

# Forest Health Technology Enterprise Team

TECHNOLOGY  
TRANSFER

*Biological Control*

## PROCEEDINGS: SYMPOSIUM ON THE BIOLOGY, ECOLOGY, AND MANAGEMENT OF GARLIC MUSTARD (*ALLIARIA PETIOLATA*) AND EUROPEAN BUCKTHORN (*RHAMNUS CATHARTICA*)



LUKE C. SKINNER, EDITOR

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Cover photo. Clockwise from upper left: *C. alliariae*, *Oberea pedemontana*, patch of *Alliaria Petiolata* (garlic mustard), closeup of *Alliaria Petiolata*, closeup of *Rhamnus cathartica* (buckthorn), *Rhamnus cathartica* under leafless canopy, *C. scrobicollis*.

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ECOLOGY, AND MANAGEMENT OF GARLIC MUSTARD  
(*ALLIARIA PETIOLATA*) AND EUROPEAN BUCKTHORN  
(*RHAMNUS CATHARTICA*)**

UNIVERSITY OF MINNESOTA, ST. PAUL CAMPUS  
ST. PAUL, MINNESOTA

For additional copies of this publication, contact:

<b>Richard Reardon</b> FHTET, USDA Forest Service 180 Canfield Street Morgantown, WV 26505 304-285-1566 rreardon@fs.fed.us	<b>Luke C. Skinner, Ph.D.</b> Minnesota Dept. of Nat. Resources 500 Lafayette Road, Box 25 St. Paul, MN 55155-4025 651-297-3763 luke.skinner@dnr.state.mn.us
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# INTRODUCTION: ABOUT THE SYMPOSIUM

**Luke C. Skinner**

Minnesota Department of Natural Resources  
500 Lafayette Road, Box 25 St. Paul, MN 55155

Invasive species are considered one of the most significant threats to biodiversity in the United States, potentially impacting about half of all rare species. Invasive plant species such as common buckthorn (*Rhamnus cathartica*), glossy buckthorn (*Frangula alnus*), and garlic mustard (*Alliaria petiolata*) are species that are invading forests throughout the Midwestern and Northeastern United States. As concerns rise over the impacts of these invasive species, resource managers and researchers need to understand the scope of potential impacts to plant and animal communities, as well as develop safe and effective management tools.

The purpose of this symposium was to get researchers and resource managers together for two days of discussions on the biology, ecology and management of garlic mustard and invasive buckthorn, with an emphasis on using classical biological control as a long-term management strategy. One day was devoted to each species. Our goal was to provide a forum where the presentations from subject experts introduced topics, gave a general overview, and ended with the most current understanding of the problems. Each day, nearly three hours were set aside for discussion of the management requirements and future research needs for each species.

Presenters for this symposium were chosen because of their current involvement in research or management of garlic mustard and buckthorn. More than 80 people from 11 different states attended the symposium with participants from local, state and federal agencies, universities, and private organizations. Three speakers came from CABI Bioscience Center in Switzerland, where overseas work is focused on biological control for garlic mustard and buckthorn; the rest came from various universities and government agencies.

The garlic mustard presentations focused primarily on issues related to the development of biological control, including host specificity testing, protocols development for monitoring future biological control releases, and ecosystem impacts. The buckthorn presentations were evenly split between talks on the biology and impacts of buckthorn, and management strategies, including the development of biological controls.

Development of biological control for garlic mustard is near completion. If favorable results from the remaining host specificity tests are obtained, we plan to pursue a permit from the USDA for introduction of the first control agent in Spring 2006. Our hope is to implement a sustainable 5-year national biological control effort against garlic mustard.

It will take more time to determine the full potential of a biological control for the two buckthorn species. Currently, the primary effort is to carry out host-specificity tests on the

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highest potential candidates. Several more years for research at CABI are planned to identify potential agents and to begin preliminary host range testing.

Symposium speakers were asked to provide summaries or short papers of their presentations and discussion points. Although the summaries may be brief, they provide state-of-the-art information related to impacts and management of garlic mustard and buckthorn. Thus, you will find these proceedings a valuable reference on the biology and management and the potential for biological control of garlic mustard and common and glossy Buckthorn.

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# SESSION 1: GARLIC MUSTARD (*ALLIARIA PETIOLATA*)

## ECOSYSTEM IMPACTS OF *ALLIARIA PETIOLATA* (GARLIC MUSTARD)

Bernd BLOSSEY<sup>1</sup>, Victoria NUZZO<sup>2</sup>, John MAERZ<sup>1</sup>, and Andrea DAVALOS<sup>1</sup>

<sup>1</sup>Department of Natural Resource, Fernow Hall, Cornell University, Ithaca, NY 14853

<sup>2</sup>Natural Area Consultants, 1 West Hill School Road, Richford, NY 13835

Invasive plants are rapidly invading North American ecosystems. Garlic mustard (*Alliaria petiolata*) (Fig. 1a) is one of the few herbaceous plants able to invade intact interior forests, and is associated with the decline of many native understory species. The rapid spread and the extensive near-monospecific stands (Fig. 1b) of garlic mustard in northeastern and mid-western forests constitute an enormous management challenge; in attempting to control the spread of the species land managers use hand pulling, herbicide and fire. However, despite widespread and aggressive control efforts the ecosystem impacts of garlic mustard on associated plant and animal communities are poorly documented (Blossey et al. 2001). Among the few documented negative effects are observations that native *Pieris* butterfly species lay eggs on garlic mustard, but larvae are unable to complete development, indicating that garlic mustard is an ecological trap and constitutes a population sink for these butterflies. (Chew 1980, Porter 1994), are.

In an attempt to better understand the ecosystem impacts of garlic mustard invasion in northeastern forests, we studied the responses of plants, invertebrates, and salamander populations to the invasion of garlic mustard.

The first study used two forests in the Fingerlakes Region of central New York to assess the impact of garlic mustard on ground beetle communities and their associated prey (Dávalos

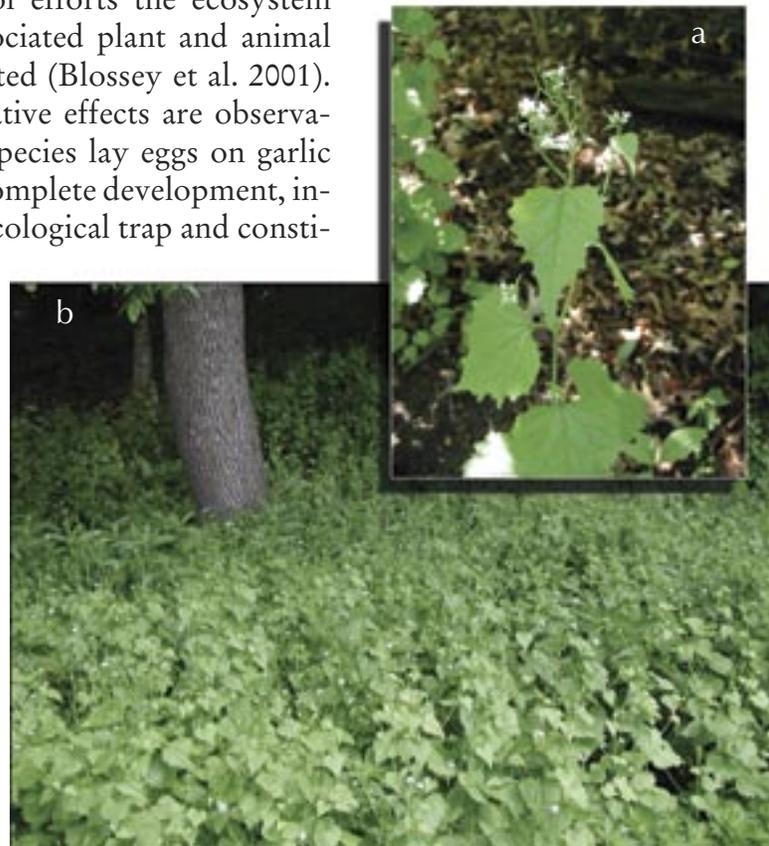


Figure 1. Garlic mustard (*Alliaria petiolata*).

and Blossey 2004). We compared carabid captures and species richness, and invertebrate abundance in invaded and non-invaded areas, and used geostatistical techniques to model ground beetle distribution and determine spatial correlation among carabids, garlic mustard, ground beetle prey availability, understory plant species richness, and litter depth. We determined that garlic mustard invasion had no effect on carabid captures and species richness, on plant species richness, or on invertebrate abundance, but did reduce litter depth in invaded areas. Ground beetles were spatially correlated, i.e. they preferred certain areas of our research plots, but their distribution was independent of garlic mustard and all other environmental factors we recorded. In the two forests we investigated, absence or presence of garlic mustard had no detectable influence on populations of organisms we investigated.

In the second study, we used 15 forests distributed south from Syracuse, New York, to just north of Philadelphia, Pennsylvania, in an attempt to assess ecosystem impacts of garlic mustard, Japanese stiltgrass (*Microstegium vimineum*) and Japanese barberry (*Berberis thunbergii*) on plant, invertebrate, and salamander populations (Maerz et al., unpublished ms). We worked at invasion fronts to avoid confounding factors of differences in prior land-use history, site conditions, etc., between invaded and non-invaded forests. We determined that across invasion fronts, garlic mustard did not have a measurable impact on species richness. In fact, species richness was higher in plots invaded by the species, suggesting that resource availability and not competition was the main variable determining plant species abundance. Similar to the first study, leaf litter depth was reduced in areas invaded by garlic mustard, and this effect was correlated with increased earthworm abundance in plots invaded by garlic mustard. All earthworm species in the study region are introduced (Bohlen et al. 2004) and their feeding greatly reduces the leaf litter layer. There appears to be a synergistic effect of invasive plant and earthworm feeding, as leaf litter depths were always lower in plots invaded by garlic mustard. We found no effect of garlic mustard on salamander populations. However, earthworms indirectly reduced the abundance of invertebrates resulting in a population decline of salamanders due to reduced leaf-litter volume.

The interactions of invasive plant and earthworms are not fully understood. We have many areas where invasive earthworms exist in the absence of invasive plants, but the reverse was never found for garlic mustard, suggesting that earthworms act as ecosystem engineers (Jones et al. 1997) and that garlic mustard appears to be a symptom of soil invasions and not the causal agent for changes in forest food webs. However, we also observed that native plants can thrive in the presence of earthworm invasions (Nuzzo and Blossey, unpublished data), suggesting that additional factors may be responsible for the observed declines of native understory species. The most likely contributing factor to the demise of native plant communities appears to be overabundance of white-tailed deer that threaten long-term survival of many native species (McGraw and Furedi 2005).

The third study was the result of observations that the vigor of garlic mustard at an invaded site declines over time; successive generations of plants become smaller with reduced seed output (Blossey and Nuzzo, unpublished data). Using transplant experiments, sterilization and fertilization of soil from across an invasion front (no garlic mustard present, recently invaded, and invaded for several years) spanning 10-15 m in a forest in Richford, New York, we found that the of accumulation of soil pathogens is most likely the cause of the decline in garlic mustard vigor. Over time, soil pathogens reduce garlic mustard vigor however, but

this effect is not obvious where control efforts remove garlic mustard. The decline in garlic mustard vigor does not result in an increase in native plant species richness or cover. Therefore, more experiments are needed to elucidate the interactions among soil microbials, garlic mustard, and other native plant species.

Overall, we were unable to find negative ecosystem effects of garlic mustard on the organisms we studied in the forests in our region. There is strong evidence that garlic mustard is a symptom of larger, but less visible, invasions by non-native earthworms. However, garlic mustard may constitute a long-term threat to existence or recovery of native plant communities that are threatened by deer herbivory through accumulation of soil pathogens. Native plant communities and the organisms associated with them are threatened by multiple factors. Thus, the implementation of a biological control program should reduce the direct threat of garlic mustard and its site occupancy. However, only a change in the overall management approach that includes developing possibilities to reduce both earthworm abundance and deer herds will allow native plant communities to recover. Implementation of biological control alone cannot accomplish this recovery.

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# BIOLOGY, IMPACT AND INTERACTIONS OF POTENTIAL BIOCONTROL AGENTS ON GARLIC MUSTARD

Esther GERBER and Harriet L. HINZ

CABI Bioscience Switzerland Centre, Rue des Grillons 1  
CH-2800 Delémont, Switzerland

In April 1998, a project investigating the potential for biological control of the alien invasive, garlic mustard (*Alliaria petiolata*) in North America, was started at the CABI Bioscience, Switzerland, Centre. An initial literature review revealed that 69 phytophagous insect species and seven fungi are associated with garlic mustard in its native range in Europe. Five insect species described as specialists in the literature were prioritized as potential biocontrol agents. Host-specificity tests showed that one, the flea beetle (*Phyllotreta ochripes*) is not specific enough and the species was removed from the list of potential agents. The four remaining species are all weevils in the genus *Ceutorhynchus* (Coleoptera, Curculionidae) and have one generation per year.



Figure 1. *C. constrictus*.



Figure 2. *C. alliariae* Brisout.

*Ceutorhynchus constrictus* (Marsham) (Fig. 1) is widely distributed in Europe and its larvae develop in the seeds of garlic mustard between May and July. Each larva destroys up to three seeds during development. Mature larvae leave the seeds to pupate in the soil, but adults delay emergence until the following year. The two shoot-mining weevils, *C. alliariae* Brisout (Fig. 2) and *C. roberti* Gyllenhal (Fig. 3), are morphologically very similar and occupy nearly identical spatial and temporal niches on garlic mustard. They differ only in their oviposition behavior and their geographic distribution. Larvae develop in the shoots of bolting plants of garlic mustard and in the petioles of rosettes from March to May. Both species frequently co-occur in locations in Central Europe, and up to 100% of plants can be attacked. Larvae of *C. scrobicollis* Beschreiber (Fig. 4) mine in the petioles, growing points, and root crowns of garlic mustard rosettes over winter. Adults emerge during May and June, aestivate during summer, and females start to lay eggs around mid September. Oviposition continues throughout the winter and under favorable conditions, until the next spring. Attack rates in the field are again high; up to 100% of plants can be attacked and up to 49 development stages (eggs and larvae) were found in one plant

(Fig. 5).

In a series of manipulative experiments we investigated the effectiveness of the two shoot miners and the root feeder in damaging garlic mustard and their potential interactions.

Competitive interactions can influence establishment of biological control agents and ultimately the overall impact on a target weed. In the worst case scenario, the more effective agent but inferior competitor, is displaced by a more competitive but less effective agent, thereby reducing the overall impact on the target weed. Our objectives were to make predictions on the effectiveness of the selected agents and make recommendations for an optimal release strategy to achieve a maximum suppression of garlic mustard with a minimum number of exotic insect species introduced.

A first experiment conducted with the two shoot miners *C. alliariae* and *C. roberti* revealed strong inter-specific competition between the two species (as expected from their high niche overlap). However, competition between the weevil species was symmetrical and equally strong as intra-specific competition. Similarly, their negative impact on garlic mustard, i.e. reduction of plant height and seed production, was equally strong. In conclusion, potentially both species could be released together without negative impact on the suppression of garlic mustard. However, our results also indicate that no cumulative effects should be expected if the two species were released together.

In a second experiment, we manipulated level and timing of attack by the root-mining weevil *C. scrobicollis* and recorded the effect on performance on potted plants of garlic mustard. Attack by *C. scrobicollis* increased mortality of *A. petiolata*, delayed the onset of reproduction, reduced seed production, and changed plant architecture, i.e. plants attacked by *C. scrobicollis* produced more shoots but these were reduced in base diameter and height. We think this change in plant architecture could negatively influence the subsequently attacking shoot-miners. Therefore, we conducted a third experiment in which we investigated the interaction between the two temporally and spatially separated *C. scrobicollis* and *C. alliariae*. As expected, due to its earlier phenological appearance on the plant, *C. scrobicollis* was not influenced by *C. alliariae*. However, overall we also found no negative impact of *C. scrobicollis* on *C. alliariae*. In



Figure 3. *C. roberti* Gyllenhal.



Figure 4. *C. scrobicollis* Bescherer.

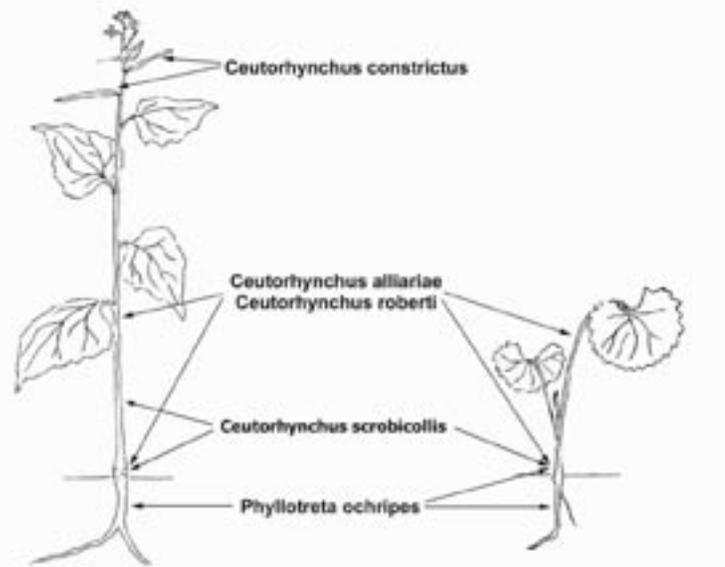


Figure 5. Locations of attack by potential control agents on garlic mustard.

Therefore, we conducted a third experiment in which we investigated the interaction between the two temporally and spatially separated *C. scrobicollis* and *C. alliariae*. As expected, due to its earlier phenological appearance on the plant, *C. scrobicollis* was not influenced by *C. alliariae*. However, overall we also found no negative impact of *C. scrobicollis* on *C. alliariae*. In

general, when releasing the same number of adults of each species, the effect of *C. scrobicollis* on garlic mustard was stronger than the impact of *C. alliariae*.

