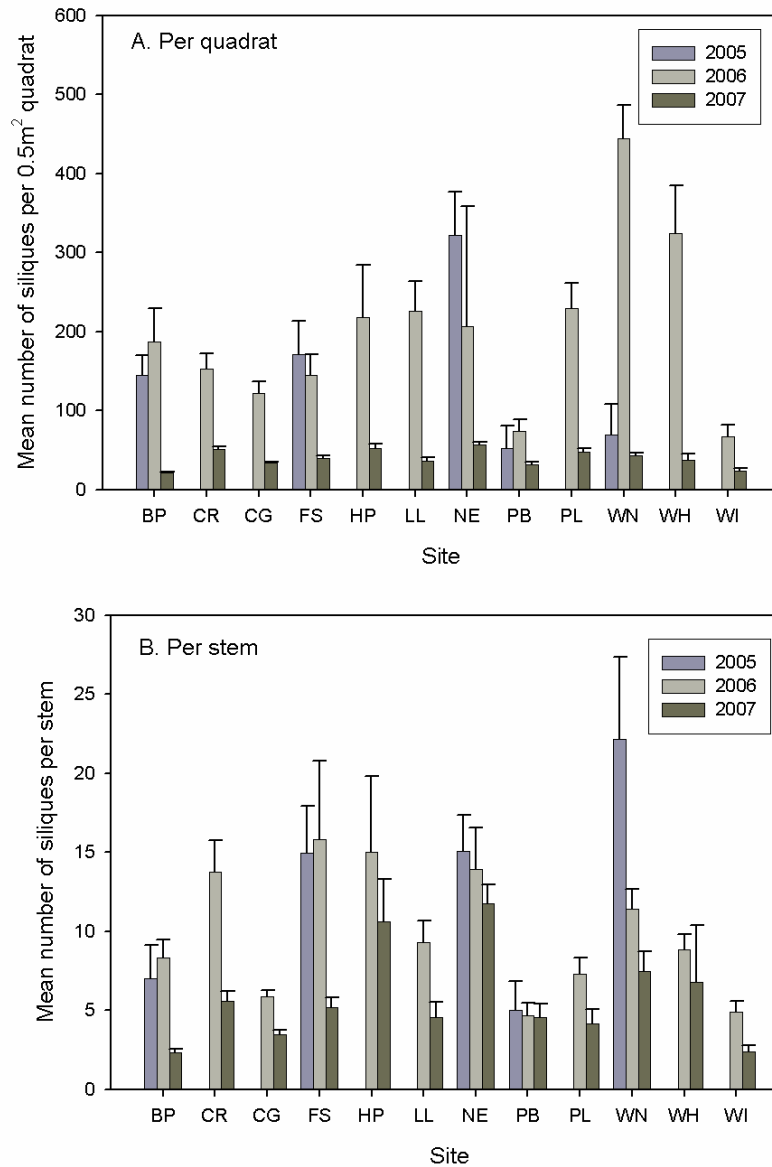WH=Westwood Hills, WI=Willmar

Figure 9. A: Regression of native species richness (mean richness per quadrat) by cover of adult + seedling garlic mustard in the spring. B: Regression of native species percent cover by cover of adult + seedling garlic mustard in the spring. Data points are the mean values for each of the 12 sites as determined from 20 quadrats at each site. Data was collected from 12 sites across Minnesota in 2006 and 2007.

Figure 10. A: Regression of garlic mustard plant population density by litter depth (spring 2006). B: Regression of garlic mustard plant population density by percent cover of bare ground (spring 2006). Data points are the mean values for each of the 12 sites in Minnesota as determined from 20 quadrats at each site.

Chapter 2

Recovery potential of garlic mustard sites: germinable seeds in the soil seed banks

INTRODUCTION

Garlic mustard (*Alliaria petiolata*) is a nonnative, invasive species which can dominate forest understories in the US (Blossey et al. 2001). The main goal of the garlic mustard biological control program is to reduce garlic mustard populations. The intent is that once garlic mustard cover is reduced, native species will be able to recover. In order to assess the effects of the biological control agent, monitoring sites were established throughout 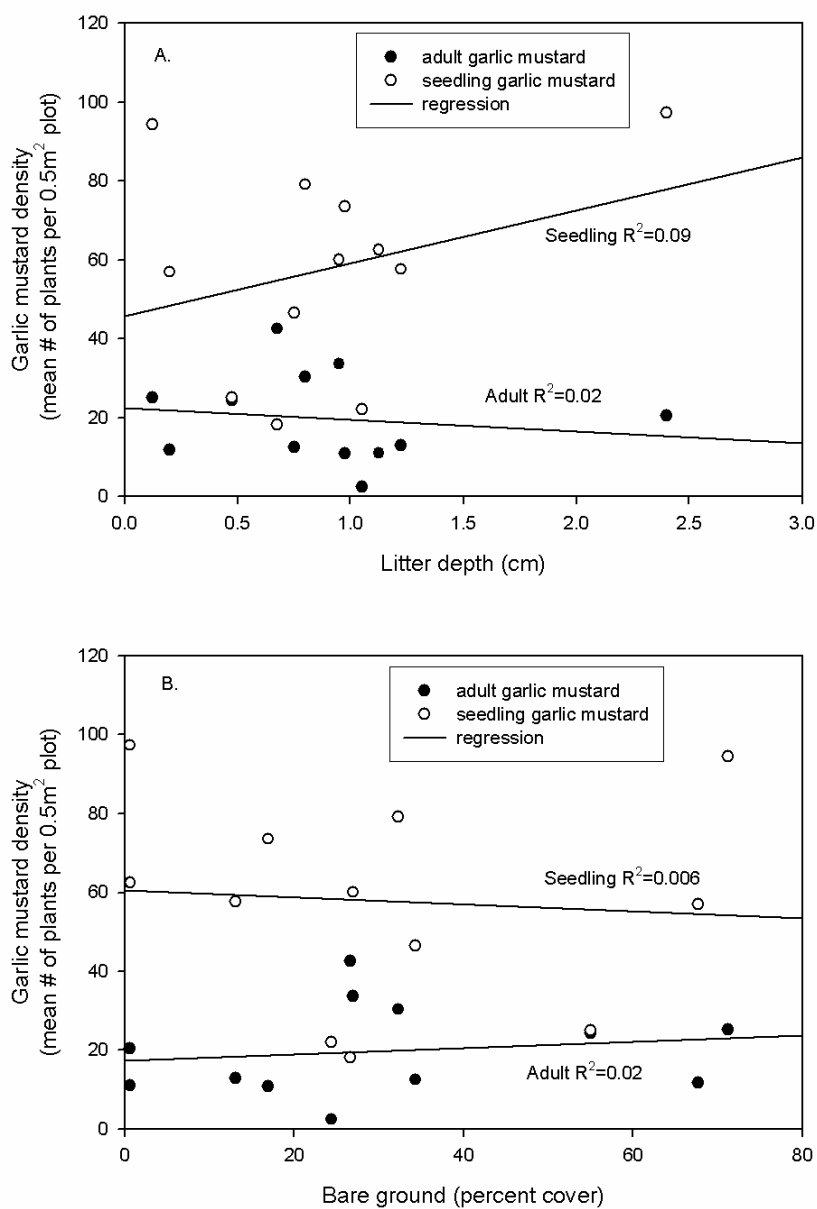Minnesota in 2005. At each site, data is collected on the species composition. If a decrease in garlic mustard opens up space in the forest floor, there are several sources for the plants that may come to occupy the space. Existing perennial plants and plants that reproduce vegetatively may expand to occupy the space. New seeds may be brought in by wind, water, or animals. Existing seeds in the soil seed bank may germinate and occupy the site. The plant composition data from the monitoring sites can be used to determine which species are already growing at the site. In order to determine what species are likely to germinate if garlic mustard is reduced, we collected soil samples to determine the plant species composition of the soil seed bank. It is important to have an adequate seed bank to reestablish native species once garlic mustard cover has decreased. By collecting seed bank data, we are also able to assess the extent of the garlic mustard seed bank. When there is little evidence of a native species seed bank then additional restorative efforts, such as seeding native species, should be considered.

