United States Department of Agriculture

TECHNOLOGY
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Biological Control

BIOLOGY AND BIOLOGICAL CONTROL OF GARLIC MUSTARD

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FHTET-2012-05
Reprint August 2015

The Forest Technology Enterprise Team (FHTET) was created in 1995 by the Deputy Chief for State and Private Forestry, USDA Forest Service, to develop and deliver technologies to protect and improve the health of American forests. This manual was published by FHTET as part of the technology transfer series.

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Cover photos

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

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Biology and Biological Control of Garlic Mustard

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ACKNOWLEDGMENTS

We wish to acknowledge the authors of preceding issues in this series for contents included in this publication. We would also like to thank all of the photographers for providing such relevant and descriptive images.

We would also like to thank Wendy W. Harding for design and layout, and Richard Reardon, U.S. Forest Service, FHTET, for providing funding for the preparation and printing of this publication.

CONTENTS

List of Figures

LIST OF FIGURES (CONTINUED)

List of Figures (continued)

List of Tables

CHAPTER 1: INTRODUCTION

Invasive Plants

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

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

Classical Biological Control of Weeds

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

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

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

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

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

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

Host Specificity Testing

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

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

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

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

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

Code of Best Practices for Biological Control of Weeds

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

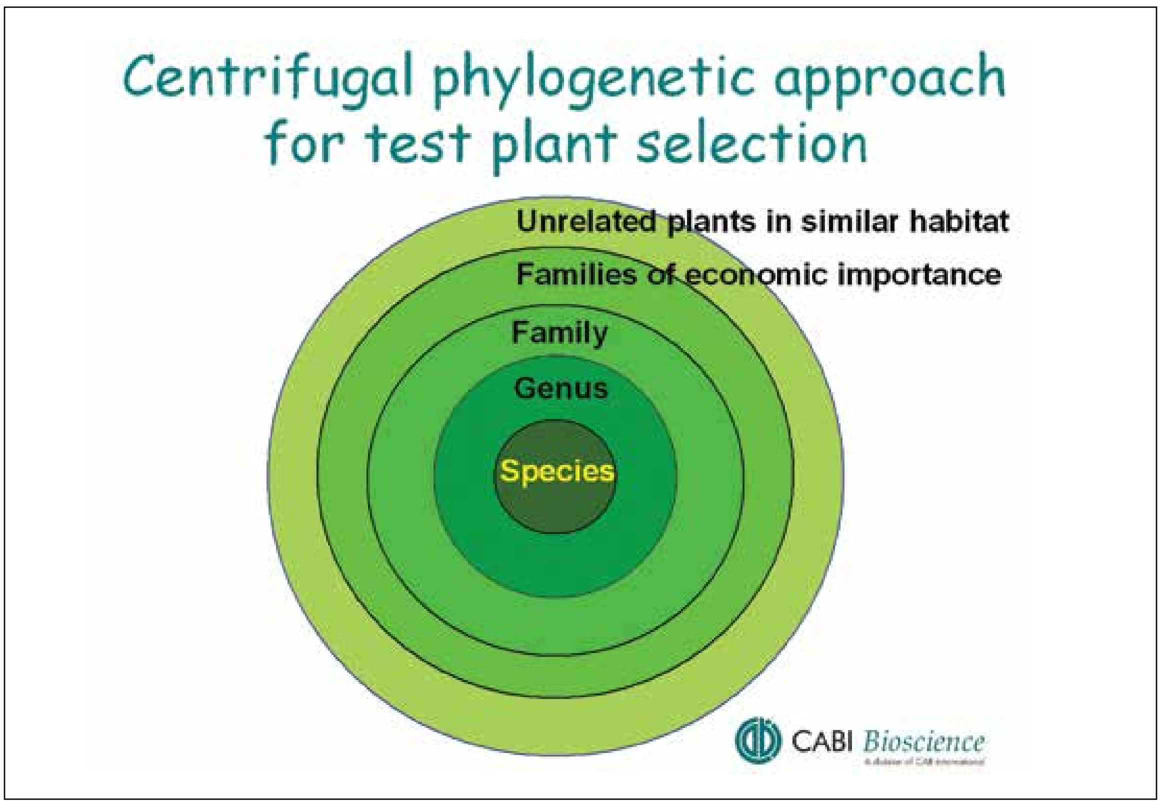

Figure 1-3. The Centrifugal Phylogenetic Approach for test plant selection provides a framework for host test plant selection. Plant species chosen for inclusion in host specificity testing start with those closely related to the invasive plant and expand to include plants less taxonomically related,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