In conclusion, results indicate that all three potential agents could be released together, provided they prove adequately host specific. However, no significant interactions between the species pairs were found and consequently, we do not expect a cumulative effect in regard to their impact on garlic mustard. From our pre-release studies we predict that *C. scrobicollis* will be the most effective agent, followed by the two stem miners, followed by the seed feeder. These predictions should be used as a basis to carefully plan well-designed release experiments, in which each individual and different combinations of all four agents should be released. The outcome will verify or contradict our predictions and thereby provide important insights into the usefulness of pre-release studies (on interaction and impact of future biological control agents) to improve the success rate and credibility of biological weed control programmes.

# PREDICTING GARLIC MUSTARD BIOCONTROL AGENT SUCCESS WITH DEMOGRAPHIC MODELING

Douglas A. LANDIS<sup>1</sup>, Adam S. DAVIS<sup>2</sup>, and Douglas W. SCHEMSKE<sup>3</sup>

<sup>1</sup>Department of Entomology, Michigan State University  
204 CIPS, East Lansing, MI 48824

<sup>2</sup>Current Address: USDA-ARS Invasive Weed Management Unit  
N-319 Turner Hall, UIUC, 1102 S. Goodwin Ave., Urbana, IL 61801

<sup>3</sup>Department of Plant Biology, Michigan State University  
166 Plant Biology, East Lansing, MI 48824

## INTRODUCTION

Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara and Grande) (Brassicaceae) is a widespread invasive plant considered to be one of the most harmful exotic invasive plants in North America. A native of Europe, *A. petiolata* invades forested communities where it can displace native herbaceous flora, compete with timber species regeneration, alter litter layer depth and composition, impact mycorrhizal associations, all of which can result in cascading ecosystem impacts (Meekins and McCarthy 1999, Blossey et al. 2001). Extensive work on conventional controls has failed to yield practical methods for large-scale suppression of garlic mustard, and biological control is viewed by many as the only effective means to manage this plant at landscape scales (Blossey et al. 2001). However, there is increasing awareness of the non-target impacts of some weed biological control efforts (Louda et al. 1997, Callaway et al. 1999). While recognizing the importance of biocontrol, there has been a call to increase the scientific rigor of these programs (Louda et al. 2003) and to assure that biocontrol agent guilds have strong impacts on target plants (Pearson and Callaway 2003, Thomas et al. 2004, Pearson and Callaway 2004). The challenge for invasive plant biologists is to predict if the likely impacts of either a single biocontrol agent or guild of agents are sufficient to produce adequate target suppression across a range of habitats and geographic areas and if so, to determine if suppression is sufficient to justify the risks (if any) of introduction(s).

In this regard, plant demographic analysis and matrix modeling have great promise to improve the efficiency, efficacy, and safety of biological control of weeds (Shea and Kelly 1998, Rees and Hill 2001, McEvoy and Coombs 1999, Meyers and Bazely 2003). By determining the level of mortality required to suppress, halt, or reverse weed populations, demographic models can provide estimates of the likelihood that single or multiple biocontrol agents will be successful in different habitats and regions (Parker 2000). While these techniques have

great potential, they have not been used in a predictive fashion to inform biocontrol agent selection.

## DISCUSSION

In its native range, *A. petiolata* is a component of forest ecosystems but does not form extensive and damaging infestations as it does in North America. In Europe, *A. petiolata* is found in similar habitats as in North America; however, populations are typically scattered and smaller in size. In 1998, work was initiated to investigate the potential for classical biological control of *A. petiolata* in North America. From an initial literature survey, 69 insects and 7 fungi attacking *A. petiolata* were identified (Hinz and Gerber 1998, 2001). Testing is currently focusing on several species of *Ceutorhynchus* weevils. Adult *C. alliariae* and *C. roberti* feed on leaves and the larvae mine in stems and leaf petioles. Each of these two species exhibits very high attack rates in the field (generally >80% of *A. petiolata* infested) and primarily impact *A. petiolata* by reducing seed set. Larval *C. scrobicollis* mine in rosette meristems and root crowns resulting in rosette death (hereafter called rosette feeding). This species also exhibits high attack rates (50-100%) in the field, with affected plants appearing water stressed and subject to premature desiccation. The remaining two species, *C. constrictus* and *C. theonae* are seed feeders that lay eggs directly in siliques (Gerber et al. 2002). Larvae consume seeds before exiting to pupate in the soil (Blossey et al. 2001).

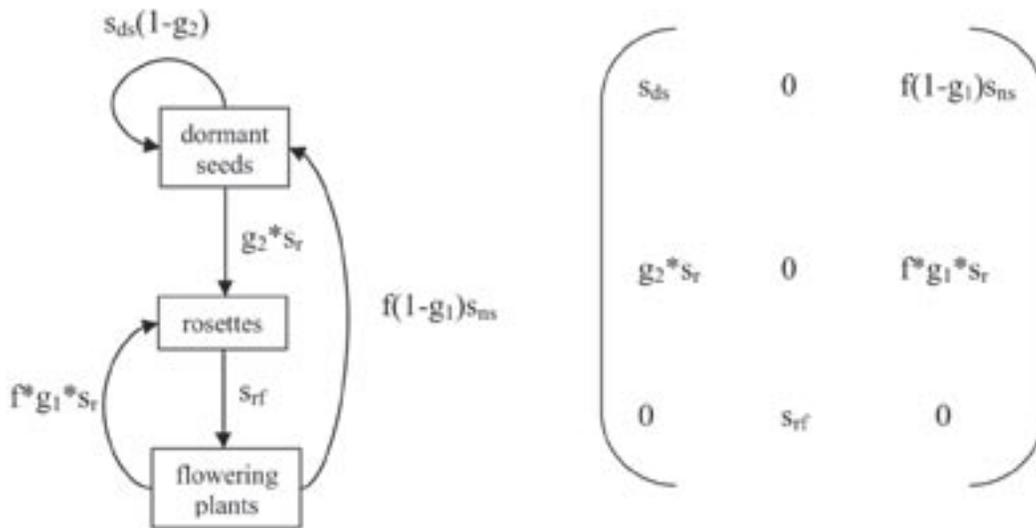


Figure 1. The life cycle of *A. petiolata* represented in a) graphical, and b) matrix format. Abbreviations for demographic parameters are as follows:  $s_{ds}$  = survival of dormant seeds,  $s_{ns}$  = survival of newly produced seeds,  $g_1$  = generation rate of newly produced seeds,  $s_r$  = survival of seedlings to rosette stage,  $s_{rf}$  = survival of rosettes to flowering stage, and  $f$  = fecundity (in seeds plant<sup>-1</sup>).

The demography of *A. petiolata* has been studied in a range of environments (Cavers et al. 1979, Byers and Quinn 1998, Drayton and Primack 1999) yet certain aspects of its life history remain poorly defined. In particular, persistence of seeds in the soil seedbank ( $s$ ) and recruitment of seedlings from newly shed seeds ( $g_1$ ) during the following growing season are variable. We modified previously existing matrix population models of *A. petiolata* (Drayton and Primack 1999, Rejmánek 2000) (Fig. 1) and parameterized it using data from Drayton and Primack (1999) and Cavers et al. (1979). By varying  $s$  and  $g_1$  within the range of variability reported in the literature we explored the implications for the success of *A. petiolata* biological control (Davis et al. in prep).

The results reveal a large impact on *A. petiolata* population growth rates is possible within the observed range of demographic parameters. If first year germination and seed survival are low, rosette mortality alone has the potential to cause declines in *A. petiolata* populations. However, if first year germination rose to 10%, or seed persistence increased to 30% (extreme values in the literature), simulated biocontrol would have little effect on *A. petiolata* population growth rates. This initial series of simulations implies that with currently available demographic data from North America, increasing rosette mortality has the potential to cause a reduction in *A. petiolata* populations. Results of a population projection analysis were somewhat less optimistic, but still suggested that biocontrol of *A. petiolata* is feasible under Michigan conditions. Rosette mortality of 70% was sufficient to signifi-

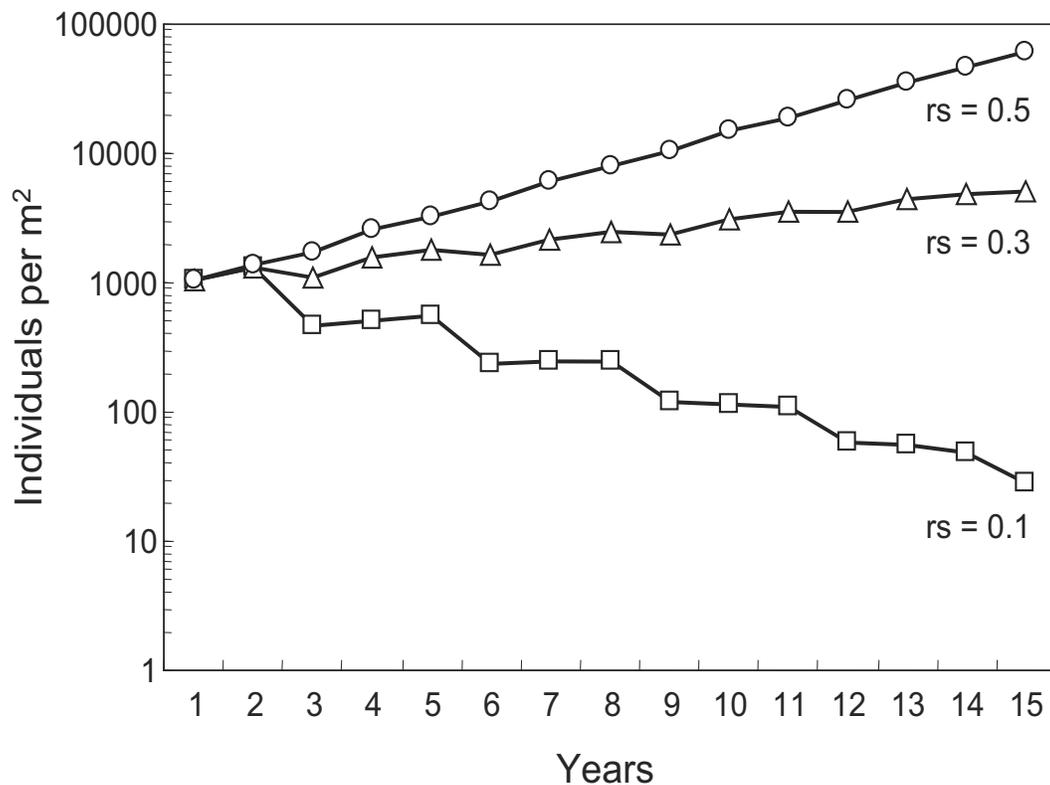


Figure 2. Population projection for *A. petiolata* under conditions typical of North America, with varying levels of rosette survival ( $rs$ ) due to simulated herbivory by a biocontrol agent.

cantly curtail *A. petiolata* population growth (Fig. 2). With 75% or greater rosette mortality, the *A. petiolata* population declined. If *C. scrobicollis* is capable of inducing this mortality under North American conditions, this would be the logical agent to introduce. However, if future testing in Europe suggests that this level of mortality is not realistic, then the joint introduction of a seed-feeding or leaf-feeding insect may need to be considered.

Taken as a whole, our preliminary analyses show that the choice of the ideal natural enemy(s) for introduction (i.e. to reduce rosette survival, seed survival, or both) is both dependent on the actual range of *A. petiolata* demographic variability and the level of agent mortality expressed in the exotic environment. Prior to an actual biocontrol introduction, the latter can only be estimated. However, plant demography is directly measurable and thus, should be the focus of a pre-introduction evaluation program. We are currently in the process of collecting demographic information from a variety of geographic locations in Michigan and from sites that vary in apparent success of *A. petiolata* invasion. With this data in hand, analyses similar to those above will be conducted and used to inform biocontrol introductions.

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# SUMMARY OF HOST-SPECIFICITY TESTING CONDUCTED IN SWITZERLAND AND BRASSICACEAE TAXONOMY

**Hariet L. HINZ and Esther GERBER**

CABI Bioscience Switzerland Centre, Rue des Grillons 1  
CH-2800 Delémont, Switzerland

## INTRODUCTION

In April 1998, a project investigating the potential for biological control of the alien invasive, garlic mustard (*Alliaria petiolata*) in North America, was started at the CABI Bioscience, Switzerland, Centre. After a literature review for insects associated with garlic mustard in Europe and preliminary field surveys, five species were prioritized as potential biological control agents based on records of their restricted host range. Host-specificity tests showed that one, the flea beetle, *Phyllotreta ochripes*, is not specific enough and the species was removed from the list of potential agents. The following summarizes results of host-specificity tests with the remaining four species, conducted since 1999/2000, as well as tests that remain to be done. All four are weevil species in the genus *Ceutorhynchus* that attack different plant parts of garlic mustard.

## SCREENING METHODS

So as to use plant and insect material in the most efficient way, we started host-specificity tests by conducting sequential, no-choice oviposition tests using cut-plant material (leaves or shoots) of test and control (garlic mustard) plants. These were offered to individual females under confined, no-choice conditions (Fig. 1), and the number of eggs laid on each



Figure 1. Testing host specificity of non-target plants under no-choice conditions.

plant species was recorded. Not surprisingly, generally many more plant species are accepted for oviposition under these artificial, confined conditions, than are used as hosts under field conditions. However, even under these conditions, all potential agents investigated only accepted plant species within the family Brassicaceae. Because larvae cannot move between plants—female oviposition determines the potential host range in these weevil species—we were able to exclude any species outside the Brassicaceae as potential host. Subsequently, we conducted no-choice oviposition and development tests using individually pot-

ted test and control plants to investigate whether species accepted for oviposition would also support larval development. All species that support development under these conditions comprise the so-called physiological or fundamental host range of a species. Subsequently, these were exposed in multiple-choice field cages (Fig. 2) or in the open field to determine the so-called ecological host range of a potential agent. This testing sequence, which progressively reduces the degree of restriction by deleting unattacked plants at each stage until only a few remain to be tested under conditions as near natural as possible, has proven a reliable way to determine the safety of potential biological control agents.



Figure 2. Field cage used for multiple choice host-specificity testing.

## STATUS QUO OF HOST-SPECIFICITY SCREENING

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### *Ceutorhynchus constrictus*

Thus far, the seed-feeding weevil *C. constrictus* is the agent with the most restricted host range. Of 54 plant species exposed under sequential, no-choice conditions as/using cut plant pieces, ten (18.5%) were accepted for oviposition, and when these were exposed under single-choice conditions, only one (*Barbarea vulgaris*) was accepted. In no-choice development tests using potted plants, only one of 12 test species exposed, the commercially grown *Brassica nigra* (black mustard), supported development to adult. Although this species was also attacked under single-choice conditions, results of open-field tests conducted in 2003 showed that *B. nigra* is not a normal field host of *C. constrictus*, i.e. no attack at all occurred. In addition, *B. nigra* grows in agricultural fields, exposed to full sun, while *A. petiolata* usually grows in shaded, natural habitats. Also, there is no record of *C. constrictus* as a pest of *B. nigra* in the European literature. According to the original test plant list, 20 plant species need further tests for *C. constrictus*. Development tests with *Brassica oleracea gemmifera* have been hampered so far, because most plants commercially available are sterile hybrids. However, the main problem testing the remaining species is lack of synchrony between the oviposition period of the seed feeder and the reproductive period of test plants.

### *Ceutorhynchus alliariae* and *C. roberti*

For *C. alliariae*, 63 plant species have been offered in sequential no-choice tests using cut leaves, 23 (36.5%) were accepted for oviposition (all within the family Brassicaceae), and

when those were exposed under single-choice conditions, 12 were accepted. In no-choice development tests using potted plants, two of 19 species exposed supported development to adult, i.e. *Nasturtium officinale* (= *Rorippa nasturtium-aquaticum*) and *Thlaspi arvense*.

Because the oviposition behaviour of the sister species *C. roberti* is more sensitive than the one of *C. alliariae*, we have concentrated our efforts on the latter and tests with *C. roberti* are therefore less advanced. Of 40 plant species offered in sequential no-choice tests so far, eleven (27.5%) were accepted for oviposition, and of 18 species tested under single-choice conditions, ten were accepted. As with *C. alliariae*, they are all in the family Brassicaceae. In subsequent no-choice development tests with 22 species, adults emerged from the same two plant species that also supported development of *C. alliariae* (see above), and in addition from *Peltaria alliacea*.

A multiple-choice field cage test established in 2003 had to be repeated in 2004, because of low attack rates. *Nasturtium officinale*, *Peltaria alliacea*, and *Thlaspi arvense* were exposed together with garlic mustard in field cages to both species. While adults of *C. alliariae* emerged from all three test species offered apart from the control, adults of *C. roberti* emerged from only two, *P. alliacea* and *T. arvense*. [However, mining in *N. officinale* could have also been due to *C. roberti* and it is suspected that probably all three species are attacked by both weevils.]. Similar to *C. constrictus*, all three plants generally grow in habitats different from the ones occupied by garlic mustard. *Nasturtium officinale*, for instance, usually grows in and along streams and ditches, and *T. arvense* in agricultural habitats, which makes it unlikely that these species would be encountered by the weevils in nature. In addition, neither *C. alliariae* nor *C. roberti* have been recorded on any of these species in the literature. While *T. arvense* and *P. alliacea* are both European species, it appears controversial as to whether *N. officinale* is indigenous to the US or not (see Gerber et al. 2004 for refs).