The existing seed banks are an important component to management of the sites. Instead of assuming the existence of a viable native seed bank, it is sensible to determine whether or not it exists (van der Valk and Pederson 1989). If it is not found to exist, then preparation can be made for additional restoration efforts. Consequently, our main research question was: Is there a native species seed bank to re-colonize the site if garlic mustard is reduced by biological control insects? A second question was: What seeds of unwanted species are present? Unwanted species are nonnative invasive species that may expand their populations and impede native species recovery if garlic mustard cover is reduced. Garlic mustard itself can have a large seed bank that will take time to deplete.

Many of the sites had high garlic mustard cover and were generally not high-quality forest. It was likely that the native seed bank would be somewhat depleted. Garlic mustard is often associated with a nonnative plant community, either because it responds to disturbance as do other nonnatives or because garlic mustard creates an unfavorable site for native species. Soil seed bank data and continued monitoring of the sites will help estimate future plant communities at the monitoring sites.

METHODS

Soil samples were collected in September, 2005 from the seven Minnesota monitoring sites which had been established by that time (Table 1). A soil corer was used to collect soil cores 8 cm in diameter by 5 cm deep (surface area = 50 cm^2, soil volume = 251 cm^3). The monitoring plots were laid out along transects, so the soil samples were collected adjacent to those same transects. Along each transect, a soil sample was collected every 5 m. A total of 40 soil samples were collected at each site (total surface area = 2011 cm^2, total soil volume = 10,053 cm^3).

Upon collection, the soil samples were placed in a cold room (2 to 4 °C) for four months to stratify the seeds. The soil sieving method of Ter Heerdt et al. (1996) was then used to reduce the bulk of the soil. Soil was sieved through a coarse (4.0 mm) sieve to remove bulky material and through a fine sieve (0.212 mm) to retain the seeds and fine soil. The remaining soil and seed mixture was spread into flats in the greenhouse. The soil was spread thinly on top of sterilized potting soil and thin layer of sand. The trays were kept moist. As seedlings emerged their identity was recorded. Once a seedling was identified it was pulled. Unidentifiable seedlings were transplanted to additional pots and grown until identification could be made. Counts were made weekly until there was no additional germination for three weeks. At this point the seed bank soil was mixed to bring any additional seeds to the surface. The flats were then monitored until there was no additional germination for three weeks. Only germinable seeds were counted, there were no additional searches or counts of non-germinable seeds.

Data was summarized by site. The number of individuals of each species was totaled to give a seed bank profile for each site. The species were also categorized as native, nonnative, or unknown (species could not be positively identified). The richness and population density of native and nonnative species seed banks were determined.

RESULTS

There was a germinable seed bank at all 7 sites, but the number of seeds found from the 10,053 cm^3 of soil per site ranged from only 6 seeds at Luce Line to 85 seeds at Hilloway Park (Table 2). Luce Line, Baker Park, Fort Snelling, and Nerstrand all had very few seeds germinate from soil collected (12 or fewer individuals, Table 2). *Pilea* sp. was the most common species to germinate. It had the greatest number of seeds and was found at six of the seven sites (Table 2). *Pilea* was most abundant at Hilloway Park, where 68 individuals germinated (Table 2). It was also common at Pine Bend and Warner Nature Center.

Seeds of native species were more common than nonnative species, although *Pilea* accounted for most of the individuals (112 individuals, Table 2). Most of the other native species were site specific; eight of the native species only occurred in one site each, and the other three only occurred at two sites (Table 2). Adjusting the data to a seeds/m^2 scale, the native species seed bank ranged from 10 to 402 seeds/m^2 (Table 3). Four of the sites have more native species seeds/m^2 than nonnative species, but three of the sites have more nonnative species seeds than native species seeds (Table 3). Native species seed bank richness was low, ranging from 2 – 5 native species per site (Table 4).

Garlic mustard was the most abundant nonnative species recovered from the soil samples (Table 2). In total, there were 14 germinable garlic mustard seeds recovered from the 70,371 cm^3 of soil collected from the 7 sites. The next most common nonnative

species were *Stellaria media* (8), *Leonurus cardiaca* (6), and *Medicago lupulina* (6) (Table 2). Garlic mustard was present in the seed bank in four of the seven sites (Table 2). *Chenopodium album* was also found in four sites. Other common nonnative species included *Medicago lupulina* (present at 3 sites), *Stellaria media* (2 sites), and *Taraxacum officinale* (2 sites) (Table 2). Pine Bend, Warner Nature Center, and Nerstrand had the highest numbers of nonnative seeds (Table 2). All of the sites, except for Hilloway Park and Luce Line, had greater nonnative species richness than native species richness, but this was generally only 1 additional species (Table 4).

DISCUSSION

A widely differing species composition between seed banks and standing vegetation has been found in forests (Leck et al. 1989). The seeds for many forest herbs are very rare in the seed bank (Leck et al. 1989). Forest seed banks often have seeds from earlier successional species as these species can have widespread seed production and dispersal (Leck et al. 1989). This was likely the case for many of the nonnative species found in the seed bank in our monitoring study. These early successional species produce large numbers of seed, some of which will fall in favorable locations. It was surprising that garlic mustard seeds weren't found in all of the sites, since garlic mustard is so abundant in all of the sites. A more intensive sampling method would likely increase the number of seeds recovered. Additionally, garlic mustard can be difficult to germinate in lab conditions (Baskin and Baskin 1992). This study used germination to determine the seed bank. This method gives an estimate of what is likely to germinate, but can underestimate the total seed bank if there are species that are difficult to germinate (Gross 1990).