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

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

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

About this Manual

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

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

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

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

Appendix 1. Monitoring Garlic Mustard Biological Agents

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CHAPTER 2: GETTING TO KNOW GARLIC MUSTARD

Description and Classification

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

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

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

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

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

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

Garlic Mustard Biology

Life History Overview

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

Description

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Garlic Mustard Distribution in North America

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

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

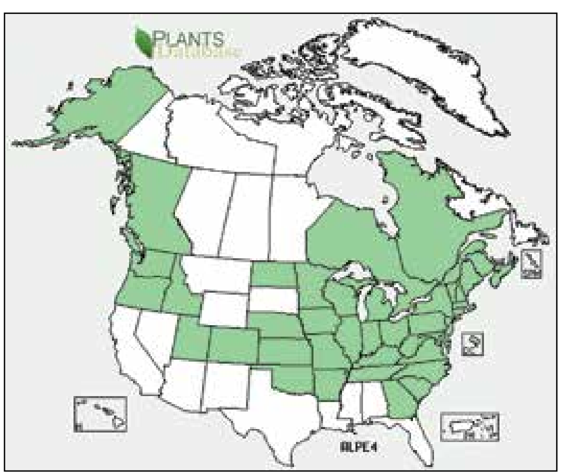

Figure 2-19. Garlic mustard is present in 37 states and 6 Canadian provinces. (USDA, NRCS. 2012. The PLANTS Database [http://plants.usda.gov], 6 November 2012. National Plant Data Team, Greensboro, NC 27401-4901 USA)

Garlic Mustard Biology and Ecology

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

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

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

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

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

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

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

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

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

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

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

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

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

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CHAPTER 3: BIOLOGY OF GARLIC MUSTARD BIOCONTROL AGENTS

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

Basic Insect Biology

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

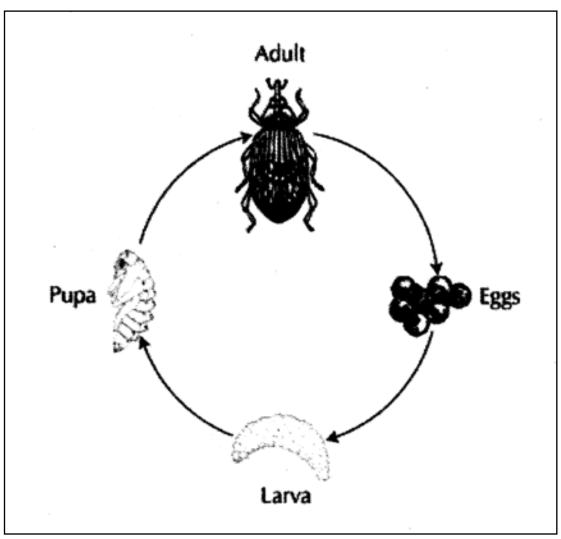

Figure 3-1. Garlic mustard biological control weevils have four life stages and complete metamorphosis. (bugwood.org)