Based on the original test plant list proposed for the project, only 10 plant species remain to be tested with *C. alliariae*. Because none of these species are currently available at the Centre or failed to grow sufficiently large to be used, they are planned to be tested under quarantine conditions at the University of Minnesota. With the exception of *Draba reptans*, all other plant species are already grown there. A first shipment of *C. alliariae* to the University of Minnesota is scheduled for April 2005. For *C. roberti*, 29 plant species remain to be tested. However, sometimes only 1-2 replicates are missing. Tests with nine species indigenous to North America are currently being conducted in quarantine.

### *Ceutorhynchus scrobicollis*

In contrast to the other agents, oviposition of *C. scrobicollis* females using cut plant pieces is less specific. Of 73 plant species and varieties exposed in sequential no-choice tests, 35 (48%) were accepted. Three of these were native North American species in families other than Brassicaceae. However, instead of eggs being inserted in plant tissue (normal oviposition behaviour), eggs were laid at the outside of plant material where they dried up prior to hatching (no larvae were produced). In subsequent development tests using potted plants, adults emerged from five of 37 species and varieties exposed, including the three species accepted by the stem miners (*N. officinale*, *P. alliacea* and *T. arvense*). In addition, one adult each emerged from a *Brassica oleracea sabauda* variety and an indigenous *Draba* species.

We exclude *B. oleracea sabauda* as a normal field host, since various varieties were not attacked when exposed in a multiple-choice, field-cage test, and *C. scrobicollis* has never been recorded as a pest of commercially grown *B. oleracea*. The *Draba* species had erroneously been identified as *Draba reptans*. Once the identity of this species is clarified, further tests under single and multiple choice conditions should be established. The other three species were exposed under open-field conditions in October 2004. Whereas *P. alliacea* and *T. arvense* were accepted to a similar degree as garlic mustard, only very few eggs were laid on *N. officinale*. As with the two shoot miners, neither of the three plant species has been recorded as a field host of *C. scrobicollis*; each generally grows in a habitat different from the those in which garlic mustard grows.

Combined with results from previous years and based on the original test plant list proposed for the project, 18 plant species need additional testing for *C. scrobicollis*. However, for some of these only two replicates are missing. Because we were not able to obtain plants or grow them large enough for testing in Switzerland, nine of these are currently tested in quarantine.

## BRASSICACEAE TAXONOMY

Recent molecular analyses revealed that the tribal classification system within the family Brassicaceae no longer holds. This makes the traditional centrifugal phylogenetic approach to the selection of test plant species and host range testing difficult, where species closely related to the target are hypothesized to be at greater risk of attack than species more distantly related.

In addition, the family Brassicaceae has a large number of native congeners; there are 95 native North American genera listed in the family Brassicaceae, 11 of which include U.S. FWS listed T or E species. Therefore, several species, most of them in the family Brassicaceae, were added to the list, which originally only contained six native Brassicaceae species, among them *Peltaria alliacea* and *Thlaspi arvense*, that supported development to adult of three of the four potential agents (see above). Whereas the genus *Peltaria* does not occur in the United States, the genus *Thlaspi* did 'traditionally' include several indigenous North American species. However, based on results of additional molecular analyses, native North American *Thlaspi* species were recently proposed to be summarized in a different genus, *Noccaea*, thus being taxonomically separated from the European *Thlaspi*. Nevertheless, some native *Noccaea* species should be tested to determine whether the host range of the potential biocontrol agents matches the proposed taxonomic separation. The taxonomic position of *Rorippa nasturtium-aquaticum* (syn. *Nasturtium officinale*), which was readily accepted and supported development by the same three potential agents, also appears to be under flux. While the United States Department of Agriculture plant database classifies *Nasturtium officinale* as *Rorippa nasturtium-aquaticum*, Al-Shehbaz and Price (1998) separate it together with four other species in the genus *Nasturtium*. The genus appears to be more closely related to *Cardamine* than to *Rorippa*, and contains two native North American species, *Nasturtium gambelli* and *N. floridanum* (Al-Shehbaz and Price 1998). Although neither of the two species

is present in the eastern U.S., where *A. petiolata* is most prevalent, we suggest testing both species and/or other native *Rorippa* species.

Even if a new phylogeny, based on molecular analyses, were soon to be available (there are several research groups in the U.S. and Europe that currently work towards that goal), evidence demonstrating that the biocontrol agents follow the new classification will be needed. Without additional tests, including at least native *Thlaspi* and *Rorippa* species, it may prove difficult to convince USDA's Technical Advisory Group overseeing biological control about the environmental safety of the proposed biocontrol agents. Also, it might be advantageous to investigate why three of the otherwise host-specific, potential biocontrol agents develop on the same non-target plants. This may be due to the each agent's degree of relatedness to garlic mustard, similar secondary compounds, or other unknown factors. Identifying mechanisms that determine the host choice of potential garlic mustard biocontrol agents could help to demonstrate their environmental safety.

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# HOST RANGE TESTING OF GARLIC MUSTARD (*ALLIARIA PETIOLATA*) BIOCONTROL INSECTS IN MINNESOTA

Elizabeth KATOVICH<sup>1</sup>, Roger L. BECKER<sup>1</sup>, David W. RAGSDALE<sup>2</sup>,  
and Luke C. SKINNER<sup>3</sup>

<sup>1</sup>Department of Agronomy and Plant Genetics  
University of Minnesota, 1509 Gortner Ave  
St Paul, MN 55108

<sup>2</sup>Department of Entomology, University of Minnesota  
1980 Folwell Ave, St Paul, MN 55108

<sup>3</sup>Minnesota Department of Natural Resources  
500 Lafayette Road, Box 25, St. Paul, MN 55155

At the University of Minnesota, we are evaluating the host range of three species of *Ceutorhynchus*, potential biological control insects of garlic mustard: *C. scrobicollis*, a crown-mining weevil, and *C. roberti* and *C. alliaris*, which are stem miners.

The initial objective of our project was to supplement the host range testing efforts by CABI personnel with North American native plants that have been difficult to obtain or propagate in Switzerland (Fig. 1). Test plants included in our initial host range testing are native to North America and include representatives of the following plant families, Brassicaceae, Fabaceae, Cyperaceae, Liliaceae, and Ranunculaceae (Table 1). Plant species included in our host range testing that are not members of the mustard family grow in similar habitats to garlic mustard.

The initial list of Brassicaceae species included in host range studies were selected on the basis of traditional mustard taxonomic classification systems. Recent studies on the molecular systematics of the Brassicaceae reveal that taxonomic subdivisions based on plant morphological characteristics do not reflect actual phylogenetic relationships (Koch et. al 2003). As a result of these findings, additional mustard species that are representative of common genera native to North America, have been included in our host range studies. Additional native mustard species from the following genera have been included: *Iodanthus*, *Physaria*, *Thlaspi*, *Rorippa*, *Draba*, and *Descurainia*.

In Minnesota, all host range testing is conducted under quarantine conditions and includes no-choice, single-choice and larval-development tests as developed by Gerber et al. (2001).



Figure 1. Growing test plants and garlic mustard for host specificity testing at the University of Minnesota.

Table 1. Initial Plant species to be tested in quarantine in Minnesota.

<b>Brassicaceae</b>
<i>Arabis canadensis</i> (sicklepod)*
<i>Arabis shortii</i>
<i>Cardamine bulbosa</i> (bulbous cress)*
<i>Cardamine heterophylla</i> (slender toothwort)
<i>Cardamine diphylla</i> (crinkle root)
<i>Dentaria laciniata</i> (cut-leaved toothwort)*
<i>Iodanthus pinnatifidus</i> (purple rocket)
<i>Physaria acutifolia</i> (sharpleaf twinpod)
<i>Thlaspi alpestre</i> (wild candytuft)
<i>Descurainia pinnata</i> (western tansymustard)
<i>Rorippa sessiliflora</i> (southern yellow-cress)
<i>Draba ventosa</i>
<b>Fabaceae</b>
<i>Amphicarpaea bracteata</i> (Hog peanut)*
<b>Cyperaceae</b>
<i>Carex laxiflora</i> *
<b>Liliaceae</b>
<i>Erythronium albidum</i> (white trout lily)
<b>Ranunculaceae</b>
<i>Ranunculus septentrionalis</i> (swamp buttercup)*
<i>Aconitum noveboracense</i> (northern blue monkshood)*
<i>Anemone canadensis</i> (Canada anemone)*

\*Host range testing completed



Figure 2. Rearing *Ceutorhynchus scrobicollis* in quarantine.

Various plant species were included in larval-development tests to determine whether *Ceutorhynchus* spp. develop on hosts other than garlic mustard.

As of July, 2005, we have established colonies of *C. scrobicollis*, *C. roberti* and *C. alliaria* in our quarantine and host range testing has been conducted with *C. scrobicollis*. Thus far in our host range tests, *C. scrobicollis* has not completed development on any plant species other than garlic mustard. Plant species that have been tested in quarantine in Minnesota at the time of this writing are identified in Table 1 with an asterisk.

In addition to host range testing activities, we are developing procedures to rear *Ceutorhynchus* spp. under quarantine conditions with the goal of establishing and maintaining colonies of each species of weevil (Fig. 2). We have developed a procedure to induce a second oviposition period in *C. scrobicollis* to reduce the time necessary to complete host range testing. To date, we have successfully reared two generations of *C. scrobicollis* in quarantine.

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# MONITORING GARLIC MUSTARD (*ALLIARIA PETIOLATA*) AS PART OF A BIOCONTROL PROGRAM

Victoria NUZZO<sup>1</sup> and Bernd BLOSSEY<sup>2</sup>

<sup>1</sup>Natural Area Consultants, 1 West Hill School Road, Richford, NY 13835

<sup>2</sup>Department of Natural Resource, Fernow Hall, Cornell University, Ithaca, NY 14853

The main question in weed biocontrol programs is whether release of control agents results in the anticipated reductions in target weed populations. Standardized long-term monitoring is the most effective approach for detecting changes in natural communities. Monitoring, defined here as the collection and analysis of repeated standardized measurements (Elzinga et al. 1998), allows identification of changes over time, and permits making inferences about the cause and effect of observed changes. In biocontrol programs, accurately relating presence, abundance, and impact of the biocontrol agent to observed changes in plant populations is critical (Blossey 1999). An apparent decline in a target plant population may be due to the biocontrol agent, climate fluctuations, attacks from other insect species, or other factors, including natural fluctuations in the target plant's population density. Data on abundance of biocontrol insects and target plant populations obtained through long-term monitoring allows investigators to assess the true impact of the biocontrol agents, the response of the target plant to the control agents, and the response of the community. These combined factors then can be used to evaluate the relative success of the biocontrol program, compare predicted to actual impacts, and gain insight into how ecosystems respond to changes in abundance of individual species (Blossey 2003).

Ideally, long-term monitoring is initiated before biocontrol organisms are released. This approach is particularly valuable when the target plant populations, such as garlic mustard (*Alliaria petiolata*), show naturally large abundance fluctuations (Nuzzo 1999). Long-term trends in the populations of target and non-target species are more easily separated from annual fluctuations when the natural population fluctuations in the absence of biocontrol organisms are known.

With the initiation of the biocontrol program targeting garlic mustard, we began development of a standardized monitoring protocol that could be implemented by scientists and resource managers. Standardization allows comparison of data collected by various investigators across the range of garlic mustard. Balancing scientific sophistication with ease of implementation of monitoring increases the ability of non-academic personnel to participate in widespread monitoring efforts, and provides them with a better understanding of the effects of biocontrol releases beyond detailed research sites. The measures proposed in the monitoring protocol were developed based on garlic mustard's biology (Nuzzo 2000), the biology of the four weevils considered as biocontrol agents (Blossey et al. 2001), and the response of garlic mustard to attack by these weevils (Gerber et al., unpublished data).

Briefly, garlic mustard is an obligate biennial that spreads only by seed (Cavers et al. 1979), which germinate in early spring and form a basal rosette by June. Plants overwinter as rosettes, produce flower stalks the following spring, and seeds ripen in summer. Each gen-

eration lives approximately 15 months, and the two age classes co-occur for approximately 3 months (April-June in NY). Adults of all four weevils produce characteristic “windowpane” feeding patterns on leaves, and “scraping” marks on stems and petioles. Adults are small and difficult to observe, as are larvae, which feed internally on seeds, stems, and root crowns. Under heavy attack by adults and larvae of one or more of the weevil species, garlic mustard plants become shorter, can produce more but thinner stems, are less robust, often have tip dieback, and produce fewer siliques (Blossey et al. 2001).

The desired outcome of biocontrol is a dramatic reduction in abundance of garlic mustard, and an anticipated recovery of the invaded communities. Therefore, the monitoring protocol was developed to capture these changes by recording: 1) Garlic mustard frequency, abundance, plant performance and seed production over time (before and after introduction of weevils); 2) Species composition and abundance of groundlayer plant communities (before and after introduction of weevils); and 3) Presence, abundance, and feeding damage of biocontrol agents (after introduction). The resulting data can be used to correlate weevil abundance to changes in garlic mustard abundance, and changes in garlic mustard abundance to changes in the native plant community.

Monitoring was implemented beginning in 2000 at four sites with established garlic mustard populations, two in New York and two in Illinois. Two to four transects were randomly established through the garlic mustard populations, and 0.5m<sup>2</sup> permanent quadrats (Fig. 1) were established at 10-m intervals along the transects, for a total of 20 to 26 quadrats/site. Garlic mustard was present in all quadrats, with initial cover ranging from 10 – 100%.



Figure 1. Monitoring garlic mustard with quadrat sampler.

Data were recorded from 2000 to 2004 in mid June and early October, to coincide with garlic mustard seed production and rosette development, respectively. Garlic mustard data consisted of estimated percent cover of each age class (seedling and adult in June, rosette in October), stem height and number of siliques/stem (June), and counts or estimates of seedling (June) and rosette density (October). Presence of external attack consisted of recording presence/absence of easily recognized insects (spittlebug and scale); insect herbivory (leaf mines, win-

dowpane feeding, edge feeding, and holes), deer browse, and disease, and then estimating percent of total leaf area removed by all forms of attack using “percent removed” classes. While this method has limitations (Louda 1984), in particular a potential to underestimate feeding attack when individual leaves are completely removed, or plants are completely de-

foliated, it is the most useful method to approximate intensity of insect attack. Community data consisted of presence and estimated percent cover of all vascular species.

Several trends were apparent after 5 years. Garlic mustard maintained presence in all quadrats in June, when both adults and seedlings were present. In October, when only rosettes were present, garlic mustard frequency fluctuated widely between years, occurring in 4 - 100% of quadrats at individual sites. Garlic mustard cover, density, and silique production varied significantly throughout the study period, both within and between sites.

Counting seedlings and rosettes was often difficult, particularly when plants were very small or densely crowded. When accurate counts could not be made, density was estimated within seven classes. The vast majority of stems were fertile, but sterile stems were present at all sites in all years, accounting for 1 - 25% of total stem density. While stem density varied from year to year within sites, mean final stem height was relatively similar at each site over the 5-year study period, as also reported by Byers and Quinn (1998). This suggests that in established garlic mustard stands, stem height may be a relatively stable measure, regardless of garlic mustard abundance in any given year. Since stem height is sensitive to attack by the proposed biocontrol insects, (E. Gerber et al., unpublished data), it is possible that a consistent and significant reduction in stem height following introduction of the weevils could be attributed to, and used as an indication of, weevil establishment and impact.

Presence of external attack was recorded to document background levels of herbivory. Relatively little leaf attack was detected, especially in June. In October leaf attack was more common, when up to 10% of leaf area was removed by disease and insect feeding. Most attacks were on older leaves that survived the summer, although deer browse was occasionally recorded on new leaves. "Windowpane" feeding was extremely rare, and likely produced by generalist weevils known to attack garlic mustard in the U.S. (B. Blossey and V. Nuzzo unpublished data). This feeding pattern is anticipated to become much more common after introduction of biocontrol insects, because it is the characteristic feeding pattern of the proposed biocontrol weevils. The frequency and extent of windowpane feeding will be used to estimate presence and abundance of the biocontrol weevils, because these small weevils cannot be easily observed or counted in the field. Community measures (mean number of species and mean percent cover) did not fluctuate significantly between years, indicating that the invaded communities remained stable over the 5-year study. Thus, a significant and consistent change in these measures following introduction of biocontrol would indicate a community response to the predicted reduction in garlic mustard. We can't predict exactly how biocontrol will impact garlic mustard in North America, nor how this will affect the associated native plant community. In Europe, garlic mustard responds to herbivore attack by decreasing in height, sometimes producing more but smaller diameter stems, sometimes producing more but smaller inflorescences and siliques, and producing fewer seeds/plant. The actual impact of biocontrol on garlic mustard in North America must be evaluated after release, and the standardized monitoring protocol will be an important tool. Only by monitoring many sites over many years will it be possible to avoid erroneously concluding an impact of biocontrol agents when in fact the decline in population could have been caused by

other (yet to be identified) factors. In addition, incorporating data from multiple locations will facilitate regional and national assessments of biocontrol effectiveness.

Every measure of garlic mustard abundance showed extreme fluctuation from year to year, reflecting the natural variation in this biennial species. As a result, identifying long-term trends, and separating the impact of biocontrol from natural fluctuations, requires more than one to a few years. Therefore, pre-release monitoring should be initiated as early as possible and be conducted annually. Initiating monitoring 1 year prior to release of biocontrol will not provide adequate baseline data to detect a biocontrol-related impact. The Garlic Mustard Monitoring Protocol, available at a <http://www.invasiveplants.net> website created and maintained by the Ecology and Management of Invasive Plants Program at Cornell University, gives detailed information on site selection, quadrat construction and layout, and data collection. Data sheets are also included, along with an equipment list, and additional information. Using this protocol, practitioners can begin monitoring garlic mustard infestations well before release of biocontrol weevils. The resulting baseline data will permit a Before and After introduction, in Control and Impact areas study design (BACI) and help to separate the impact of biocontrol insects from other factors. Once insects are released, practitioners will be able to determine if the insects have established, document the relative success of a release at a site, and help track changes in the natural community following biocontrol. After weevils have been approved for release as biocontrol agents, additional monitoring methods will be field tested for usefulness in detecting presence and abundance of weevils, and incorporated into an updated version of the monitoring protocol.