There are several sites that may need additional restoration help because of a lack of native seeds in the seed bank. Baker Park, Fort Snelling, Luce Line, and Nerstrand all had low native seed density. However, Nerstrand had one of the higher covers of native species in the above ground vegetation (see monitoring summaries) and so may be able to compensate more quickly than the other three sites. It will be important to continue monitoring. If native species are not recruiting into spaces opened up by reduced presence of garlic mustard, additional restoration efforts may be necessary. It is also possible that there may be a flush of early successional nonnative species if garlic mustard populations are reduced. However, these species may be replaced over time by native species that do better in the forest environment than the more disturbance-oriented species. The nonnative species that were most commonly expressed in the seed bank were generally not species known to dominate forest understories.

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TABLES

Table 1. Identification and latitude and longitude of the garlic mustard monitoring sites from which soil samples were collected in Minnesota in September 2005.

ID	Site Name	City	County	N deg	N min	W deg	W min
BP	Baker Park Preserve	Maple Plain	Hennepin	45	19.356	94	59.667
FS	Fort Snelling State Park	Saint Paul	Ramsey	44	52.373	93	11.634
HP	Hilloway Park	Minnetonka	Hennepin	44	57.552	93	26.098
LL	Luce Line	Long Lake	Hennepin	44	58.441	93	35.137
NE	Nerstrand State Park, Prairie Creek SNA	Nerstrand	Rice	44	21.527	93	5.809
PB	Pine Bend Bluffs SNA	Inver Grove Heights	Dakota	44	47.076	93	1.732
WN	Warner Nature Center	Marine on St. Croix	Washington	45	10.583	92	49.641

Table 2. Number of seeds found per site by species (seeds per 0.2011 m^2 surface area). Soils were sampled at seven garlic mustard monitoring sites in Minnesota in September 2005.

Species	BP	FS	HP	LL	NE	PB	WN	Total seeds	Site occurrences
Native species:									
Desmodium sp.	0	0	0	0	0	0	1	**1**	**1**
Erechtites hieracifolia	0	0	1	0	0	0	0	**1**	**1**
Erythronium albidum	0	0	0	0	2	0	0	**2**	**1**
Eupatorium rugosum	0	1	0	0	0	0	0	**1**	**1**
Galium sp.	0	0	1	1	0	0	0	**2**	**2**
Parietaria pensylvanica	0	0	0	0	0	10	0	**10**	**1**
Pilea sp.	1	0	68	2	1	23	17	**112**	**6**
Pinus strobus	0	0	9	0	0	0	0	**9**	**1**
Ranunculus abortivus	0	0	0	0	0	1	0	**1**	**1**
Sambucus canadensis	0	0	0	1	0	1	0	**2**	**2**
Typha sp.	1	1	0	0	0	0	0	**2**	**2**
Viola sp.	0	0	2	0	0	0	0	**2**	**1**
Total native seeds	**2**	**2**	**81**	**4**	**3**	**35**	**18**	**145**	**7**
Nonnative species:									
Alliaria petiolata	0	0	1	0	6	5	2	**14**	**4**
Chenopodium album	0	1	0	1	2	1	0	**5**	**4**

Leonurus cardiaca	0	0	0	0	0	6	0	**6**	**1**
Medicago lupulina	2	3	0	1	0	0	0	**6**	**3**
Robinia pseudoacacia	0	0	1	0	0	0	0	**1**	**1**
Silene vulgaris	0	1	0	0	0	0	0	**1**	**1**
Solanum nigrum	0	0	0	0	0	2	0	**2**	**1**
Stellaria media	1	0	0	0	0	0	7	**8**	**2**
Taraxacum officinale	0	1	0	0	1	0	0	**2**	**2**
Urtica dioica	0	0	0	0	0	0	1	**1**	**1**
Verbascum thapsus	1	0	0	0	0	0	0	**1**	**1**
Total nonnative species	**4**	**6**	**2**	**2**	**9**	**14**	**10**	**47**	**7**
Unknown	**2**	**2**	**2**	**0**	**0**	**0**	**0**	**6**	**3**
Total seeds	**8**	**10**	**85**	**6**	**12**	**49**	**28**	**198**	

BP=Baker Park, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, WN=Warner Nature

Table 3. The number of native, nonnative, and unknown seeds per site on a seeds/m^2 scale. Soils were sampled at seven garlic mustard monitoring sites in Minnesota in September 2005.

Site	BP	FS	HP	LL	NE	PB	WN
Total native	10	10	402	20	15	174	90
Total nonnative	20	30	10	10	45	70	50
Total unknown	10	10	10	0	0	0	0
Total	40	50	423	30	60	244	139

BP=Baker Park, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, WN=Warner Nature

Table 4. Seed bank species richness per site. Soils were sampled at seven garlic mustard monitoring sites in Minnesota in September 2005.

Site	BP	FS	HP	LL	NE	PB	WN
Native richness	2	2	5	3	2	4	2
Nonnative richness	3	4	2	2	3	4	3

Unknown species richness	1	1	1	0	0	0	0
Total	6	7	8	5	5	8	5

BP=Baker Park, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand,
PB=Pine Bend, WN=Warner Nature

Chapter 3

Competitive and allelopathic effects of garlic mustard

INTRODUCTION

The competitive relationships between native species and garlic mustard (*Alliaria petiolata*) are not well understood. Garlic mustard is able to invade and displace native vegetation, even in relatively undisturbed forests (McCarthy 1997). Garlic mustard's ability to be a strong competitor is a likely reason why native species are displaced when garlic mustard invades (Meekins and McCarthy 1999). In addition, garlic mustard roots exude allelochemicals which can negatively affect native species by decreasing germination rates (Prati and Bossdorf 2004). The active allelopathic compound in garlic mustard is allyl glucosinolate (sinigrin) (Vaugh and Burhow 1999). This study addresses the potential for competition between garlic mustard and native species as well as the role of allelopathy.

Previous studies have had conflicting results on the importance of allelopathic effects of garlic mustard. McCarthy and Hanson (1998) found little evidence that allelopathy impacted other species, but Vaughn and Berhow (1999) pointed out that in McCarthy and Hanson's method was potentially flawed. Prati and Bossdorf (2004) did find evidence of a negative impact on germination due to allelopathy. Previous studies have looked at the impact of garlic mustard allelopathy on standard assay plants (McCarthy and Hanson 1998, Vaughn and Berhow 1999) or *Geum* species (Prati and Bossdorf 2004). In this study we address the effects of allelopathy on species native to Minnesota.

Experiment 1 was designed to determine if the effect of garlic mustard on native species is due primarily to competition or allelopathy and to determine the relative influence of each on native species. Experiment 2 provides information on the recovery potential of sites invaded by garlic mustard. It addresses whether allelochemicals left behind by the garlic mustard could inhibit germination and growth of key native species even after the garlic mustard is removed. If there is little residual effect of garlic mustard in the soil, then it is unlikely that there will be long-term direct allelopathic effects on plant species that attempt to reestablish in sites that had been heavily invaded by garlic mustard.