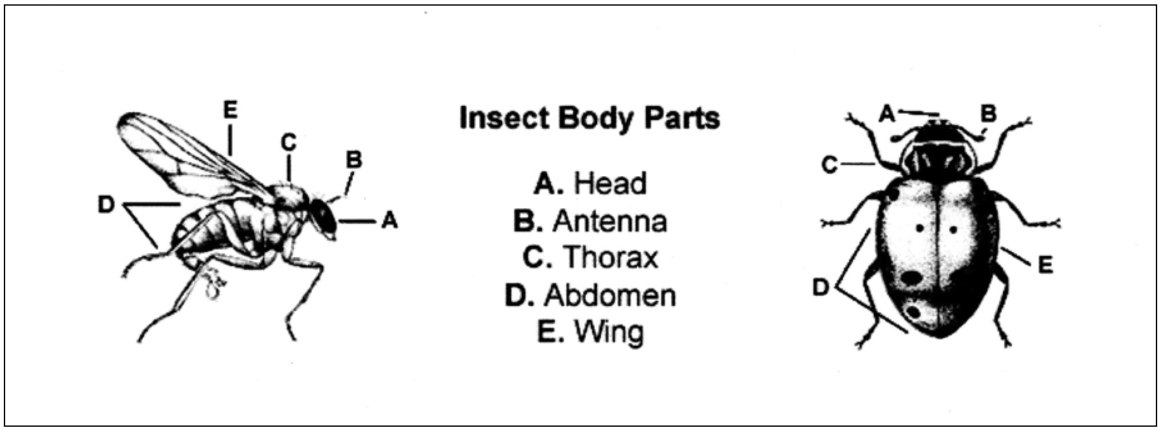

Figure 3-2. Generalized adult insect anatomy. (bugwood.org)