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# PRE-RELEASE MONITORING OF GARLIC MUSTARD IN MICHIGAN

Jeffrey A. EVANS<sup>1</sup>, Douglas A. LANDIS<sup>1</sup>, and Douglas W. SCHEMSKE<sup>2</sup>

<sup>1</sup>Department of Entomology, Michigan State University  
204 CIPS, East Lansing, MI 48824

<sup>2</sup>Department of Plant Biology, Michigan State University  
166 Plant Biology, East Lansing, MI 48824

*Alliaria petiolata* (Bieb.) Cavara and Grande (Brassicaceae) is a globally distributed, cool-weather biennial plant that is highly invasive in forest understory and edge communities in North America. In Michigan, *A. petiolata*'s distribution is primarily restricted to the southernmost four to five tiers of counties, although it occurs in isolated locations elsewhere and appears to be spreading northward through the state (Reznicek et al. 2005, pers. obs.) (Fig. 1). In 2003 we launched research efforts to quantify the impacts of *A. petiolata* on Michigan native communities and to evaluate its potential for biological control in anticipation of the future availability of biocontrol agents (Landis et al. 2004, Evans et al. 2005).

Eight research sites were selected in the southern Lower Peninsula of Michigan (Landis et al. 2004) (Fig. 1). Sites were selected based on the following criteria:

1. State, federal or other lands under long-term conservation management.
2. Forested communities of >2 ha in size with *A. petiolata* populations established for more than three years.
3. Areas protected from disturbance or future *A. petiolata* management for a period of at least 10 years.

At each site we established 20 permanent 0.5-m<sup>2</sup> sampling quadrats (0.5 x 1 m) spaced 10 m apart along transects and recorded GPS coordinates for each site.

Data were collected according to a nationally standardized protocol (Nuzzo and Blossey unpublished). Overall site inventories were conducted to characterize forest type, maturity (maximum diameter at breast height of principal canopy trees) and total plant community composition. In spring (June) and fall (Sept. - Nov.) 2003 and fall (Sept. - Oct.) 2004, data collected from each quadrat included:

1. Vegetation cover (total, *A. petiolata* total and *A. petiolata* by seedling and rosette stage plants).
2. Number of *A. petiolata* seedlings and rosettes litter depth.
3. Cover by other species, bare soil, wood, leaves and rocks.

Data were also collected on percent of plants damaged by herbivores (by species or damage type) and area of *A. petiolata* leaf surface removed. During the spring sample we also recorded the height and number of siliques produced for each mature plant of *A. petiolata*. In contrast to standardized sampling methods outlined by Blossey (unpublished), not all

sampling quadrats contained *A. petiolata* at the initiation of the study. This was done to allow us to measure spread of *A. petiolata* populations within sites.

Several problems arose while working at these sites. During the spring sampling period seedling densities can be overwhelmingly high. The protocol put forth by Nuzzo and Blossey (unpublished) recommends estimating seedling density categorically under these circumstances. Because we intend to incorporate these data into population models of *A. petiolata* biocontrol, it is important to have the most accurate estimates of seedling density possible. Rather than estimate density categorically, we divided our 0.5 x 1 m sampling quadrat into quarters or eighths, depending on the density of *A. petiolata*, and counted representative subplots within the larger quadrat. We were then able to scale-up this density to estimate seedling density in the overall quadrat. We feel that this method is more accurate than making categorical estimates and still is more efficient than counting whole quadrats at high densities.

We found that identifying the many forbs, grasses, sedges, seedlings and saplings was beyond our abilities on many occasions. We always travel to field sites with botanical manuals and field guides but were still unable to identify many species in the field. For these we identified species types in the quadrats and quantified cover as per the standard protocol (Nuzzo and Blossey 2002). Representative specimens from outside the sampling quadrats were collected and preserved with a flower press for later identification. We employed assistance from expert plant taxonomists at several university herbaria and were able to put species names on our unknowns for which we had quantified cover.

Finally, one of our sites was vandalized during hunting season. The conduit we used to mark sampling areas was removed or bent over at some quadrats. The first line of defense against vandals is to drive the conduit far enough into the ground that it is not easily removed or bent. As an additional precaution, we recommend establishing the sampling transects relative to permanent benchmarks such as trees, large boulders, or other immovable objects from

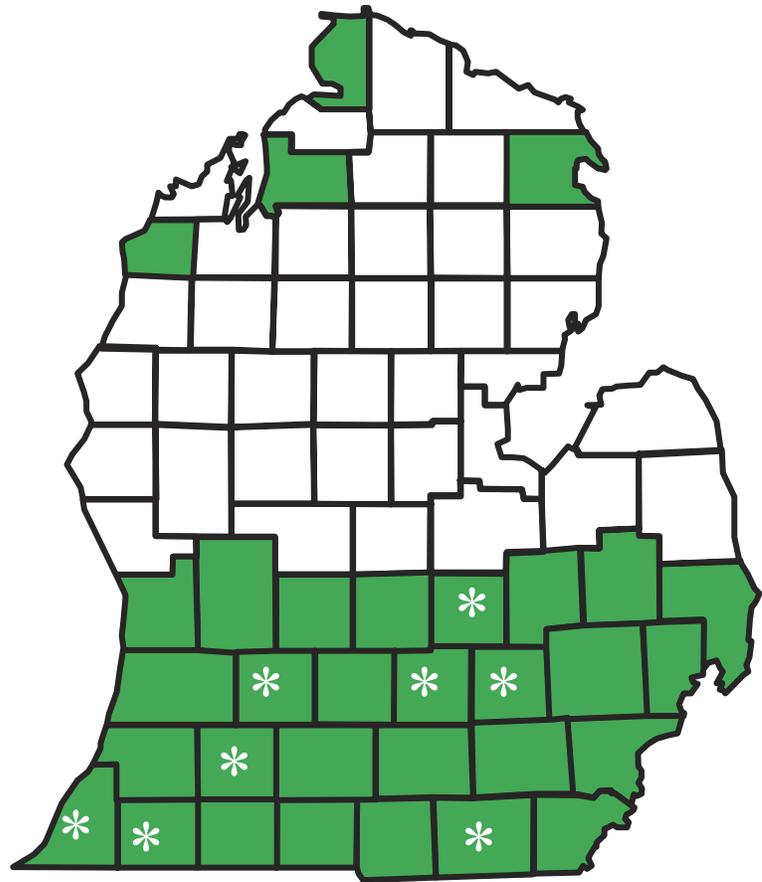


Figure 1. Approximate distribution of *A. petiolata* within Michigan's lower peninsula (shaded counties) and locations of study sites (stars).

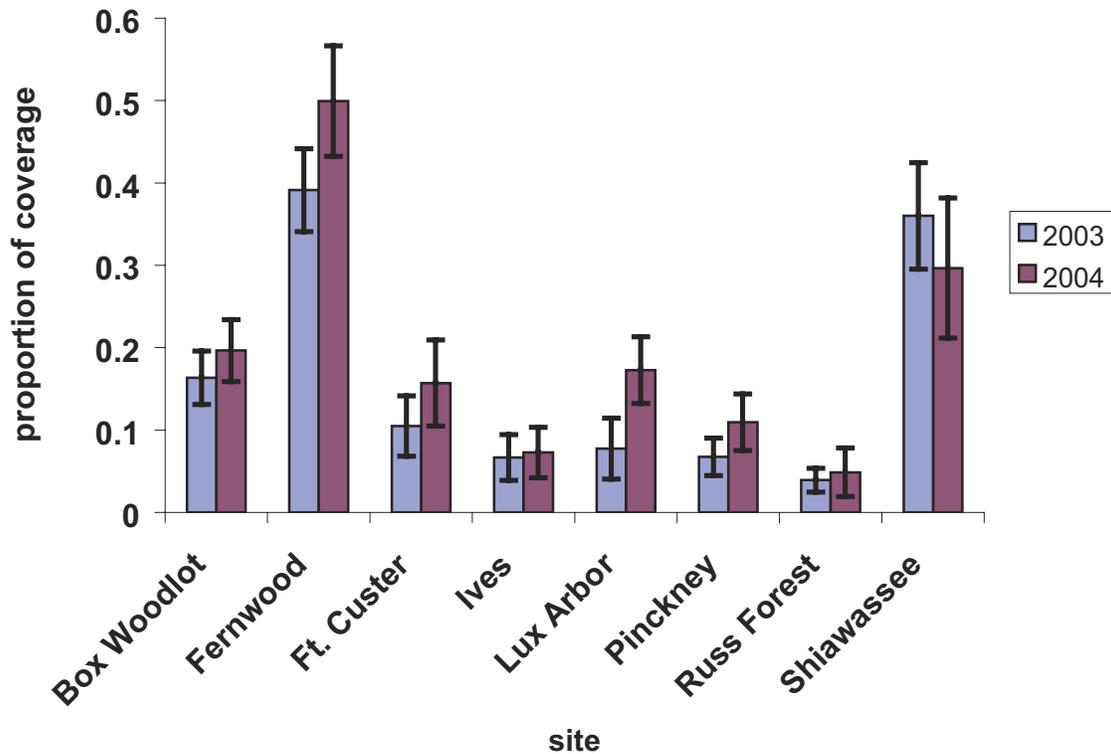


Figure 2. Proportion of sampling area covered by *A. petiolata* foliage  $\pm$  1 SE in spring.

which the locations of the sampling quadrats can be re-established in the event that plot markers are moved.

Across the eight sites in 2003 and 2004, mean *A. petiolata* cover within sampling quadrats ranged from 3.9 – 50% of total area in spring and from 0.1 – 18.9% of total area in fall. The mean area occupied by *A. petiolata* was numerically greater in 2004 than in 2003 at seven of the eight sites in both spring and fall (Fig. 2), although the increase is not statistically significant at most sites. Shiawassee, the only site whose mean *A. petiolata* cover decreased, was subjected to heavy flooding in spring of 2004 which destroyed nearly all seedlings and rosettes.

The number of plots containing second-year *A. petiolata* plants does not consistently increase annually, although this is likely an artifact of the plant's biennial life cycle as plots early in the invasion process alternate between containing first- and second-year plants in successive years. Sites in the later stages of invasion are less likely to exhibit this pattern, because the development of a seed bank moderates this alternation. A better measure of the spread of *A. petiolata* at a site is the count of quadrats containing either or both first- and second-year plants, which indicates change in its distribution across a site. From 2003 to 2004 *A. petiolata* increased or maintained its within-site distribution at seven of the eight sites in the

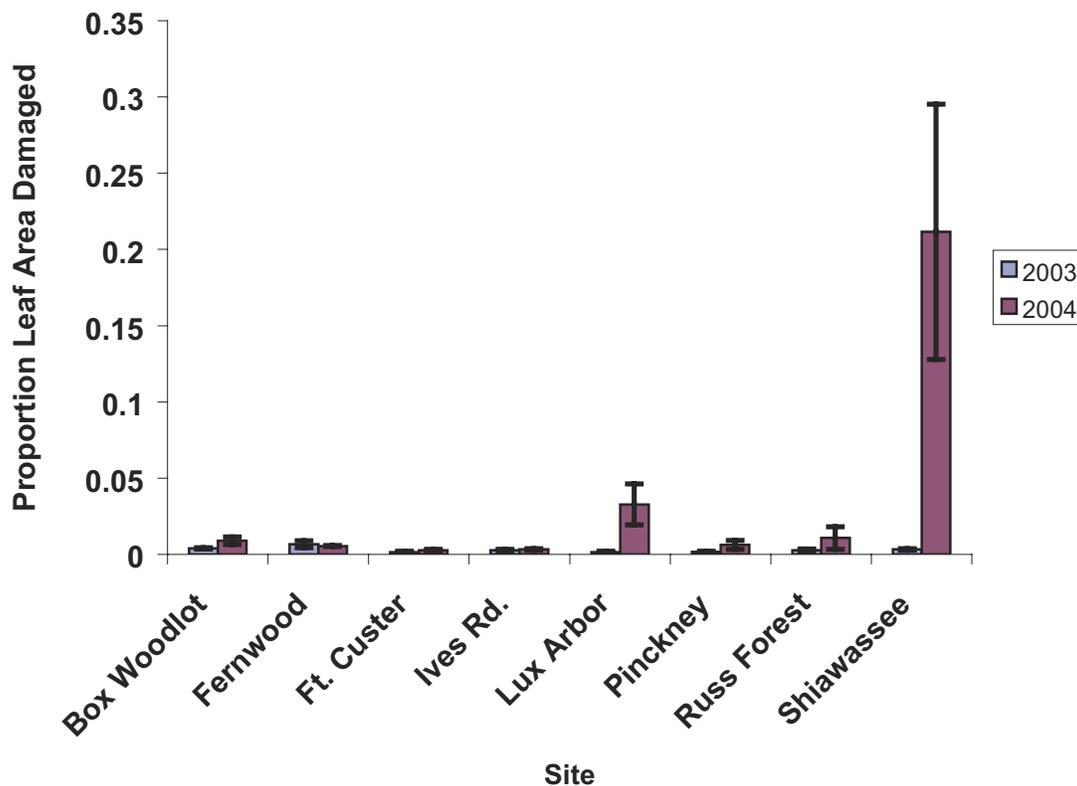


Figure 3. Proportion of leaf area damage recorded during spring sampling in 2003 and 2004. Only quadrats containing *A. petiolata* are included. Higher damage rates observed at Shiawassee in spring 2004 reflect extensive spring flooding. Fall damage rates are similar.

spring and at five of the eight sites in the fall. Again, the decrease observed in the fall could likely be attributed to *A. petiolata*'s biennial lifecycle.

We recorded types of damage caused to plants in several categories. In 2004 we observed edge feeding damage in 10 - 90% of quadrats and damage caused by creation of leaf holes in 10 - 90% of quadrats. However, the amount of leaf area removed only ranged from 0.1% - 3.3% (excluding extensive flood damage at the Shiawassee site in spring 2004) (Fig. 3). Thus, there is evidence that organisms are sampling *A. petiolata* plants at all sites but are not causing significant damage. We saw evidence of deer browsing at four of the eight sites in spring of 2004, but again, little leaf area was removed.

Using the spring data on *A. petiolata* abundance, species richness, composition and abundance of other species and ground cover at the eight sites, we asked which variables were the best predictors of *A. petiolata* abundance at the quadrat level. We excluded all variables that contained direct or indirect measures of *A. petiolata* abundance, i.e. seedling count or damage measurements, from the analysis.

Because our data were not normally distributed, we chose to use a regression tree analysis, a nonparametric multivariate technique. Classification and regression tree (CART) analy-

ses make no assumptions about the distribution of the data and are therefore appropriate in situations where the assumptions of traditional statistical analyses would be violated (De'ath and Fabricius 2000).

Litter depth and proportion of other vegetation cover were the factors most significantly correlated with *A. petiolata* cover. Quadrats with low leaf litter, low coverage of non-*A. petiolata* vegetation, higher species richness, and a high proportion of bare soil had the greatest proportion of *A. petiolata* coverage. The majority of quadrats with low *A. petiolata* cover (n = 189) are separated by litter depth alone in this analysis, suggesting that managers may want to pay particular attention to sites with low litter depth that could be more invulnerable. These relationships are strictly correlative, and we do not propose that any of these site features are driving *A. petiolata* invasion dynamics.

## CONCLUSIONS

Monitoring of *A. petiolata* populations in Michigan is being conducted in advance of implementation of biocontrol efforts. These baseline data will provide a measure of the relative success of any biocontrol efforts made in the future and allow us to test hypotheses about *A. petiolata* invasion processes.

*We have shown that A. petiolata* populations are expanding at most sites. This finding is consistent with our understanding of *A. petiolata*'s invasive properties. Because we have not found evidence of any significant herbivory or other natural controls on *A. petiolata*, and because conventional control methods have failed at all but the smallest scales (Blossey et al. 2001), classical biological control may be necessary for regulating Michigan populations.

Across the eight study sites, *A. petiolata* abundance is negatively correlated with abundance of other vegetation and litter depth, but positively correlated with bare soil substrate. Although some correlations appear strong, it is too early to assume causality of any of these relationships.

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## SESSION 2: BUCKTHORN (*RHAMNUS CATHARTICA*)

### BUCKTHORN BIOLOGY AND INVASION HISTORY

Kathleen S. KNIGHT

College of Natural Resources, University of Minnesota  
1530 N Cleveland Ave, St Paul, MN 55108

#### BIOLOGY OF *RHAMNUS CATHARTICA*

*Rhamnus cathartica* (common buckthorn) can grow either as a bush with many stems or a single-stemmed tree up to 30 feet tall (Godwin 1943). It has spines and serrate, shiny green leaves with converging veins. The leaves appear in April and May, and fall during October and November (Godwin 1943) (Fig. 1). In forest understories in North America, this allows shrubs to photosynthesize while the canopy trees and many native shrubs are leafless (Fig. 2). In one study, 38% of *R. cathartica*'s annual carbon gain occurred while a native shrub, *Cornus racemosa*, was leafless. *R. cathartica* also exhibited a high photosynthetic rate and a high rate of carbon gain compared to *C. racemosa* (Harrington et al. 1989). These traits may allow *R. cathartica* to grow very quickly, and compete effectively with native plants.