METHODS

Experiment 1: Growing with garlic mustard

This experiment tests the hypothesis that growing with garlic mustard reduces native species biomass more than growing with a conspecific. We hypothesized that the allelochemicals produced by garlic mustard would give garlic mustard an advantage. To differentiate effects due to competition from effects due to allelopathy, activated carbon was used as a treatment to ameliorate the effect of the allelochemicals (Prati and Bossdorf 2004). Carbon itself has been found to have little effect on plant growth (Ridenour and

Callaway 2000, Inderjit and Callaway 2003). We predicted that native plant biomass would be greatest in pots without garlic mustard regardless of carbon presence/absence and would be the least in pots with garlic mustard and no carbon, since these would experience allelopathic effects (Fig. 1). If allelopathy was a strong mechanism, then carbon addition should counteract the effects, so plant biomass should be similar in pots without garlic mustard and pots with garlic mustard and carbon addition. This amelioration of allelopathic effects would be visible in interaction plots and in ANOVAs with an interaction term.

To test these hypotheses, pots with native species were established in a full factorial, completely randomized block design with the following treatments: 1) no carbon addition or activated carbon added at a 1:50 carbon:soil ratio (Ridenour and Callaway 2000, Siemens et al. 2002) and 2) no garlic mustard present (planted with a conspecific plant instead) or garlic mustard present. To test the impact of carbon addition on garlic mustard itself, pots with the treatment of either no carbon addition or carbon addition were established and garlic mustard sown into them. All pots contained a 2:1 MetroMix 200 potting soil: steamed soil mixture and 15-9-12 (NPK) fertilizer, applied at the label's medium rate of 3.6oz/ft^3 so nutrients would not be limiting.

The response to the treatments was measured by the aboveground biomass of the species of interest. Garlic mustard and five native species were seeded, but only garlic mustard, *Solidago flexicaulis* (zig-zag goldenrod, Asteraceae), and *Thalictrum dioicum* (early meadow rue, Ranunculaceae) germinated in sufficient numbers for data analysis. Plantings of *Geranium maculatum* (wild geranium, Geraniaceae), *Impatiens* capensis (spotted touch-me-not, Balsaminaceae), and *Erythronium albidum* (white trout lily, Liliaceae) were unsuccessful. Pots were overseeded to try to achieve the target number of replicates (10 per treatment). If more than one seed germinated in a pot, additional seedlings were removed. The target number of plants was not achieved, but enough germinated to justify analyzing the results (Table 1). The native species were acquired as seed from Prairie Moon Nursery. Seeds of garlic mustard were collected in July 2005 from Baker Park, Fort Snelling State Park, and Warner Nature Center. All seeds (garlic mustard and natives) were in cold storage (2 to 4 °C) from August 2005 to January 2006.

For each native species there were 2 carbon treatments x 2 garlic mustard treatments x 10 replicates = 40 pots. "Garlic mustard only" pots had 2 soil treatments x 10 replicates = 20 pots. Each pot was 13.5 cm x 13.5 cm x 13.5 cm. After planting, the pots were placed in a greenhouse in a randomized block design. The photoperiod in the greenhouse was 16 hours light/8 hours dark and the temperature ranged from 15 to 21 °C. Pots were watered with hoses every other day to maintain moist soil. The plants were allowed to grow for 11 weeks and then they were harvested. The plants were dried for 48 hours at 60 °C to determine the aboveground biomass of natives and garlic mustard.

ANOVAs were used to analyze the effect of garlic mustard and carbon (roles of competition and allelopathy). The model for the ANOVA was the effect of block, garlic mustard presence/absence, carbon presence/absence, and garlic mustard – carbon interaction on the biomass of the species in question. For each species, the biomass was compared among the treatments. For the pots that contained only garlic mustard, the final biomass of garlic mustard was compared among the soil treatments to determine if the carbon had an impact on garlic mustard growth.

Soils from the greenhouse pots and garlic mustard shoot tissue were analyzed to examine the effects of fertilizer, carbon, and garlic mustard plants on soil properties and the effects of fertilizer and carbon on garlic mustard shoot tissue. Soil was taken from pots that were not seeded to native plants or garlic mustard, but had either 1) no fertilizer and no carbon, 2) fertilizer only, 3)carbon only, or 4) both fertilizer and carbon. Four pots with garlic mustard were also analyzed; all four contained fertilizer, but only 2 had carbon added. The University of Minnesota Research Analytical Lab performed the analyses. Soils were tested to determine pH and macro and micronutrient levels (pH, P, K, Zn, Fe, Cu, and Mn are discussed in the results). Descriptions of soil analysis methods can be found at http://ral.cfans.umn.edu/soil.htm#1. Shoot tissue from garlic mustard plants grown with and without carbon were also analyzed by the University of Minnesota Research Analytical Lab to determine if there were differences in micro and macronutrients due to carbon addition. The lab used the elemental analysis by inductively coupled plasma (ICP) method - dry ashing method (485°C ashing temperature) to determine Al, B, Ca, Fe, K, Mg, Mn, Na, P, and Zn (methods at http://ral.cfans.umn.edu/plant.htm). Four plants grown in carbon and four plants grown without carbon were analyzed. These small samples of soil and shoot tissue were analyzed to alert us to any major impacts of carbon on macro and micronutrients. These analyses were not meant to be an exhaustive study of the effects of carbon, fertilizer, and garlic mustard on soil and shoot properties.

Experiment 2: Growing on soil conditioned by garlic mustard

This experiment addresses the recovery potential of native species seeded in to soils in which garlic mustard had been previously grown. Presence of garlic mustard can inhibit germination of some species through allelopathic chemicals from root exudates, although species tested differed in sensitivity (Prati and Bossdorf 2004). Our experiment addresses the hypothesis that native species will have less aboveground biomass in pots that contained garlic mustard due to allelochemicals. We hypothesized that the natives would grow larger in pots where allelochemicals from garlic mustard are minimized by the presence of activated carbon. Garlic mustard was also re-seeded into soils previously conditioned by garlic mustard to determine if new garlic mustard plants are impacted by soil conditioning of previous garlic mustard plants.