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

Insects and Garlic Mustard

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

Garlic Mustard Biocontrol Agents

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

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

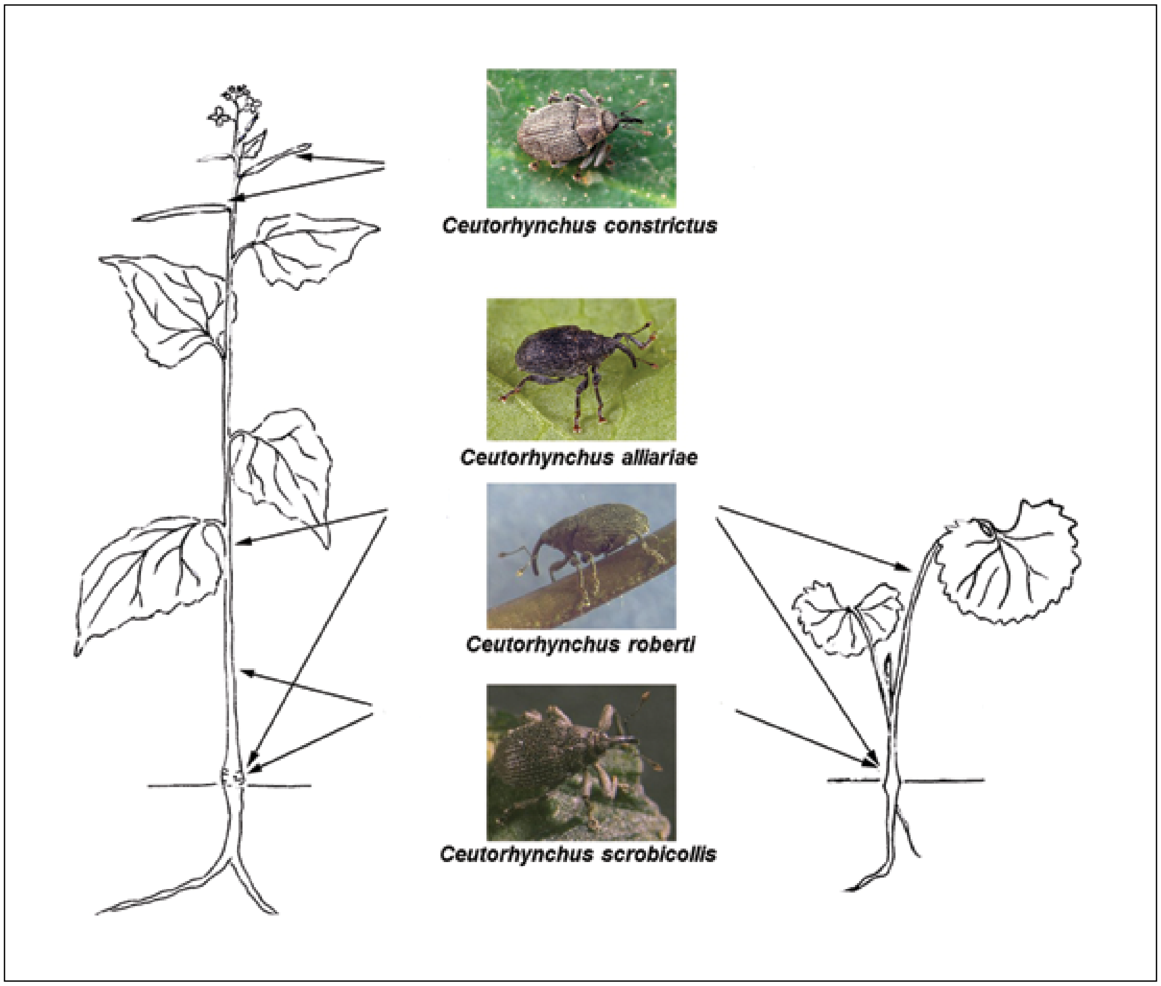

Figure 3-3. Four weevil species selected for biological control and their feeding niche on bolting plants (left) and rosettes (right) of garlic mustard. *Ceutorhynchus alliariae* and *C. roberti* have identical feeding niches.

Ceutorhynchus scrobicollis Nerensheimer & Wagner

Order: Coleoptera

Family: Curculionidae

Native Distribution

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

Original Source

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

Description

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

Life History

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

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

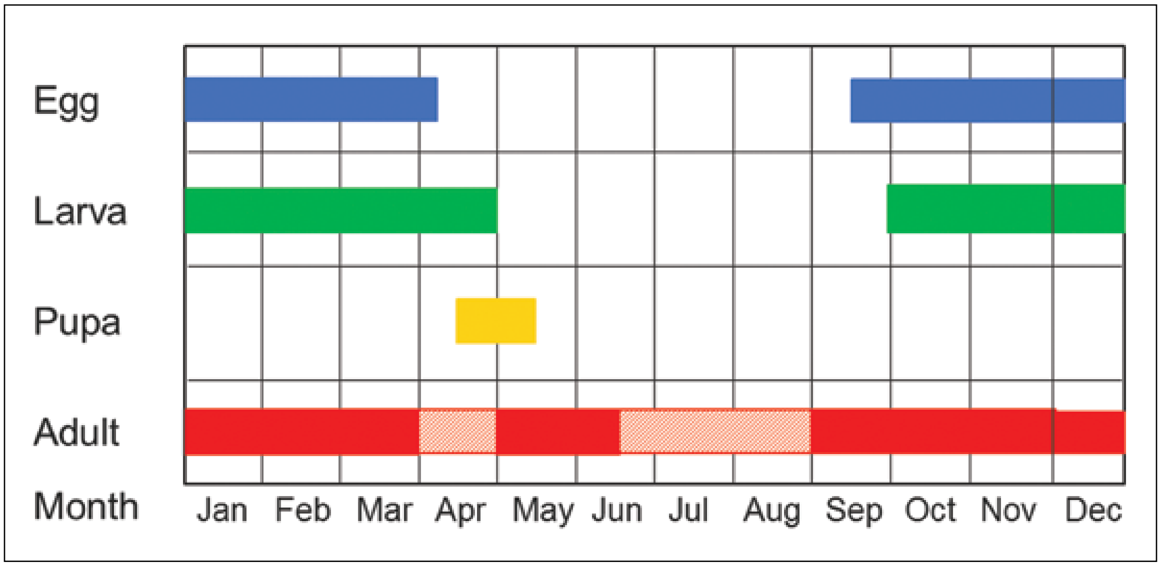

Figure 3-5. Life cycle of *Ceutorhynchus scrobicollis*. Bars indicate the approximate length for each life stage. Patterned bar for adults indicates periods without activity. (Esther Gerber, CABI)