*R. cathartica* is dioecious, with yellowish-green insect-pollinated flowers appearing in May or June. Fruits mature in September, but they remain on the plant through much of the winter. *R. cathartica* reproduction is prolific, and can occur early. *R. cathartica* has been known to reproduce in its fourth year (Grubb et al. 1999). After it begins to reproduce, it continues to do so every year (Godwin 1943), and berries are spread by several species of birds (Godwin 1943). The berries contain secondary metabolites that have a laxative effect. Many berries simply fall to the ground, creating dense seedling patches beneath the adult bushes (Godwin 1943). The seeds overwinter and germinate the following summer.



Figure 1. *Rhamnus cathartica* (common buckthorn).

*R. cathartica* is suspected to be allelopathic. However, studies with buckthorn have failed to show effects of allelopathy on other plants (Archibold et al. 1997). *R. cathartica* is a mycorrhizal plant, and associates with arbuscular-vesicular fungi (Godwin 1943) in both its native and invaded range (Knight and Reich, unpublished data).

## R. CATHARTICA IN EUROPE AND ASIA

The earliest evidence of *Rhamnus* species is found in charcoal and pollen cores in Europe. Early humans burned buckthorn and other woody species, and the charcoal discovered by archaeologists aids in reconstruction of the vegetation present in early history. In one study, in the French Pyrenees, charcoal samples showed that several species of *Rhamnus* existed between 13,000 and 11,000 y.b.p. These species were associated with an open shrub community. In the post-glacial period, deciduous oak forests became established and these *Rhamnus* populations began to decline (Heinz and Barbaza 1998).

Today, *R. cathartica* is found north as far as Norway and Sweden, across Russia into western Siberia, in Afghanistan, and in southern Europe to middle and eastern Spain.



Figure 2. *Rhamnus Cathartica* under leafless canopy of trees.



Figure 3. Understory showing *Rhamnus cathartica* as dominant species.

In its European range, *R. cathartica* is characteristic of scrub areas undergoing succession, hedgerows, and as an understory shrub in ashwoods or oakwoods (Godwin 1943). Its frequency in hedgerows may be due to dispersal by birds. In its native range, *R. cathartica* grows singly or in very small groups (3-6 trees), and does not form large thickets of young seedlings.

## R. CATHARTICA IN NORTH AMERICA

*R. cathartica* was brought to North America as an ornamental shrub. The green leaves that last from early spring to late fall made it very desirable for hedges. It became naturalized in many areas in the upper midwest and the northeast parts of the U.S., and some adjacent areas in Canada (Gourley 1985) in the early 1900s. It invaded oak woodlands, fencerows and forest edges, becoming the dominant understory species in some cases (Fig. 3). Over some large areas, it makes up more than 90% of the plant biomass. Age classes of buckthorn populations show that once a few plants are established, the population can grow very quickly (Archibold et al. 1997). European starlings and other bird species disperse the berries.

*Frangula alnus*, also known as *R. frangula*, is another invasive buckthorn from Eurasia that is invading many areas of North America. Both its native (Godwin 1943) and invasive ranges (Catling and Porebski 1994) overlap with *R. ca-*

*thartica*, although it seems to prefer different habitat types. Several native *Rhamnus* sp. and closely-related *Frangula* sp. inhabit North America. *R. alnifolia*, *R. lanceolata*, and *F. caroliniana* exist in many of the same regions of North America as *R. cathartica*.

*R. cathartica* leaves are nutrient-rich (Harrington et al. 1989), and buckthorn may change soil nitrogen pools (Heneghan et al. 2002). This changing of ecosystem properties may have indirect effects on other species. *R. cathartica* may also directly affect native plant species. However, published scientific literature describing these effects is lacking. Anecdotal evidence suggests that, due to shading in dense stands of *R. cathartica*, plant diversity, spring ephemeral populations, and native tree regeneration may decline as invasion of *R. cathartica* progresses (Apfelbaum and Haney 1987) (Fig. 4).

*R. cathartica* also affects native bird species. When robins nest in *R. cathartica* rather than native shrub species, the nest predation rate increases (Schmidt and Whelan 1999). Anecdotal evidence suggests that bird diversity may also decline as invasion of *R. cathartica* progresses (Apfelbaum and Haney 1987). This may be due to nest predation, as with the robins, or to the quality of berries as food. The laxative effects of buckthorn berries may be detrimental to native birds that eat them. *R. cathartica* is also an agricultural problem in North America. It is an alternate host for some crop pests, including potato aphids, soybean aphids, oat crown rust (*Puccinia coronata*) and other cereal rusts (Leonard 2003, Ragsdale et al. 2004, Catling 1997, Archibold et al. 1997).

Buckthorn and other invaders may have positive effects on each other, causing an “invasion meltdown”. Earthworms are non-native in Minnesota, and many other areas of the northern U.S. that were glaciated. European earthworms have invaded these areas (James 1995), and they cause devastating effects in forests by eliminating the leaf litter (Bohlen et al. 2004, Hale et al. 2005). *R. cathartica*’s high-nitrogen leaves are an excellent food source for earthworms (Hendriksen 1990), potentially facilitating denser earthworm populations. The bare soil conditions that earthworms create are ideal for *R. cathartica* seed germination, which is inhibited by leaf litter (Bisikwa 2005). Both of these invaders are thought to have large effects on native plant populations, and it is difficult to separate the effects of *R. cathartica*, earthworms, and native deer populations.

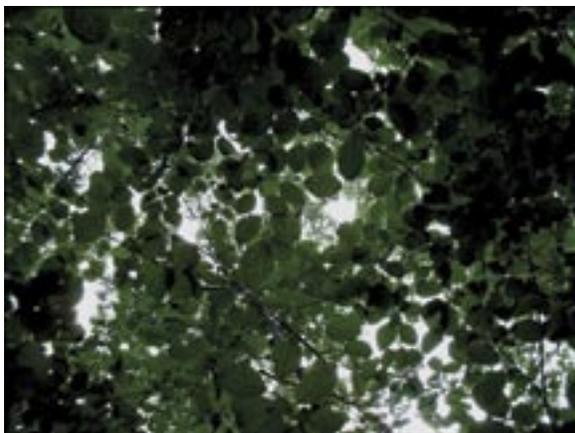


Figure 4. Shading in dense stands of *Rhamnus cathartica*.

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# BIOTIC AND ABIOTIC INFLUENCES ON *RHAMNUS CATHARTICA* (COMMON BUCKTHORN) ESTABLISHMENT

Kathleen S. KNIGHT

College of Natural Resources, University of Minnesota  
1530 N Cleveland Ave, St Paul, MN 55108

Understanding the factors that influence the successful establishment and growth of young buckthorn (*Rhamnus cathartica*) seedlings is an important step toward controlling buckthorn invasion. This research uses both experimental and observational approaches to address biotic and abiotic factors that affect buckthorn seedlings. Specifically, we examined the effects of competition by overstory and understory native plants, self-facilitation of buckthorn, and effects of soil biota from Europe and North America.

Our survey of 20 forested areas in central and southeastern Minnesota revealed relationships between buckthorn seedling cover, overstory canopy openness, and understory native plant cover and diversity (Knight and Reich, 2005). Within 1-m<sup>2</sup> plots, *R. cathartica* seedling cover was positively related to overstory canopy openness, and negatively related to understory native plant cover and diversity. In 1-ha plots, the negative relationship between *R. cathartica* seedling cover and understory plant cover was also present. However, in the 1-ha plots, *R. cathartica* seedling cover was positively related to understory plant diversity and had a more complicated relationship with light. This demonstrates how relationships can change depending on the size of the area observed.

In 2003, we began a field experiment to further explore the effects of overstory and understory plants on buckthorn seedlings. Other small-scale experiments such as ours, in which propagule pressure is directly controlled (Foster et al. 2002; Naeem et al. 2000, Dukes 2001; Tilman 1997) or assumed to be similar in all plots (Stachowicz et al. 1999; Kennedy et al. 2002; Knops et al. 1999), have demonstrated negative effects of native diversity on invasive species. The effect of diversity on invasion has been attributed to competition for resources, including nutrients (Naeem et al. 2000; Knops et al. 1999), water (Dukes 2001), light (Naeem et al. 2000; Knops et al. 1999), and physical space (Stachowicz et al. 1999). Experiments that affect resources through direct additions or disturbance have shown that invasibility increases with gross resource supply (Davis & Pelsor 2001; Burke & Grime 1996).

At the Warner Nature Center in east central Minnesota, we established plots across a light gradient (overstory competition) in an oak-dominated forest. In each plot, we created subplots with different levels of native understory plant diversity and competition. Subplots contain no plants, ten plants of three, six or ten native species, ten plants with shoots tied back, or a shade structure. In each subplot, we planted *R. cathartica* seeds, and recorded their germination, survival, and growth (leaf number). *R. cathartica* seedlings will be harvested in August 2005, and biomass will be measured. This experiment will also test for facilitation effects of mature *R. cathartica*, as well as soil moisture and fertility effects.

The preliminary results of this experiment show effects of both overstory and understory competition on the growth and survival of the *R. cathartica* seedlings. In the sub-

plots with no competition from understory plants, the *R. cathartica* seedlings show a strong positive response to light. As competition for light with the overstory trees decreases, the seedlings grow faster and have better survival. However, in the subplots with aboveground or belowground competition, the *R. cathartica* seedlings are not able to take advantage of higher light in canopy gaps. These seedlings only have a small increase in growth as light competition with overstory trees decreases. All subplots with ten native plants had similar detrimental effects on the *R. cathartica* seedlings, regardless of the number of species or whether the shoots were tied back, and the shade structure effect was similar as well. The *R. cathartica* seedlings that were in plots near mature *R. cathartica* trees had increased growth and survival. This may be due to increased fertility of these plots, caused by the mature *R. cathartica* trees.

In another experiment, we examined relationships between invading plants, the soil microbial community, and light. In August 2004, we collected soil at each of four forested sites in Minnesota and Poland. Half of the soil was autoclaved to kill any soil organisms and served as a control. *R. cathartica* and *Prunus serotina* seeds were planted in these soils, and placed in growth chambers with three light levels. The plants were harvested, dried, and weighed after 70 days of growth, and roots will be stained and examined for infection by soil symbionts.

Preliminary results show that, whereas *P. serotina* conformed to our “escape-from-natural-enemies” hypothesis, with decreased growth in live soil from its native range (consistent with Reinhardt et al. 2003), *R. cathartica* showed a very different pattern. In high and medium light, European soil biota did not have a negative effect on *R. cathartica*. In low light, there was a small negative effect of European soil biota. However, North American soil biota had a large negative effect on *R. cathartica* in all light levels. This pattern was consistent for *R. cathartica* from four different populations (Minnesota, Massachusetts, Hungary, and Ukraine). These results suggest that, if *R. cathartica* has escaped from natural enemies, these natural enemies are not soil pathogens from Poland. In fact, the natural enemies in Minnesota seem to be much more detrimental to the *R. cathartica* seedlings.

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# THE PHYLOGENETICS AND STRESS TOLERANCE OF *RHAMNUS CATHARTICA* L.

J. Ryan STEWART

Department of Horticulture, Iowa State University, Ames, IA 50011-1100

## THE PHYLOGENETICS OF *RHAMNUS CATHARTICA* L.

Common buckthorn (*Rhamnus cathartica* L.) is a member of the Rhamnaceae (Buckthorn family), which is a cosmopolitan family comprised of over 50 genera and about 900 species (Richardson et al., 2000). Recent work utilizing plastid DNA sequences has placed Rhamnaceae within the order Rosales along with other families such as Elaeagnaceae, Barbeyaceae, Dirachmaceae, Urticaceae, Ulmaceae, Moraceae, and Rosaceae (Chase et al., 1993; Judd et al., 1994). The Rhamnaceae is an old lineage; a rhamnaceous flower has been found that dates to 94-96 Myr B.P. (Basinger and Dilcher, 1984). The family is characterized by flowers with petal-opposed stamens.

Worldwide, the *Rhamnus* L. genus is comprised of 125 (Johnston and Johnston, 1978) to 142 (Grubov, 1949) species that are mainly found in the temperate and subtropical areas of the Northern hemisphere. However, some species are found in South America and even southern Africa (Grubov, 1949; Johnston and Johnston, 1978). (The name of the genus, *Rhamnus*, was originally used by Theophrastus (300 B.C.) (Grubov, 1949) to describe a low, thorny thicket (probably *R. oleoides*) in the Greek archipelago.)

Prior to Grubov's (1949) intrageneric division of the *Rhamnus* s.l. genus, most classifications of the genus were basic, e.g., presence/absence of winter bud scales and thorns, type of inflorescence, number of petals, etc. (Weberbauer, 1895). In addition to elevating the subgenus *Frangula* to generic rank, Grubov (1949) divided the *Rhamnus* s.s. clade into numerous sections. The sections include (North American and well-known European and Asian representatives of some sections are in parentheses): *Pseudofrangula* (*R. alnifolia*), *Tetrarhamnus*, *Pseudoceanothus*, *Eurhamnus* (*R. alpinus*), *Pseudalaternus* (*R. lanceolata*, *R. smithii*, *R. serrata*, and *R. crocea*), *Alaternus* (*R. alaternus*), and *Cervispina* (*R. cathartica*, *R. utilis*, and *R. davurica*).

Of the 142 species of this genus recognized by Grubov (1949), 88 (62%) are in the section *Cervispina*. Forty-nine (55%) of the species in this section are indigenous to East Asia. The remaining species are of Eurasiatic origin. Grubov (1949) considered *R. utilis* (Chinese buckthorn) and *R. davurica* (Dahurian buckthorn) to be ancestral to *R. cathartica*. *R. utilis* is a large-leaved tree that is native to the mountain forests of southern and central subtropical provinces of China (Grubov, 1949). *R. davurica* is found in the northwestern provinces of China as well as in Korea and Japan (Grubov, 1949). Both of these taxa have been introduced into the United States.

Grubov hypothesized that that the section *Cervispina* originated in East Asia and migrated westward through the Angara-Sayan mountain system of Russia (near Lake Baikal).

It was in this mountain system that two subsections, *Cathartiformes* and *Petrophilae* separated. Members of *Cathartiformes* have the same ancestral origin as *R. cathartica*. *Cathartiformes* continued to proceed along the northern route in Russia and Europe along the ascending alpine system. *Petrophilae* probably progressed in the area of the Irano-Turanian highland into the mountain systems of the Mediterranean. According to Grubov (1949), there are 42 species that are connected to, or are within the range of, *R. cathartica*. Many of these species have been documented to yield hybrids with *R. cathartica*, including: *R. utilis* (Gil-Ad and Reznicek, 1997; Grubov, 1949); *R. tinctoria* (Dyer's buckthorn) (Grubov, 1949); *R. saxatilis* (Avignon berry) (Grubov, 1949); *R. pallasii* (long leaf buckthorn) (Grubov, 1949); and *R. spathulifolia* (Grubov, 1949).

One interesting thing to note is there is a large territorial break between the native ranges of *R. cathartica* and *R. davurica* (Grubov, 1949). Although I believe it is very likely that these two species can hybridize, I have yet to find anything in the literature to verify this. Further work needs to be done to document current ranges of these species in the areas of their natural distributions.

It would be prudent for horticulturists, land managers, and botanists to gain a better understanding of the species that can hybridize with *R. cathartica*. Hybridization between two species may serve as a catalyst for the evolution of invasiveness (Ellstrand and Schierenbeck, 2000). Additionally, should hybridization occur between an introduced species and a native species, the resulting populations likely would be more genetically diverse than their progenitors (Ellstrand and Schierenbeck, 2000). Many of the species in the section *Cervispina* may be considered to have ornamental or even medicinal value. Zhang et al. (1997) suggested several plants from China of ornamental and economic value that may merit introduction into the southeastern United States. Of the 22 *Rhamnus* L. species listed, at least ten belong to the section *Cervispina* and may be able to hybridize with *R. cathartica*.

Although Johnston and Johnston (1978:1) felt the work of Grubov (1949) had "languished in well-deserved obscurity," Grubov did provide some salient information; many of his hypotheses have withstood the test of time. For example, although Philip Miller (1691-1771) elevated the subgenus *Frangula* Miller to generic rank, over the past several hundred years it has been demoted and elevated many times (Kartesz and Gandhi, 1991). However, Grubov (1949) and more recently others (Bolmgren and Oxelman, 2004; Kartesz and Gandhi, 1991) have provided strong evidence that *Frangula* Mill. is a monophyletic sister clade to the rest of *Rhamnus* s.l. In particular, it is important to note that there has been no documentation of hybridization between members of *Rhamnus* L. s.s. and *Frangula* Mill. Some examples of North American taxa of *Frangula* Mill. include *F. caroliniana* (Carolina buckthorn), *F. betulifolia* (birch-leaf buckthorn), *F. californica* (California coffeeberry), and *F. purshiana* (cascara buckthorn).

Knowledge of the phylogenetics of *R. cathartica* is beneficial for many reasons. For example: In summer 2000, an agricultural pest, *Aphis glycines* Mats. (soybean aphid), was discovered in North America. As part of its complex life cycle in its native range in Asia, it overwinters on *R. davurica* and *R. japonica*. Recently, Voegtlin et al. (2004) found that *R. cathartica* and *R. alnifolia* were accepted by *A. glycines* in North America and that eggs successfully overwintered. I believe it is important to understand the relationships among

*Rhamnus* s.l. to predict which species will likely be successful hosts for overwintering *A. glycines*. Furthermore, recent work has revealed that at least two *Frangula* species, *F. purshiana* and *F. californica*, are associated with *Phytophthora ramorum* (sudden oak death) in California (COMTF, 2005). Further work needs to be done to understand which *Rhamnus* s.l. species can serve as alternate hosts to this pathogen.