This experiment used a full factorial, randomized complete block design. The treatments were 1) no carbon addition or activated carbon added at a 1:50 carbon:soil ratio (Ridenour and Callaway 2000, Siemens et al. 2002) and 2) garlic mustard never present or garlic mustard previously grown in the pot. As in experiment 1, all pots were 13.5 cm x 13.5 cm x 13.5 cm and contained a 2:1 MetroMix 200 potting soil: steamed soil mixture and 15-9-12 (NPK) fertilizer, applied at the medium rate of 3.6oz/ft^3. The pots were placed in a greenhouse in a randomized block design. As in experiment 1, the photoperiod in the greenhouse was 16 hours light/8 hours dark and the temperature ranged from 15 to 21 °C and pots were watered with hoses every other day to maintain moist soil. In pots with the garlic mustard conditioning, the garlic mustard was allowed to grow for 3 months and then it was removed from the pots by cutting it below the crown. Pots were left un-watered for two weeks to kill any remaining parts of the garlic mustard plant. After this period seeds of native species or garlic mustard were added to the pots. Seeds of *Solidago flexicaulis, Thalictrum dioicum*, and garlic mustard

established. Few *Allium canadense* (wild garlic, Liliaceae) bulbs and *Isopyrum biternaturm* (false rue anemone, Ranunculaceae) seedlings established, so those results are not reported. Plants were allowed to grow for 14 weeks after emergence and then shoots were harvested for aboveground biomass measurements (dried at 60 °C for 48 hours). For each species there were 2 soil treatments (carbon present or absent) x 2 garlic mustard treatments (garlic mustard conditioned soil or no garlic mustard ever present) x 10 replicates = 40 pots. There was enough germination to reach the expected 10 replicates for each treatment for *Solidago, Thalictrum,* and garlic mustard.

ANOVAs were used to analyze the effect of presence of garlic mustard and effect of carbon (potential presence of allelochemicals). As in experiment 1, the model for the ANOVA was the effect of block, garlic mustard presence/absence, carbon presence/absence, and garlic mustard – carbon interaction on the biomass of the species in question. For each species, the biomass response was compared among the treatments. A presence of garlic mustard by presence of allelochemicals interaction would indicate that the allelochemicals have an impact on native species and can affect the plant growth during the recolonization of garlic mustard sites.

RESULTS

Experiment 1: Growing with garlic mustard

None of the species had interaction plots (Fig. 2) similar to the pattern expected if allelopathy was driving the effects of garlic mustard on other species (Fig. 1). The interaction plots for *Thalictrum* showed that biomass varied little in the carbon addition pots with and without garlic mustard, but biomass was greater in the no-carbon pots with no garlic mustard than the no-carbon pots with garlic mustard (Fig. 2A, B). This pattern suggests that carbon addition had a negative impact on *Thalictrum* growth. However, in the ANOVA of *Thalictrum* biomass, there was no statistically significant effect of block ($F_{8,7}=2.30$, P=0.14), garlic mustard presence or absence ($F_{1,7}=3.49$, P=0.10), carbon presence or absence ($F_{1,7}=0.60$, P=0.37), or an interaction between the two ($F_{1,7}=0.36$, P=0.57). Only one *Thalictrum* plant was able to grow in a pot with garlic mustard and no carbon, so statistical tests lack the replication needed to determine significance (Table 1). While the ANOVA did not show statistically significant effects of garlic mustard competition or allelopathy, the fact that only 1 plant was actually able to grow in the garlic mustard/no carbon treatment (versus 4 in the garlic mustard/carbon treatment, and 6 and 8 in the treatments without garlic mustard, Table 1) indicates that allelopathy may hinder the ability of *Thalictrum* to grow with garlic mustard.

Solidago plants were affected by garlic mustard and carbon presence or absence, but the expected interaction (the presence of carbon counterbalancing the negative effect of garlic mustard) was not observed (Fig. 2C, D). In the ANOVA of *Solidago* biomass, garlic mustard presence or absence ($F_{1,25}=5.14$, P=0.03) and carbon presence or absence ($F_{1,25}=8.66$, P=0.007) were both statistically significant. However the interaction between the two ($F_{1,25}=0.01$, P=0.90) was not. There was also no statistically significant

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effect of block ($F_{9,25}$=1.01, P=0.46). *Solidago* biomass was greater in pots where garlic mustard was absent than when garlic mustard was present and when carbon was absent versus when carbon was present (Fig. 2C, D).

Garlic mustard growth was not hindered by carbon addition (Fig. 2E). The ANOVA showed no effect of block ($F_{4,2}$=0.38, P=0.82) or carbon ($F_{1,2}$=0.27, P=0.65). Data analysis was hindered by the small sample size. The lack of carbon effect supports the data that show that carbon does not affect plant growth. Results for all species are summarized in Table 2.

Soil analysis of pots with no plants present showed the degree to which fertilizer increased phosphorus (P), potassium (K) and manganese (Mn) (Fig. 3). Carbon presence resulted in lower levels of P, Mn, zinc (Zn), and copper (Cu) and higher levels of K. Of course, without replication, it cannot be determined if these values are all within the same range of values or if there is a statistically significant difference between them. Iron (Fe) did not vary greatly among pots without plants. Fertilizer tended to decrease pH and carbon did not change this effect. Pots with garlic mustard and fertilizer had similar amounts of P and K, regardless of the presence or absence of carbon (Fig. 3). There was variation in iron among pots with garlic mustard although the variation did not relate to the presence or absence of carbon (Fig. 3). Zn, Cu, and pH did not vary dramatically among the different treatments. The shoot tissue from garlic mustard plants grown without carbon was compared to those grown with carbon (Fig. 4). Sodium (Na) and boron (B) were slightly higher in plants grown with carbon, Mn was slightly lower with carbon, but for most elements there was little difference due to carbon (Fig. 4). Overall, there was no indication that carbon presence reduced any nutrient level so low that it was insufficient to meet the nutrient needs of the plant.

Experiment 2: Growing on soil conditioned by garlic mustard

For *Thalictrum*, there was an effect of carbon presence ($F_{1,27}$=4.97, P=0.03), but there was no effect of previous garlic mustard presence ($F_{1,27}$=0.06, P=0.80) or an interaction between previous garlic mustard presence and carbon ($F_{1,27}$=0.02, P=0.88) (Fig. 5A, B). *Thalictrum* biomass was higher in pots with no carbon. Similarly, for garlic mustard, there was an effect of carbon presence ($F_{1,27}$=14.56, P=0.0007), but there was no effect of previous garlic mustard presence ($F_{1,27}$=0.20, P=0.65) or an interaction between the previous garlic mustard presence and carbon ($F_{1,27}$=0.44, P=0.51) (Fig. 5 E,F). For *Solidago* there was no effect of previous garlic mustard presence ($F_{1,27}$=0.11, P=0.74), carbon presence ($F_{1,27}$=2.5, P=0.12), or an interaction between the two ($F_{1,27}$=0.05, P=0.83) (Fig. 5C, D). Although not statistically significant, *Solidago* biomass tended to be greater in pots with no carbon. Unlike *Solidago* and *Thalictrum*, garlic mustard plants had higher biomass in pots that had carbon (Fig. 5E, F). For all three species, previous soil conditioning by garlic mustard had no impact. Since there was no legacy effect of garlic mustard, there was no interaction with carbon (no amelioration of garlic mustard's impact). Results for all species are summarized in Table 2.

DISCUSSION

Strength of competition with garlic mustard

When plants were grown with garlic mustard, the impact of garlic mustard on those plants were likely due to its impact as a competitor and not from allelopathy. Garlic mustard and carbon treatments had direct effects, but with no interaction of the two treatments, there is little evidence for an allelopathic effect (Table 2). Garlic mustard showed a trend toward decreasing *Thalictrum* biomass. Garlic mustard is likely having an effect on *Thalictrum* through competition, although the increased germination in carbon soils means that allelopathy may potentially play a role. Garlic mustard did decrease *Solidago* biomass, but carbon did not ameliorate that effect, so in this case the effect of garlic mustard on *Solidago* was due solely to competition and not to allelopathy. Competition with garlic mustard is likely having a stronger impact on native plants than any direct impacts due to allelopathy. This, however, does not exclude the potential for garlic mustard to have strong indirect effects due to allelopathy. Garlic mustard's allelopathic exudates have been found to be detrimental to mycorrhizal fungi in the soil (Stinson et al. 2006, Callaway et al. 2008). Many native species are dependent on mycorrhizae and grow poorly when mycorrhizae populations are suppressed. The importance of allelopathy in garlic mustard is likely through this indirect mechanism of altering soil biota as to be unfavorable for native species (Stinson et al. 2006, Callaway et al. 2008).

Effects of garlic mustard soil conditioning

Growing in soil conditioned by garlic mustard did not cause a decrease in *Thalictrum, Solidago,* or garlic mustard biomass (Table 2). There was little evidence that allelochemicals left behind by garlic mustard had any impact on the growth of the target species. This bodes well for restoration attempts in soils which had garlic mustard. Barring other changes to soil chemistry and soil biota, native plants should be able to grown on soils where the previous presence of garlic mustard may have released allelchemicals. Again, direct allelopathic effects on native plants is likely less important than potential negative impact of allelochemicals on mycorrhizae. If allelochemicals from garlic mustard have degraded the native soil biota then native species dependent upon that soil biota may have difficulty reestablishing in sites with garlic mustard infestations (Stinson et al. 2006, Callaway et al. 2008). Our study used greenhouse soils and was not designed to test this indirect mechanism of impact on native species.

Effects of carbon addition

Carbon had a greater effect than anticipated. It was anticipated that carbon addition alone would have little impact. In several cases, carbon addition caused a decrease in biomass, for some it had no effect, and in one case carbon increased biomass (Table 2). It is not clear why this occurred. The limited data collected on soil nutrients indicate that carbon additions weren't dramatically changing the soil nutrients, but the small differences in nutrients such as Mn and P may have had an impact. Carbon may also have altered soil moisture which resulted in the varying impacts on different species and studies (Inderjit and Callaway 2003).

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TABLES

Table 1. The number of replicates of each treatment for each species in experiment 1 (growing with garlic mustard or a conspecific). The goal was 10 replicates per treatment. Treatments varied in the presence or absence of garlic mustard (GM) and carbon (C).

	GM absent C absent	GM absent C present	GM present C absent	GM present C present
Thalictrum	6	8	1	4
Solidago	9	9	10	10
Garlic mustard	NA	NA	6	2

Table 2. Summary of effects of the garlic mustard and carbon treatments on the test species in experiments 1 and 2. Garlic mustard presence caused a decrease in *Solidago* biomass, but did not affect *Thalictrum* in experiment 1. Conditioning soil with garlic mustard did not have an impact any of the species in experiment 2. Carbon presence tended to decrease biomass of *Solidago* in both experiments. Carbon presence decreased *Thalictrum* biomass, but increased garlic mustard biomass in experiment 2. There was no evidence for a garlic mustard – carbon interaction in either experiment.

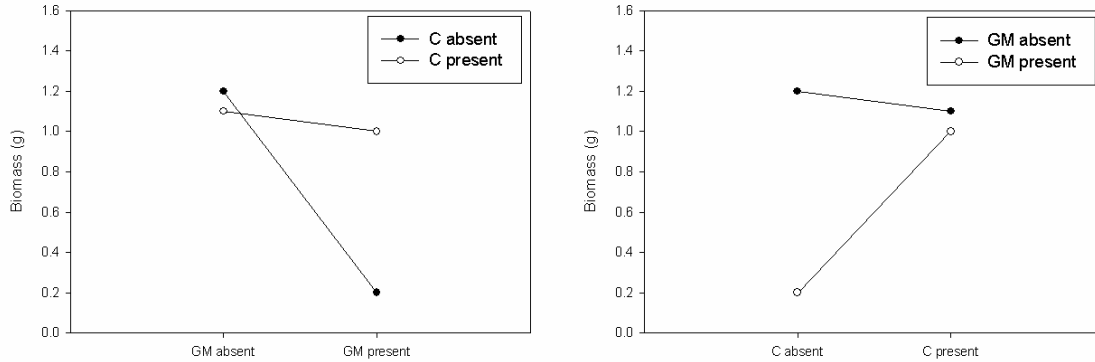|	Garlic mustard effect	Carbon effect	Garlic mustard - carbon interaction
Experiment 1: Growing with garlic mustard			
Thalictrum	decrease biomass (trend in graph)	none	none
Solidago	decrease biomass*	decrease biomass*	none
Garlic mustard	NA	none	none
Experiment 2: Growing on soil conditioned by garlic mustard			
Thalictrum	None	decrease biomass*	none
Solidago	None	decrease biomass (trend in graph)	none
Garlic mustard	None	increase biomass*	none