### STRESS TOLERANCE OF *RHAMNUS CATHARTICA* L.

Although the focus of the study by Stewart and Graves (2004) was to provide horticulturists and ecologists information concerning the preferred soil conditions of *F. caroliniana* (Walt.) Gray, using *R. cathartica* as a comparison allowed the researchers to glean information concerning traits that may contribute to the invasiveness of *R. cathartica*. The specific objectives of Stewart and Graves (2004) were to determine how root-zone water content affects gas exchange and growth of Carolina buckthorn compared to that of common buckthorn.

Three irrigation treatments, drought, control, and partial-flood, were randomly assigned to 90 plants (experimental units), 45 each of Carolina buckthorn and common buckthorn (Fig. 1). For Carolina buckthorn, among the nine plants in each irrigation treatment were three plants native to each of three states, Missouri, Ohio, and Oklahoma. The common buckthorns were grown from seeds collected in Iowa. Net photosynthesis and soil moisture content of all plants were determined when the soil moisture of plants in the drought treatment declined to 10%. There were six such irrigation cycles. Leaf area and dry mass of roots, stems, and leaves of each plant were measured at end of the sixth irrigation cycle.

Stewart and Graves (2004) found that root, stem, and total plant dry weights of common buckthorn were greater than those of Carolina buckthorn. Also, they found that the ratio of root-to-leaf dry weight of common buckthorn was 31% greater than that of Carolina buckthorn. Leaf area and root, stem, and total plant dry weights of control-treated common buckthorn were greater than those of Carolina buckthorn, regardless of treatment. They concluded that common buckthorn appears to use carbon more efficiently than does Carolina buckthorn.

Although Carolina buckthorn had a photosynthetic rate that was 26% greater than that of common buckthorn, the low dry matter accumulation of Carolina buckthorn suggested that much of its fixed carbon was used for maintenance respiration. Furthermore, mean photosynthetic rates did not differ even though the accumulation of dry matter of the control-treated common buckthorn was 56% greater than that of Carolina buckthorn. The



Figure 1. Water stress tolerance study of *R. caroliniana* and *R. cathartica*.

31% greater leaf area of common buckthorn may explain this finding, but it also may indicate that common buckthorn has a higher resource-use efficiency.

Additionally, partially flooded common buckthorn initially showed leaf epinasty, but recovered as manifested by normal shoot development. Further work needs to be done to understand the adjustments made in the root zones of common buckthorn under extremely wet conditions.

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# IMPACT OF *RHAMNUS CATHARTICA* (EUROPEAN BUCKTHORN) ON MIDWESTERN WOODLAND ECOSYSTEMS AND THE IMPLICATIONS FOR RESTORATION MANAGEMENT

Liam HENEGHAN

Environmental Science Program, DePaul University  
2325 North Clifton Ave, Chicago, IL 60614

## INTRODUCTION

Removal of *R. cathartica* and other woody shrub invasives is a typical prelude to the ecological restoration of Midwestern woodlands. Such management is likely to affect the availability of light within infested woodlands, and may positively contribute to the succession of a rehabilitated understory plant community. However, it is important to consider the possibility that *R. cathartica* invasion imposes a persistent impact on the physical and biological properties of soil. If this is the case, although managed woodlands would have a restored light gradient, they may retain modified ecosystem properties, which could constrain the ultimate success of subsequent restoration.

The results reported here demonstrate that *R. cathartica* is indeed associated with significant modifications to a variety of ecosystem properties in an urban woodland. I discuss several of these impacts, and conclude with a summary of the implications for future management.

## CHARACTERIZING THE INVASION

In the Midwest, *R. cathartica* has a patchy distribution within invaded woodland stands. We have demographically characterized two 100m<sup>2</sup> thickets at each of two locations close to Chicago Illinois: Mary Mix McDonald Woods in Glencoe, and the East Woods at the Morton Arboretum in Lisle (Fig. 1). The average age of an individual in a thicket at McDonald woods was 34 years (the eldest individual was 72); 100m<sup>2</sup> plots had 21 individuals greater than 5cm DBH. The invasion was more recent at the East Woods, where the average age was 22 and 100m<sup>2</sup> plots had 12 individuals greater than 5 cm DBH.

The high density of buckthorn contributes to a significant shading of the woodland floor (Fig. 2). We compared three replicate buckthorn thickets to two managed areas (three replicated areas within each site) using fisheye lens photography of the canopy. The digital images were analyzed using the Gap Light Analyzer program, available through Simon Fraser University, Burnaby, British Columbia, Canada, and the Institute of Ecosystem Studies, Millbrook, N.Y., USA. The removal of buckthorn resulted in an increase of more than 10% in the openness of the canopy, measured at 1.5 m above the woodland floor (Heneghan and Umek, *in prep*).

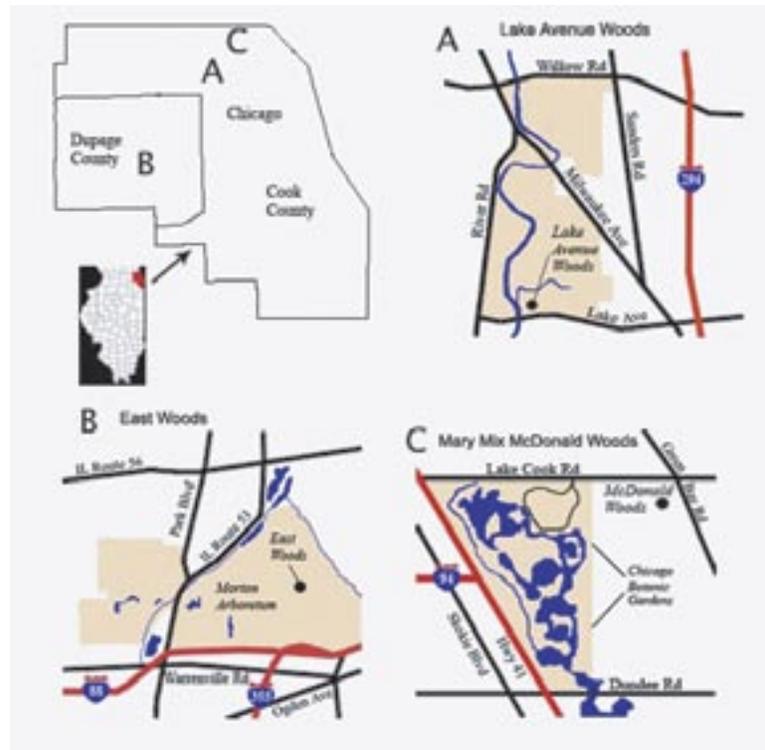


Figure 1. Locations of research sites in the Greater Chicago area, Illinois.

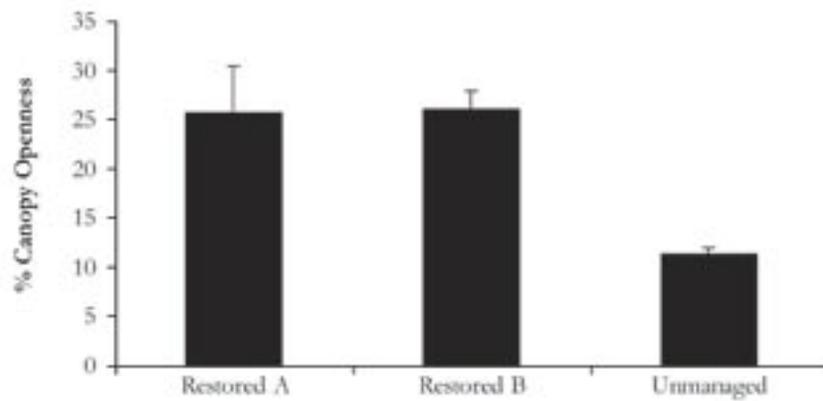


Figure 2. Comparison of canopy openness between an unmanaged buckthorn thicket and areas from which buckthorn has been removed (restored).

## SUCCESS OF *R. CATHARTICA* AND ITS IMPACT ON SOME ECOSYSTEM PROPERTIES

The success of buckthorn in ecosystems of the Midwest may be accounted for, among other factors, by virtue of its escape from herbivory, by alterations of soil processes including decomposition in the woodland floor where they grow, and by its synergy with other invaders. I discuss each of these factors in turn.

### European buckthorn escape from herbivory

In September 2002 we collected leaves from 5 individuals of 9 species: European buckthorn (*R. cathartica*); sugar maple (*Acer saccharum*); red oak (*Quercus rubrum*), bur oak (*Quercus macrocarpa*); basswood (*Tilia americana*); hickory (*Carya ovata*), ironwood (*Ostrya virginiana*); wild cherry (*Prunus serotina*); and witchhazel (*Hamamelis virginiana*). Scans were made of 1135 leaves and the digital images were analyzed for leaf loss using ImageJ (<http://rsb.info.nih.gov/ij>), which was modified with a plug-in written in Java that allowed us estimate leaf area lost (Fig. 3). The analysis revealed that average herbivory on all species was 4.3%, and that herbivory on *R. cathartica* was significantly lower than all other species examined (0.8% loss to herbivores) (Grady, Heneghan and Jabon, *in prep*).

### Rapid decomposition of buckthorn litter and elevated decomposition rates within invaded areas

*R. cathartica* litter has high nitrogen levels (2.2% N) and this contributed to its rapid decomposition. Decomposition, measured using litterbags (which may underestimate decomposition rates), was rapid compared to *Populus deltoides*, *Q. ellipsoidalis*, and *Prunus serotina*. A negative exponential decay model fitted to data collected over a 300-day period predicted that less than 25% of *R. cathartica* litter would remain after a year, compared to more than 50% of all other litters measured. Additionally, this litter bag experiment revealed that mixed litter (when all litter types were combined in the same litterbags) decomposed more rapidly than would be predicted for each type of litter decomposing, separately. This suggests that, when *R. cathartica* is present, the litter decomposition on the woodland floor occurs more rapidly (Heneghan *et al.* 2002). We used a cotton strip assay to test the hypothesis that overall rates of decomposition were elevated in invaded areas. This assay uses a standard decomposition substrate and thus facilitates reliable inter-site and inter-year contrasts. This experiment has not been completed, but early results appear to confirm that decomposition rates are significantly elevated in invaded areas of two woodlands (Fig 4) (Heneghan and Iatropulos, unpublished).

The rapid decomposition of forest floor material associated with buckthorn has at least two significant consequences.

1. Areas invaded by *R. cathartica* are associated with some modified soil properties including elevated soil N, (also elevated pH and soil moisture levels). The rapid decomposition of litter with elevated N concentrations has led to N enrichment of



Figure 3. Contrast of leaf loss from a) Hammemlis versus b) *R. calthartica*. The area lost of herbivory was given a false color (yellow) before analysis.

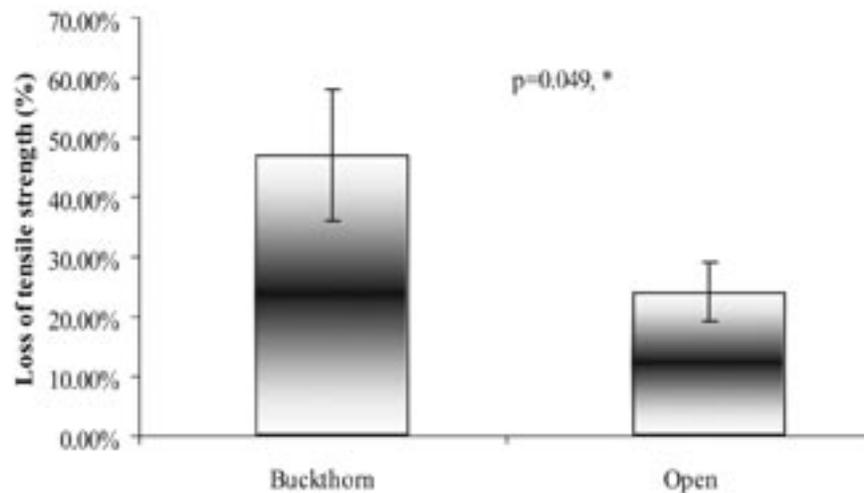


Figure 4. Loss of tensile strength of cotton strips in invaded (buckthorn) versus uninvaded (open) areas after 28 days – this experiment is being conducted in replicated plots in each of two woodlands north of Chicago (Heneghan and Iatropulus, unpublished).

the upper soil horizon. Both the total %N and %C is consistently higher in invaded areas of woodland. Laboratory measurement of nitrogen mineralization over a 28-day period revealed lower N mineralization in soils from under buckthorn than in soils collected from adjacent areas. This suggests that changes in the functioning of the soil microbial community may be responsible for the modified N associated with dynamics in buckthorn thickets, contributing to an accumulation of N (Heneghan et al. 2004). Results of microbial analysis using Ecoplates (from Biolog) revealed that there were consistent differences in microbial activity between soils associated with *R. cathartica* (Fig. 5) (Heneghan *et al.* 2004.).

2. There is a collapse of the soil arthropod community in woodland areas associated with *R. cathartica*. We sampled these communities in 255 cm<sup>2</sup> quadrats in invaded areas and compared them to litter samples taken from uninvaded areas in June and December of 2002, and April 2003. Consistently, there was a lower abundance in *R. cathartica* plots. For instance, in June 2002 there was an average of 83 individuals per quadrat in uninvaded areas, and 6 individuals per quadrat in invaded plots. To a large extent this was a function of the amount of dead organic matter (DOM) in the plots. However, even when abundance was expressed per unit mass of DOM there were differences found between plots in the April samples (Heneghan and Bernau, *in prep.*).

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### Interactions between *R. cathartica* and invasive earthworms

There is a strong relationship between the presence of *R. cathartica* and the abundance of earthworms of Eurasian origins in Mary Mix McDonald woods (Jim Steffen, unpublished). We exploited this gradient of invasive earthworm density to examine the effect of earth-

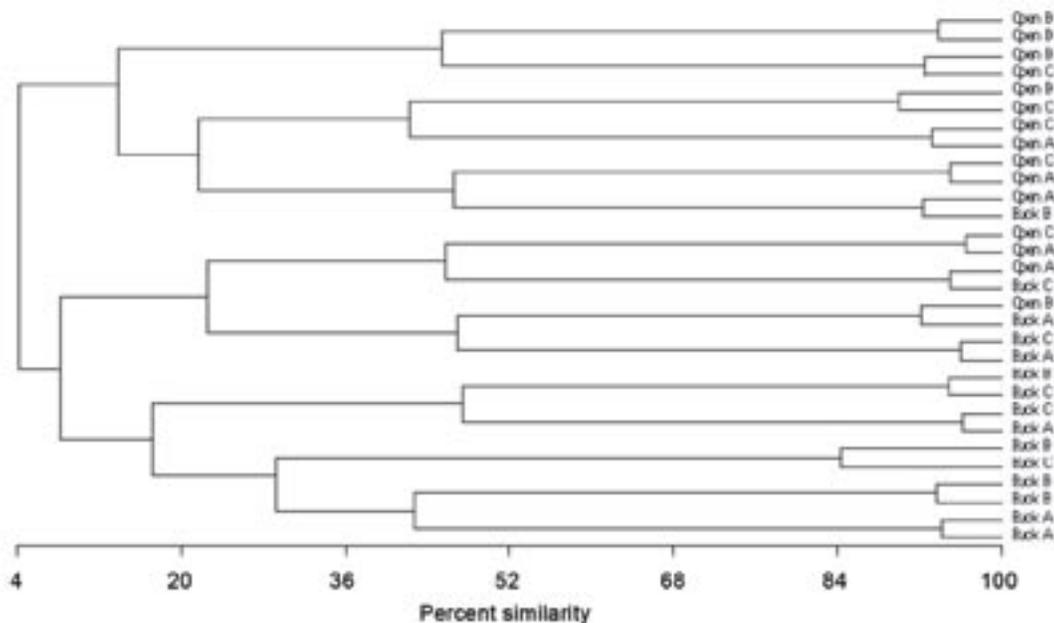


Figure 5. Similarity between microbial substrate utilization in random samples of soils associated with *R. cathartica* ("Buck") versus soils from un-invaded areas ("Open").

worms on litter dynamics on woodland floors. Using *R. cathartica*, *Q. rubrum*, *Q. alba*, and *A. saccharum* litter, we demonstrated that decomposition rates were very high in buckthorn thickets where earthworm populations attain the highest mass litter loss in bags to which worms had free access. After four weeks no *R. cathartica* litter remained, although litters of low quality (*Quercus spp.*) were little affected by earthworm presence (Fig. 6) (Heneghan, 2003, Heneghan, Steffen and Fagen, *in prep.*).