*= P value <0.05 in ANOVA

Figure 1. Hypothetical interaction plots if garlic mustard has an allelopathic effect on test plant biomass that is ameliorated by the addition of carbon. In the absence of carbon, plants have lower biomass when grown with garlic mustard. By examining the biomass of the test plant in the presence of carbon it can be determined whether this is due to either competitive or allelopathic effects. If the biomass of the test plant when grown with garlic mustard is greater when carbon is added (as pictured in the figure), then this indicates that the carbon is ameliorating the effect of the garlic mustard allelopathic chemicals. If carbon had little impact on test plant biomass, and biomass was less in the presence of garlic mustard irrespective of carbon, then that would indicate that the impact of garlic mustard is more heavily due to competition and not allelopathy. The pictured interaction plot is a hypothetical example of results that would support an allelopathic effect of garlic mustard. The numbers are made-up to demonstrate the pattern and to show that some variation in biomass is expected (for example, biomass would not be *exactly* the same between the garlic mustard absent pots with and without carbon due to normal variations in plant growth among the replicates).
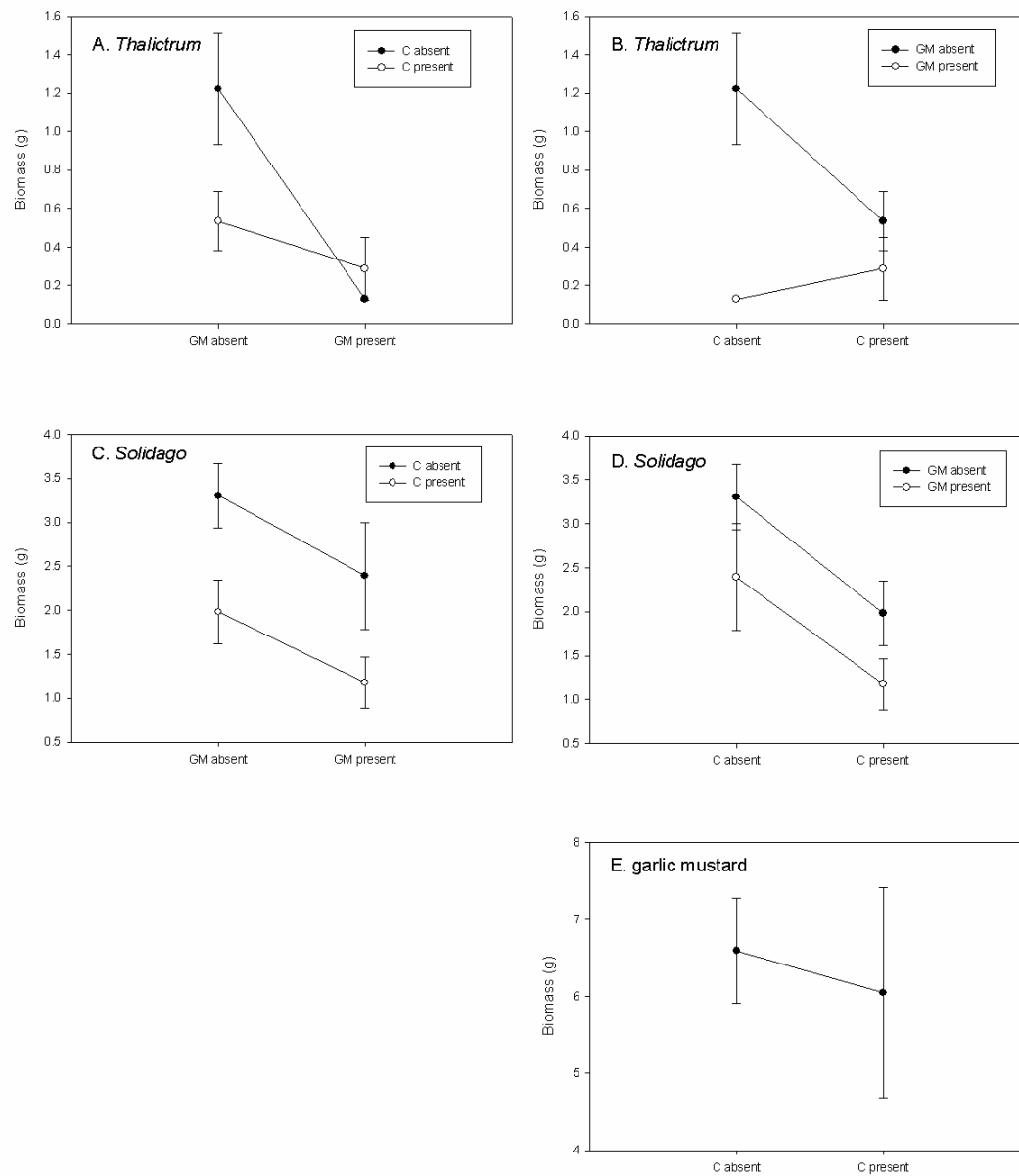
Figure 2. Experiment 1 (growing with garlic mustard) results: Interaction plots for *Thalictrum dioicum* (A,B), *Solidago flexicaulis* (C,D), and garlic mustard (E). Test plants were grown in the greenhouse for 11 weeks, growing with or without garlic mustard (GM) and with or without carbon (C). Plants were then harvested and the dry weight of their aboveground biomass was determined. Mean biomass of the shoots are graphed along with their standard error.
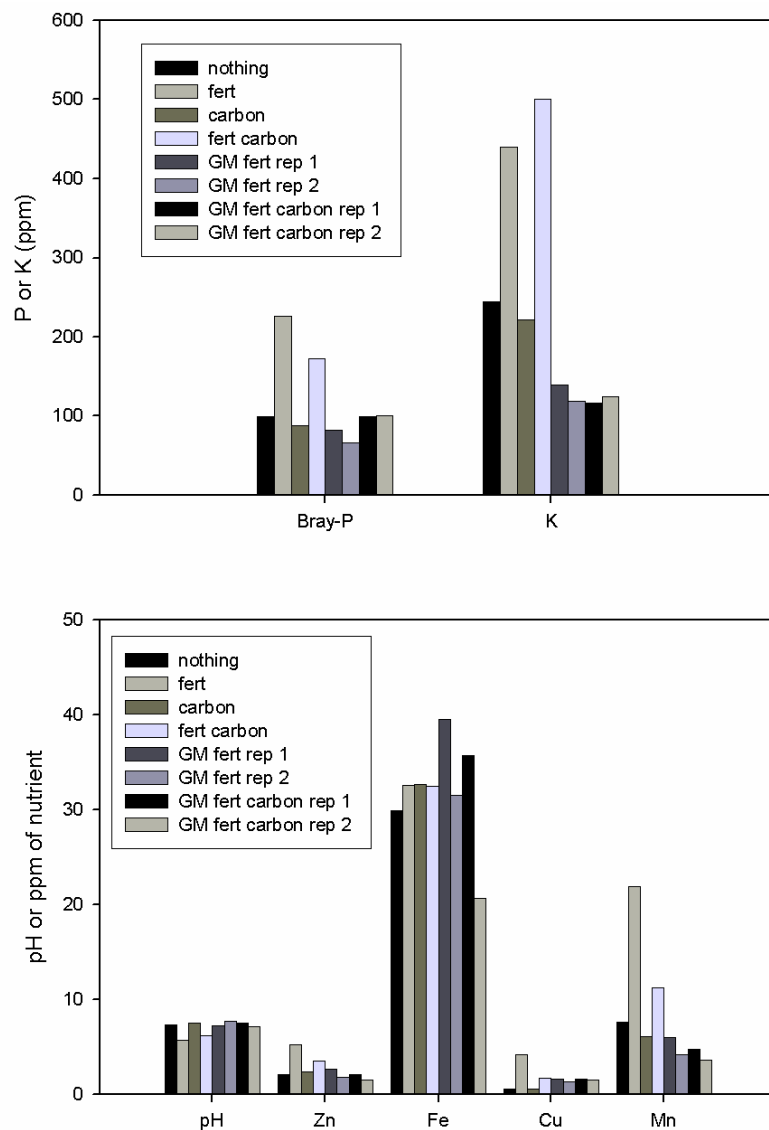
Figure 3. Properties of soils from pots in garlic mustard greenhouse experiments. The first four soils were from pots with no plants growing in them: 1) nothing additional added, 2) fertilizer added (fert), 3) carbon added, and 4) fertilizer and carbon added. The second four soils were from pots in which garlic mustard (GM) had grown for 3 months. There were two replicates of garlic mustard soils with fertilizer only and two replicates of garlic mustard soils with fertilizer and carbon present. All soils had been watered every other day for 3 months. After 3 months the soils were collected and the analyses performed.
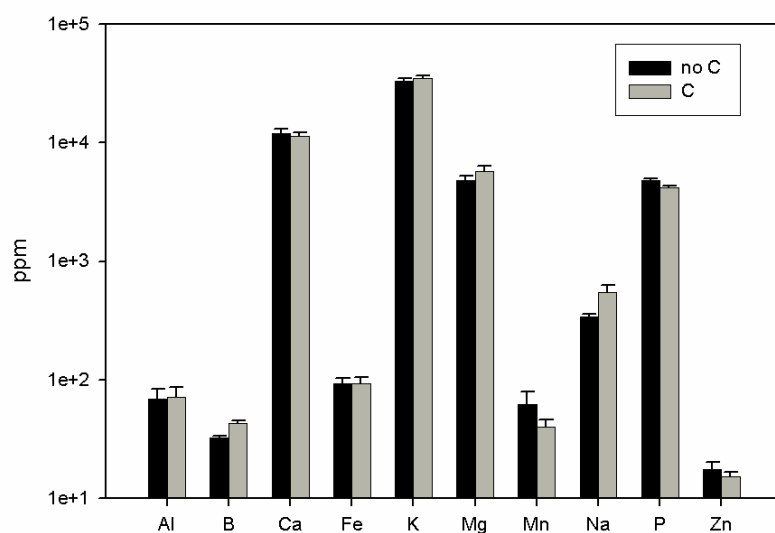P=phosphorus, K=potassium, pH, Zn=zinc, Fe=iron, Cu=copper, Mn=manganese