## Implications

The results of our studies on *R. cathartica* in urban woodlands in the Chicago area indicate that this invader has impacts both above and below ground. Management that aims at rehabilitating woodland light regimes can be successful as revealed by analysis of canopy openness in invaded versus managed plots. The success of *R. cathartica* as an invader may be attributed to its escape from herbivory, but also to its impacts on the soil. There has been no attention paid to “legacy” effects of the invasion on the soil properties. We have demon-

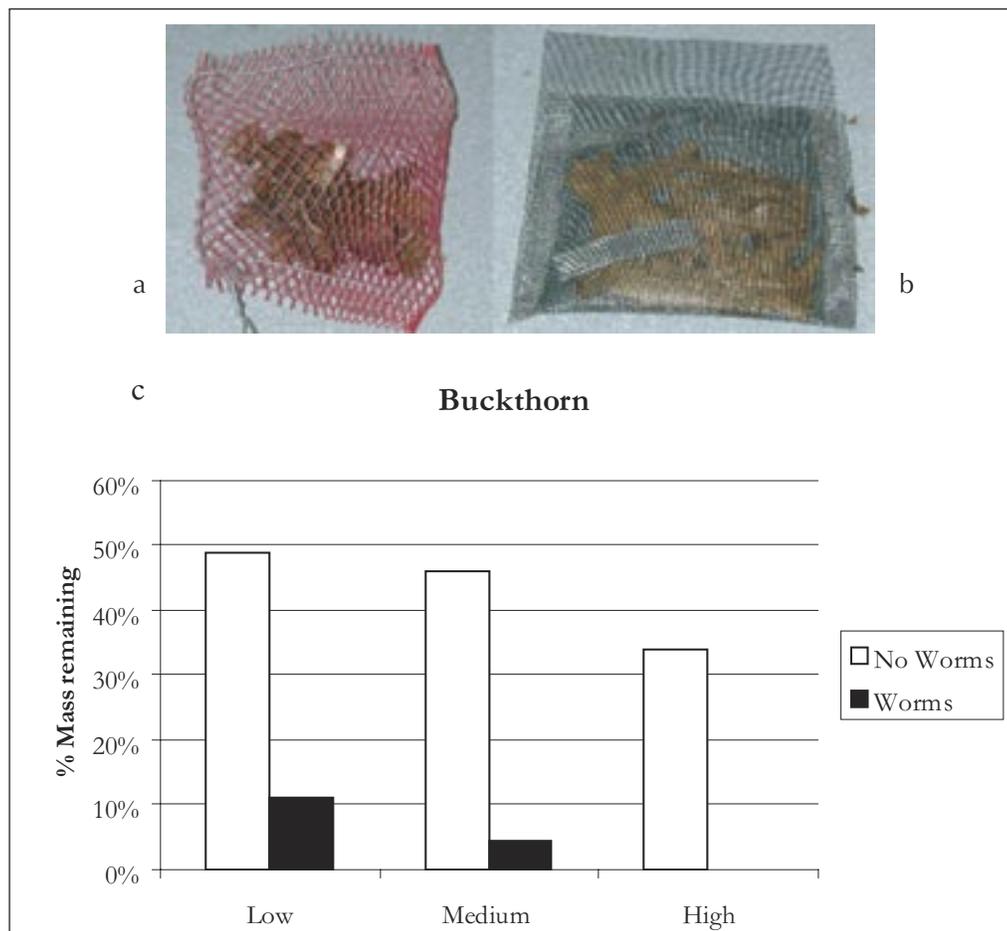


Figure 6. Effect of invasive earthworms on litter decomposition. Litterbags were constructed to permit access by invasive earthworms a) or prevent earthworm access b). In all plots (low, medium or high earthworm abundance) *R. cathartica* litter was significantly effected by earthworm. Impact is highest in thickets of *R. cathartica* where earthworm abundance peaks.

strated that invasion by *R. cathartica* has dramatic effects on the critical ecosystem process of decomposition. Not only is the litter of *R. cathartica* rapidly decomposed, the overall rate of decomposition of invaded plots is elevated. As a result, many associated soil characteristics, including pH, nitrogen cycling, and moisture levels, are different in invaded plots compared with adjacent uninvaded areas. Soil biological characteristics are also modified, including microbial community functioning and the abundance and diversity of litter dwelling arthropods (these communities collapse in areas with high *R. cathartica* density). No systematic evaluation of the persistence of these effects has been undertaken. However, it seems likely that the legacy effects of *R. cathartica* invasion persist after the plant has been physically removed. Since many of the impacts are associated with conditions and resources important in determining plant community structure and function it is possible that the chronic failure of restoration, subsequent to management of buckthorn, is associated with these modified soil properties. The soil subsequent to invasion may be primed for the successful reinvasion of these woodlands by the same problematic species which were removed by management.

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# THE EUROPEAN BUCKTHORN (*RHAMNUS CATHARTICA*) INVASION IN WEST CENTRAL MINNESOTA

Peter WYCKOFF, Rick JANSEN and RUTH PATTEN

University of Minnesota, Morris, 600 E 4th St, Morris, MN 56267

## SUMMARY

We find European buckthorn (*Rhamnus cathartica*) to be ubiquitous at seven study sites in west central Minnesota (three State parks, two city parks and two private forests). Overall, buckthorn is the most abundant tree species, and at one site buckthorn represents over 50% of all stems in the herbaceous layer. Compared to bur oak (*Quercus macrocarpa*- the only other species with substantial regeneration at our study sites), buckthorn saplings are found in significantly darker microsites with higher soil moisture. Light and soil moisture explain approximately half of the variation in growth seen among buckthorn saplings.

## INTRODUCTION

Anecdotally, European buckthorn (*Rhamnus cathartica*) is known to be ubiquitous throughout the upper Midwest. However, hard data on buckthorn distribution and abundance are hard to come by; the Forest Inventory and Analysis (FIA) and other vegetation monitoring programs often fail to track the species. Buckthorn is of special concern in west central Minnesota because of the association between buckthorn and soybean aphid; soybean production is a major component in the regional economy.

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## BUCKTHORN IN WEST CENTRAL MINNESOTA

In 2001, we established vegetation monitoring plots in three state parks, Maplewood, Glacial Lakes, and Sibley, along the prairie-forest border in west central Minnesota (Fig. 1). Buckthorn is found at all three sites (Fig. 2), although composite data masks substantial variation. Buckthorn is a minor component at Maplewood State Park but extremely common at Glacial Lakes State Park.

To examine buckthorn's impact on regional forest composition, we added four supplemental study sites: two private forests and two forested city parks. Unlike our state park sites, these supplemental sites were specifically selected because of abundant buckthorn, and represent the extreme of the regional buckthorn invasion, not typical conditions. Nevertheless, we do not feel that these supplemental sites deviate substantially from typical regional conditions, and the amount of buckthorn at these sites is truly impressive (Figs. 3 and 4).

### **Buckthorn regeneration as a function of light and soil moisture:**

In 2002 and 2003, nested plots were used to examine the relationship between overstory and understory composition at our four supplemental sites (60, 3-m radius circular overstory



Figure 1. Location of study sites in Minnesota.

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plots centered around 1 m<sup>2</sup> understory plots). Understory light levels also were measured, using a densitometer. We had hoped to compare tree recruitment in areas with and without significant buckthorn infestation, but our efforts have been thwarted by an almost complete lack of non-buckthorn advanced regeneration anywhere at our supplemental sites.

Buckthorn seedlings are common everywhere at our supplemental sites, but seedling abundance increases exponentially with overstory buckthorn abundance. In this initial study, buckthorn seedling abundance peaked at intermediate light levels. Conversely, herbaceous species diversity peaked at low and high light levels.

In 2004, we conducted a more in-depth buckthorn growth and microsite utilization study at one of our supplemental sites, Niemackl Park, in Herman, Minn. Sixty randomly sampled buckthorn saplings were tagged along with forty bur oak saplings. Bur oak regeneration is confined to the eastern side of the park, so these saplings were clustered. Soil moisture available to each tagged sapling was repeatedly measured using TDR rods. Light availability was assessed using fish-eye photos and PAR meters (above sapling PAR compared to open field PAR). Saplings were sacrificed in late 2004 and growth was assessed by measuring annual rings.

Compared to bur oak, buckthorn saplings are found in significantly darker microsites (Fig. 5) with higher soil moisture (Fig. 6). Light and soil moisture explain approximately half of the variation in growth seen among buckthorn saplings. The relationship between growth and microsite condition is less clear for bur oak. Limited success of buckthorn in

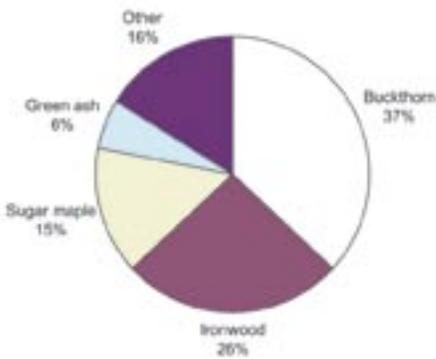


Figure 2. Small tree composition (> 2m tall, <10cm dbh) at three state parks in west central Minnesota. Buckthorn control is attempted at these parks.

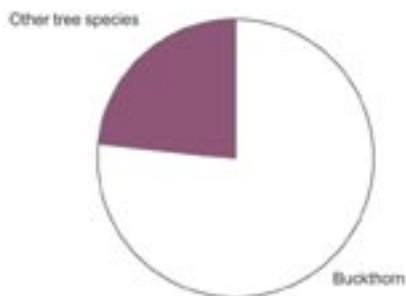


Figure 3. Small tree composition (> 2m tall, <10cm dbh) at four forested sites near Morris, Minnesota with no buckthorn control.

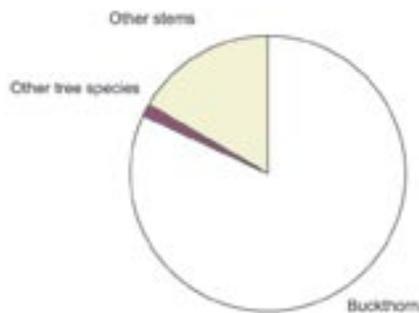


Fig. 4. Herbaceous layer (< 1m tall) stems in three forested parks near Morris, Minnesota with no buckthorn control.

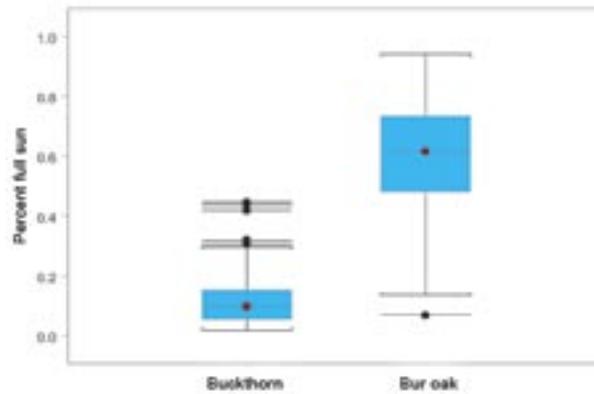


Figure 5. Light availability for buckthorn and bur oak saplings.

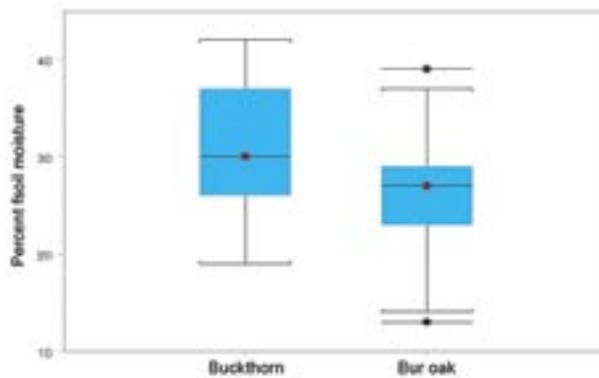


Figure 6. Soil moisture for buckthorn and bur oak saplings.

light, dry microsites suggests that, although the invasive European tree will not lead the charge as forest moves out into the prairies of western Minnesota, it might subsequently follow as a new forest develops.

### **FUTURE DIRECTIONS**

We plan to follow up on initial laboratory experiments that suggest buckthorn exudates can inhibit the germination of seeds from other species. Also, we are working with plant services at the University of Minnesota, Morris, to assess the effectiveness of management alternatives for controlling buckthorn populations. Although buckthorn is doing well in west central Minnesota, we do not feel that invasive buckthorn alone can be blamed for the regeneration failure of other tree species. To further examine regional forest dynamics, we will study the impacts of deer and earthworm herbivory on regional forest dynamics.

# CONVENTIONAL MANAGEMENT OF BUCKTHORN SPECIES

**John MORIARTY**

Ramsey County Parks and Recreation Department,  
2015 N. Van Dyke St., Maplewood, MN 55109

Over the last ten years, the control of invasive species has become a major part of a resource manager's job. Buckthorn (*Rhamnus cathartica* and *Fangulus alnus*) is the main species in our region actively being controlled. To some level, the majority of woodlands in the Minneapolis-St. Paul, Minn., Metro Area have been invaded by these trees. Control efforts are being taken with several techniques. Four such techniques covered in this presentation are hand-pulling, root wrenches, basal bark herbicide treatment, and cut stump herbicide treatment.

Hand-pulling is a technique that works well for small saplings (Figure 1). (Trees over 0.5 inches caliper are difficult for most people to pull up.) This technique works great with children and volunteers. There is no use of herbicides with hand-pulling. The pulled up trees are small enough to leave onsite without interfering with other management activities. Although this technique does not work on larger trees, it works very well as a follow up to large tree removal. Also, this technique is not suited for large areas (>5 acres) because of the time and labor involved. It is an important tool in areas where prescribed burns are not allowed.

Root wrenches are a mechanical levers designed to pull up larger buckthorn ( $\leq 2$  inches DBH) (Figure 2). The lever action enables most people to remove the trees, making it good work for volunteers. The levers can make for slow work when pulling trees at the upper size limit. There are no herbicides involved with pullers. The root systems of these trees are large enough that there can be significant soil disturbance on a site if this technique is widely used. As with hand-pulling, this technique is not suitable for large areas.

Cutting and stump treating with herbicides is the technique used by most resource managers when working on larger sites. Cutting can be done with handsaws, but chainsaws are normally used (Figure 3). Stumps of cut trees must be treated with an herbicide (Figure 4): failure to do so will lead to vigorous resprouts. Glyphosate or Triclopyr are the most commonly used herbicides. The herbicides are mixed to label specifications for cut stumps and applied by sprayer or painting. This technique works well on trees from 1 inch to >15 inches DBH. Loppers or branch pruners can be used to cut trees <1 inch DBH. Volunteers trained in this technique are used by some agencies: However, in general



Fig. 1. Hand pulling small buckthorn plants.

chainsaws and herbicides are not appropriate for use in large volunteer groups.

This technique works well on large areas. For example, we have used this technique to treat over 300 acres in Ramsey County Parks. If not removed, large cut trees can cause a problem of site access for future management activities. Cut trees should be piled and burned on site, or if onsite burning is not an option, removed for chipping or burning offsite.

Basal bark herbicide treatments is a technique where Triclopyr in a basal bark oil is sprayed on the lower 12 inches of the trunk using an ultra-low volume sprayer. This kills the tree and leaves it standing. This technique works best in areas with low tree densities or in areas well away from public use. Large numbers of standing dead trees can look bad (not aesthetically pleasing) and limit future management activities.

Treating a site with one or a combination of these techniques should be seen as the first step in a long process to control buckthorn on a particular site. In areas that allow it, prescribed burns work well to kill buckthorn seedlings and stimulate native herbaceous understory. Fire may kill larger buckthorn saplings, but normally they will re-sprout. Burns conducted on a 2- to 3-year cycle can control buckthorn, but it also will control other woody species seedlings.

The control of buckthorn is a continual process. Sites need to be visited on an annual basis, checked for missed trees, and searched for re-

established saplings. The amount of time and effort needed for re-treatments will decline over time, but should not be stopped: Buckthorn will reestablish if not monitored and controlled.

Buckthorn cannot be eliminated from all areas. When starting a buckthorn removal project consider the long-term commitment of the agency and/or volunteers. Prioritize your sites so that the highest quality habitats are treated first. It does no good to remove buckthorn from highly degraded sites if you do not work on the least degraded first.



Fig. 2. Root (or weed) wrench used to remove buckthorn.



Fig. 3. Cutting a large buckthorn with a chainsaw.

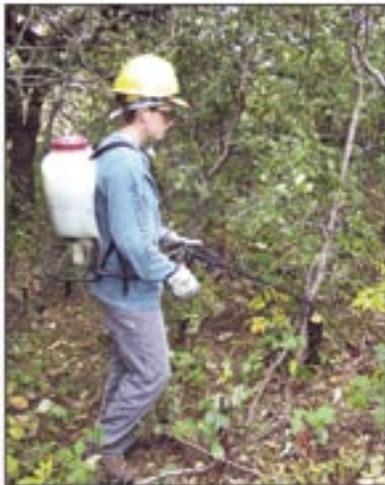


Fig. 4. Treating cut stumps with herbicide.

# DEVELOPING BIOLOGICAL CONTROL OF BUCKTHORNS

**André GASSMANN**

CABI Bioscience Switzerland Centre, Rue des Grillons 1  
CH-2800 Delémont, Switzerland

*Rhamnus cathartica* is a Eurasian species found throughout most of Europe, excluding most parts of Scandinavia and the Iberian Peninsula, and the extreme south. *Rhamnus cathartica* is also present in European Russia, in south-western Siberia, in the northern Caucasus, and in some parts of China. *Rhamnus cathartica* has been found mainly along forest margins and hedges in mesic to mesic-dry conditions. In Europe, *R. cathartica* prefers mesic to mesic-dry, warm, open or half-shaded habitats. It grows best in calcareous alkaline or neutral soils, but it can be found in humid or swampy areas.

*Frangula alnus* also is a Eurasian species, and is found in most parts of Europe, except the extreme north and part of the Mediterranean region. It is present in European Russia, in the southwestern and northern Caucasus, and in some parts of China. Thus, *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.

*Frangula alnus* grows in various open to half-shaded habitats. It prefers mesic to mesic-moist acid soils but it can be found in dry calcareous stands. *Frangula alnus* has been found almost exclusively in peat bogs and open acidiphilous forests. It is very rarely found in calcareous, drier stands. In general, the large-scale climate regions seem to play a much smaller role in defining the distribution of common and glossy buckthorns than local conditions and soil type. Neither common buckthorn nor glossy buckthorn are reported to be invasive in Europe.

Our project started in 2001 with an assessment of the potential for further work on biological control of buckthorn on behalf of the Minnesota Department of Natural Resources, and was based on work done by CABI Bioscience Switzerland Centre between 1964-66. Field work was carried out between 2002-04 in over 80 buckthorn sites in Europe and 900 individual field samples have been reared (Fig. 1).

The community of specialized arthropods associated with *R. cathartica* and *F. alnus* in Europe is largely dominated by Lepidoptera (22 species), followed by Hemiptera (6 species), Diptera (4 species), Coleoptera (1 species) and mites (2 species). One of the most conspicuous results is that the complex of specialized arthropods is much higher on *R. cathartica* than on *F. alnus*. Several species associated with *R. cathartica* have



Figure 1. Leaf damage by *Philereme vetulata* (Lep. Geometridae).

never been recorded on *F. alnus*, including: leaf mining moths *Calybites quadrisignella*, *Stigmella catharticella* and *S. rhamnella*; leaf feeding moths *Sorhagenia lophyrella*, *S. rhamniella* and *Philereme vetulata* (Fig.1, page 57); and jumping plant-lice *Cacopsylla rhamnicolla* and *Trichohermes walkeri* (Fig. 2). Other species have been reared only very occasionally from *F. alnus*. This is the case with the defoliating moths *Philereme transversata* and *Triphosa dubitata*.

In contrast, few species are exclusively associated with *F. alnus*, e.g. the leafhopper *Zygina suavis* and probably the fruit gall midges *Contarinia rhamni* and *Dasyneura frangulae* (literature records). Two other species, the defoliating Lepidoptera *Gonopteryx rhamni* and *Ancylis apicella*, prefer *F. alnus* to *R. cathartica*. A few species have been equally recorded on the two target buckthorn species, i.e. the leaf-mining moth *Bucculatrix frangutella*, the shoot-tip mining moth *Sorhagenia janiszewskae* and the stem-boring beetle *Oberea pedemontana* (Fig. 4). The root-boring moth *Synanthedon stomoxiformis* probably is associated with both target weeds in Europe as well.



Figure 2. Leaf margin gall by *Trichohermes walkeri* (Hom., Triozidae).

Several insects associated with *R. cathartica* also have been recorded on other buckthorn species, but either not, or only rarely, on *F. alnus*, including: *Sorhagenia lophyrella*, *Stigmella rhamnella*, *Philereme vetulata* and *Triphosa dubitata*). This suggests these species reject *F. alnus*, which has also been observed in sites where *R. cathartica* and *F. alnus* are present. Of the four buckthorn species which have been covered in our surveys in Europe, only *F. alnus* prefers moist habitats and acid soils. This suggests that habitat preference may have played a role in the evolutionary history of host plant preference of several insect species.

Several species seem to be specific to *R. cathartica*, including leaf mining moths *Calibytes quadrisignella* and *Stigmella catharticella*, the leaf feeding moth *Sorhagenia rhamniella*, and jumping plant lice *Trichohermes walkeri* and *Cacopsylla rhamnicolla*.

Insect species have been prioritised for further studies and preliminary host specificity studies according to their food niche, period of attack, visible damage to the host plant, potential availability, potential specificity, and ease of rearing (Figs. 3 and 4). Preliminary host suitability tests for larval development have been carried out with the leaf feeding moths *Ancylis apicella*, *A. derasana*, *Triphosa dubitata* and *Philereme vetulata*. In general, the assessment of field occurrence of the species tested matches the results of larval host suitability. For example, *Frangula alnus* and *F. caroliniana* were not suitable hosts for the leaf feeding geometrids *Triphosa dubitata* and *P. vetulata* which have not been found on *F. alnus* in most recent surveys.

In contrast, both *Rhamnus* and *Frangula* species are suitable hosts for the leaf feeding tortricid *Ancylis apicella*, which may have a slight preference for *F. alnus* in Europe. Larval development on *F. alnus* and *F. caroliniana* was very low for the congeneric species *A. derasana*, which has a clear preference for *R. cathartica* in Europe. Recent work by Bolmgren & Oxelman (2004) supports the generic separation of *Frangula* which represent a well-sup-

ported monophyletic sister clade to the rest of *Rhamnus* in its widest sense. Our preliminary host range studies with several insect species confirm that species in the genus *Frangula* are clearly separated from species in the genus *Rhamnus*.

## REFERENCES

Bolmgren, K. and B. Oxelman (2004) Generic limits in *Rhamnus* L. s.l. (Rhamnaceae) inferred from nuclear and chloroplast DNA sequence phylogenies. *Taxon* **53**: 383-390.



Figure 3. *Frangula alnus* damaged fruits.



Figure 4. *R. cathartica* attacked by *Oberea pedemeontana* (Col., Cerambycidae).

# SURVEY OF INSECT FAUNA ON COMMON BUCKTHORN, *RHAMNUS CATHARTICA*, IN MINNESOTA

Michele VAN VELDHUIZEN<sup>1</sup>, David W. RAGSDALE<sup>1</sup>, and Luke C. SKINNER<sup>2</sup>

<sup>1</sup>Department of Entomology, University of Minnesota  
1980 Folwell Ave, St Paul, MN 55108

<sup>2</sup>Minnesota Department of Natural Resources  
500 Lafayette Road, Box 25, St. Paul, MN 55155

## INTRODUCTION

Common buckthorn (*Rhamnus cathartica*) has become an invasive species in Minnesota forests and is categorized by the Minnesota Department of Agriculture as a restricted noxious weed. Managing buckthorn by cutting and treating stumps with herbicide and burning are too labor intensive to apply on a large scale basis and cannot be expected to control the spread of buckthorn, statewide. As a result, the Minnesota Department of Natural Resources has initiated a search for biological control agents for common buckthorn with the help of colleagues from the CABI Bioscience, Switzerland, Center. In Minnesota during the summer of 2004, we conducted a survey of the native or naturalized insect fauna associated with common buckthorn. The principal objective of the survey was to determine if a particular feeding niche on buckthorn is occupied by native or naturalized insects in North America. A second objective was to identify and quantify the density of generalist predators that might pose a biological impediment to successful establishment of candidate biological control agents through predation or parasitism. We collected all insects found on buckthorn and noted if they were consuming foliage or were simply present.

## MATERIALS AND METHODS

Seven sites in southeastern Minnesota were selected, three in an urban landscape, two in the Mississippi river basin, and two in agricultural landscapes. The three urban landscapes included: University of Minnesota St. Paul Campus (Ramsey County), Tierney Wood's and Hyland Park in the Three Rivers Park District (Hennepin County). The two Mississippi river basin sites were Battle Creek Park (Ramsey County) and Frontenac State Park (Goodhue County). The agricultural landscapes were University of Minnesota Outreach, Research- Experiment station (UMORE Park, Dakota County), and Courthouse Park (Waseca County), both located south of the Twin Cities metro area.

We repeatedly sampled the same 12 common buckthorn plants at each site throughout the summer by selecting four specimens in each of three size categories, small (<1m in height), medium (1-3m), and large (>3m). All branches or all reachable branches for large trees were visually surveyed every two weeks for insect fauna. Also at the same sites we surveyed 50 consecutive trees; 25 along each of two perpendicular transects. The first transect consisted of buckthorn trees growing along a path, roadway, or other opening where buckthorn had

full exposure to the sun. The second transect was perpendicular to the first transect, and sampled plants were growing in the under-story in shade or filtered sunlight. All trees selected were visually sampled for approximately one minute and all insects found were collected and identified.

Any immature insect or eggs found in either survey were returned to the laboratory for rearing. Any emerging adult was pinned for later identification. Records were maintained for each specimen that included the specific site, date collected, and individual tree number. Soft bodied insects and any immature insects that failed to reach the adult stage during rearing were preserved in vials containing 70% ethanol.

## RESULTS AND DISCUSSION

From all locations we collected approximately 500 adult insects encompassing 13 orders and 65 families. In an attempt to classify all specimens we categorized specimens into 174 unique morpho-species; species determination is ongoing. Homoptera and Hymenoptera were the most common orders, with 204 specimens and 61 morpho-species. Hymenoptera consisted mostly of parasitoids simply using common buckthorn as a resting spot or searching for their host. Out of the 174 morpho-species, 68 are labeled as herbivores with only 5 species reared solely on buckthorn (Table 1). These five species are *Acanalonia conica* (Homoptera: Acanoloniidae), *Metcalfa pruinosa* (Homoptera: Flatidae), *Neoxabea bipunctata* (Orthoptera: Gryllidae), Tortricidae spp. (Lepidoptera), and *Gyponana quebecensis* (Homoptera: Cicadellidae). All five species are categorized as generalist herbivores that include Rhamnaceae in their host range. There was no substantial plant damage observed at any site. It appears that common buckthorn in Minnesota is a resource that could be exploited if a sufficiently host-specific herbivore could be identified in Europe.

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Table 1: Feeding type categories for insects collected on common buckthorn.

	Number of Families*	Number of Morpho-species
Herbivores	29 (6**)	68 (6**)
Predators	13	26
Scavengers/ Fungal feeders	13	22
Parasitoids	10	21
Unidentified or Unknown feeding preference		37
Total	65	174

\*Excludes some unsorted Psocoptera, Collembola, Lepidoptera.

\*\*Herbivores that do not feed on buckthorn.

Some of the more abundant species identified to date include *Metcalfa pruinosa* (Homoptera: Flatidae) (Fig. 1), *Harmonia axyridis* (Coleoptera: Coccinellidae) (Fig. 2), *Graphocephala coccinea* (Homoptera: Cicadellidae) (Fig. 3), and *Acanalonia conica* (Homoptera: Acanoloniidae) (Fig. 4). The three homopterans are herbivore generalists. *Harmonia axyridis* is a predator and is known to prefer an arboreal habitat.

Based on a single season of study, there does not appear to be any major herbivore present and common to all sites surveyed. Thus, we conclude that any biological control candidate from Europe will not have to compete for its niche if released in North America. However, given that one of the most abundant arthropods collected from buckthorn is an exotic Coccinellid, there is the potential of significant biotic resistance with the introduction and establishment of a biological control agent, depending on whether or not a vulnerable life stage is present during times of *H. axyridis* high density (Fig. 5). How much of an impediment *Harmonia axyridis* may be needs further study and analysis.



Figure 1. *Metcalfa pruinosa* (Homoptera: Flatidae), the most abundant species collected on common buckthorn.



Figure 2: *Harmonia axyridis* (Coleoptera: Coccinellidae), the second most abundant species and most abundant predator collected on common buckthorn.



Figure 3: *Graphocephala coccinea* (Homoptera: Cicadellidae), an abundant species collected on common buckthorn,.



Figure 4: *Acanalonia conica* (Homoptera: Acanaloniidae), and abundant insect collected on common buckthorn.

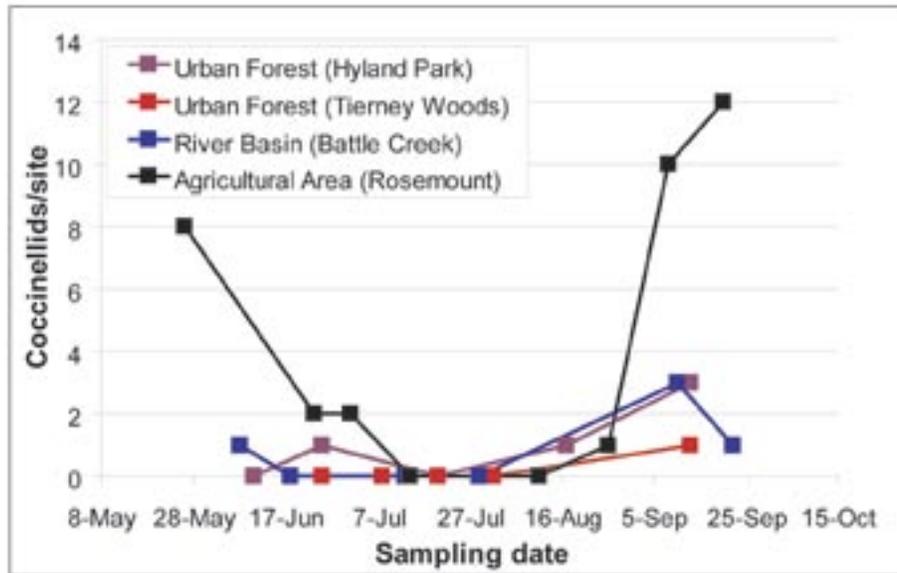


Figure 5: Abundance of Coccinellids collected at 4 sites (28 May – 25 Sep) .

# FUTURE NEEDS IN DEVELOPING BIOLOGICAL CONTROL FOR BUCKTHORN

Luke C. SKINNER<sup>1</sup> and David W. RAGSDALE<sup>2</sup>

<sup>1</sup>Minnesota Department of Natural Resources  
500 Lafayette Road, Box 25, St. Paul, MN 55155

<sup>2</sup>Department of Entomology, University of Minnesota<sup>1</sup>  
980 Folwell Ave, St Paul, MN 55108

To ensure that the current biological control research ongoing at CABI Bioscience Center in Switzerland (see chapter 14) continues to make progress, North American collaborators need to prioritize research needs. Currently these needs include:

1. Prioritizing which buckthorn species and associated insects are to be studied first.
2. Select and ship test plants in Rhamnaceae to CABI.
3. Continue host range testing of candidate insects found on buckthorn in Europe (see Gassmann, page 55).
4. Expand survey of insect fauna on buckthorn in North America (see VanVeldhuizen et al., page 58).

Initially, insect surveys in Europe were conducted simultaneously on common buckthorn, (*Rhamnus cathartica*), and glossy buckthorn (*Frangula alnus*). As these surveys were completed, it became evident that in Europe the insect communities are distinctly different between these two species, with more insect species associated with *R. cathartica* than *F. alnus*. A decision was made to concentrate further surveys on common buckthorn.

Choosing which insects to test first is not a simple task. With limited resources, care should be taken both to select insects that show selectivity towards the target plant, and to focus efforts on insects that might cause sufficient plant injury to result in control. To make this determination, we used data on the feeding niche, period of attack (Figure 1) and selectivity for the host plant. Of particular interest are internal and flower and fruit feeding insects. In reviewing the literature on control of woody plants it appears that mere defoliation may not be sufficient to control plants, but by reducing seed production we may be able to slow the spread of common buckthorn in North America. At this early phase of discovery of potential biocontrol agents we will not rule out any of the potential agents if they are sufficiently host specific.

Another urgent need is to develop a test plant list for host specificity work. Currently, CABI has only one North American *Rhamnus* species, *R. alnifolia*, and a single species of *Frangula*, *F. caroliniana*, for use in host range testing. The literature shows a total of eleven *Rhamnus* species and 21 *Frangula* species native to North America. All of these should be considered as high priority for host-range testing, along with many other closely related species. Andre Gassmann, CABI Bioscience, has proposed a test plant list, which provides a foundation for our current testing. One of the more difficult tasks is locating and collecting the test plant from North America. We rely on collaborators from various states to collect

and ship plants to us, which we in turn ship to CABI in Switzerland. In particular, there will be a need to collect multiple species in 2005-07.

Currently, we rely on CABI to carry out host specificity testing. As the program develops and as insects continue to be screened, there will be a need to expand host specificity testing, which must be conducted in a certified biosafety level 2 quarantine facility. As host specificity tests progress, it will be important to discuss with stakeholders what is acceptable and what is not acceptable in terms of potential feeding and development on non-target plants. These discussions will provide guidance to future research on potential control agents.

Lastly, faunistic studies need to be conducted to support the development of biological control of common buckthorn. It is important to document insect fauna found utilizing *R. cathartica* in North America. The main objective of these studies would be to determine whether particular feeding niches on buckthorn are occupied by native or naturalized insects. Surveys of *R. cathartica* currently ongoing in Minnesota (see chapter 15); however, it is important both to include *F. alnus* in the survey and expand the survey's geographical reach.

Although there is much work to be done, progress on all objectives is being made. Because less biological control of woody ornamentals has been attempted, a less robust track record of success is available when deciding which herbivores to use first. However, there is a wealth of information on perennial herbaceous plants that researchers can use to guide their decisions. We have come a long way in understanding what is possible for buckthorn biological control. It is anticipated that another 3-5 years of research will be needed before it is known how many insect herbivores can be considered as potential candidates for biological control of common buckthorn.

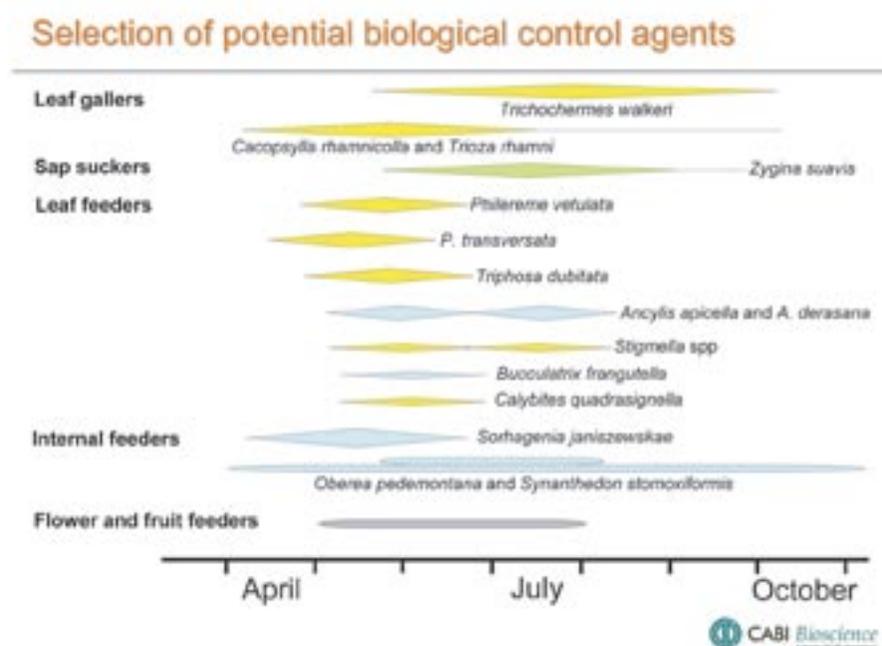


Figure 1. Potential buckthorn biological control agents by feeding niche and period of attack on buckthorn species in Europe.