Figure 4. Analysis of garlic mustard shoot tissue. Garlic mustard plants had been grown in pots either with or without carbon (C) present. Plants were grown in the greenhouse for three months. Shoot tissue was analyzed to determine nutrient levels (note the log scale).
Al=aluminum, B=boron, Ca=calcium, Fe=iron, K=potassium, Mg=magnesium, Mn=manganese, Na=sodium, P=phosphorus, Zn=zinc
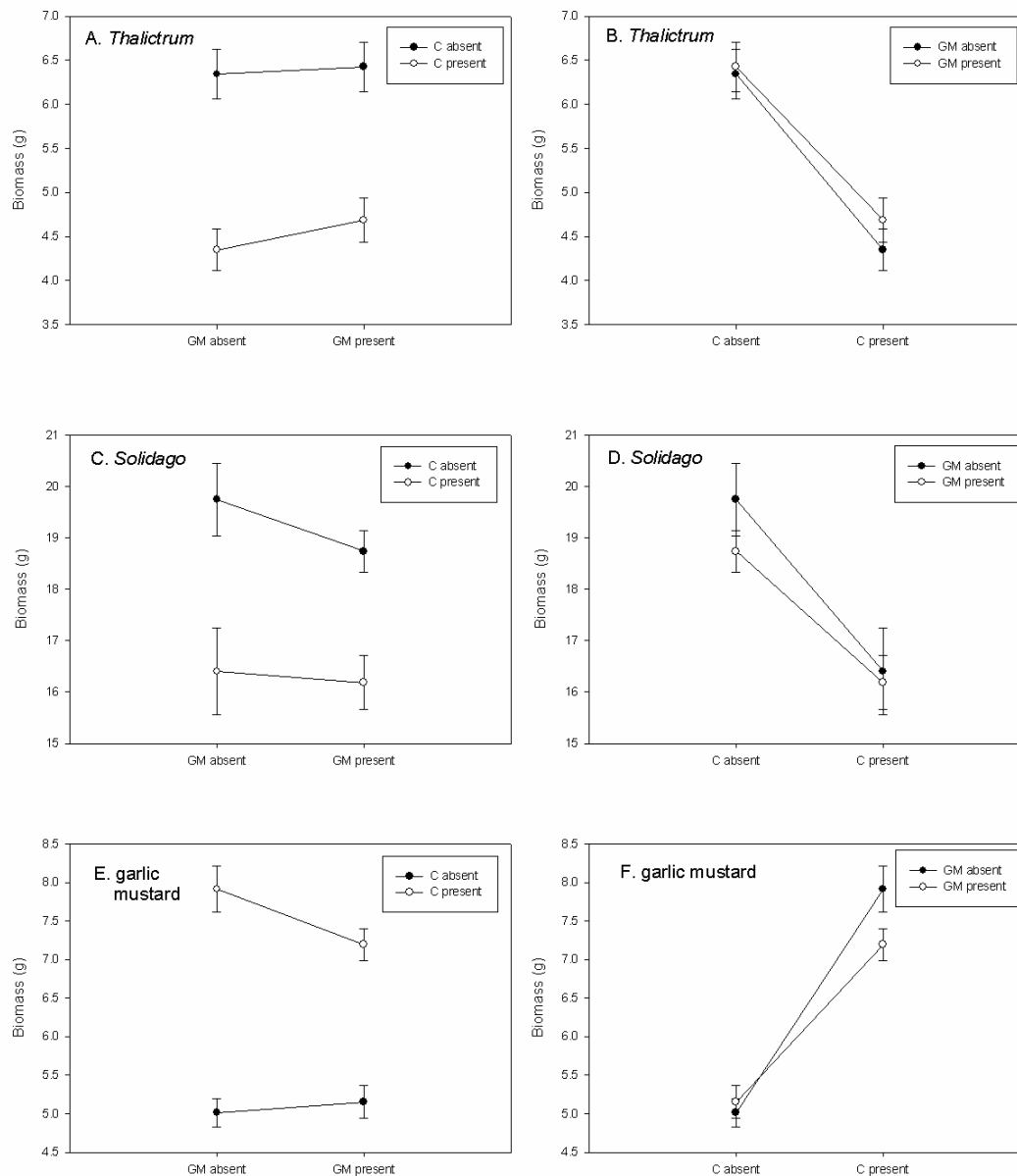
Figure 5. Experiment 2 (growing on soil conditioned by garlic mustard): Interaction plots for *Thalictrum dioicum* (A,B), *Solidago flexicaulis* (C,D), and garlic mustard (E, F). Half of the soils were conditioned by having garlic mustard grow in them for 3 months (GM present) and the other half of soils received the same amount of water, light, etc. for three months, but had no garlic mustard (GM absent). For each garlic mustard treatment, half of the pots had carbon (C) present and in half carbon was absent. After 3 months, garlic mustard was removed and the test plants (*Thalictrum*, *Solidago*, and garlic mustard) were seeded in to the soils with the 4 treatments. Test plants were grown in the greenhouse for 14 weeks. They were then harvested and the dry weight of their aboveground biomass was determined. Mean biomass of the shoots are graphed along with their standard error.