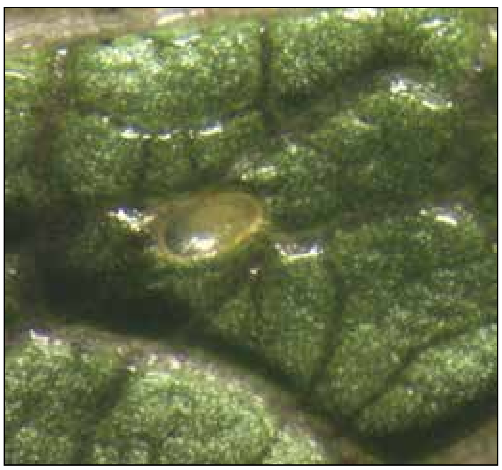

Figure 3-6. *Ceutorhynchus scrobicollis* egg laid into the leaf surface of garlic mustard. (Elizabeth Katovich, University of Minnesota)

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

Feeding Stage and Host Impact

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

Host Specificity

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

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

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

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

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

Ceutorhynchus constrictus (Marsham)

Order: Coleoptera

Family: Curculionidae

Native Distribution

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

Original Source

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

Description

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

Life History

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

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

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

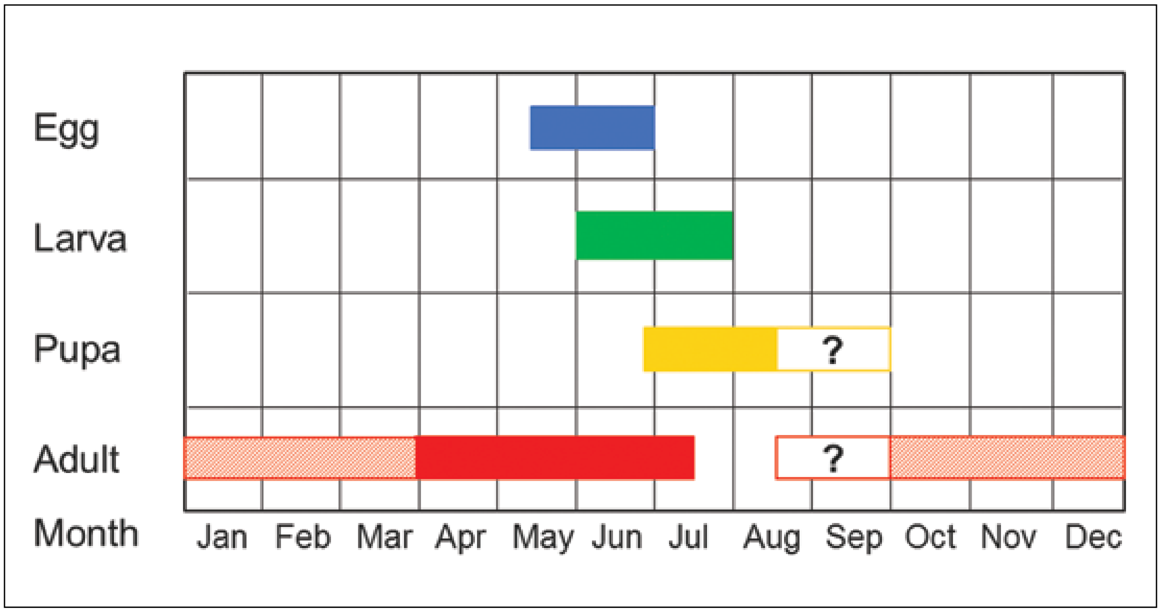

Figure 3-10. Life cycle of *Ceutorhynchus constrictus*. Bars indicate the approximate length for each life stage. Patterned bar for adults indicates period when fully developed adults remain inactive in the soil. (Esther Gerber, CABI)

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

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

Feeding Stage and Host Impact

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

Host Specificity

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

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

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

Comments

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

Ceutorhynchus alliariae Brisout

Order: Coleoptera

Family: Curculionidae

Native Distribution

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

Original Source

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

Description

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

Life History

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

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

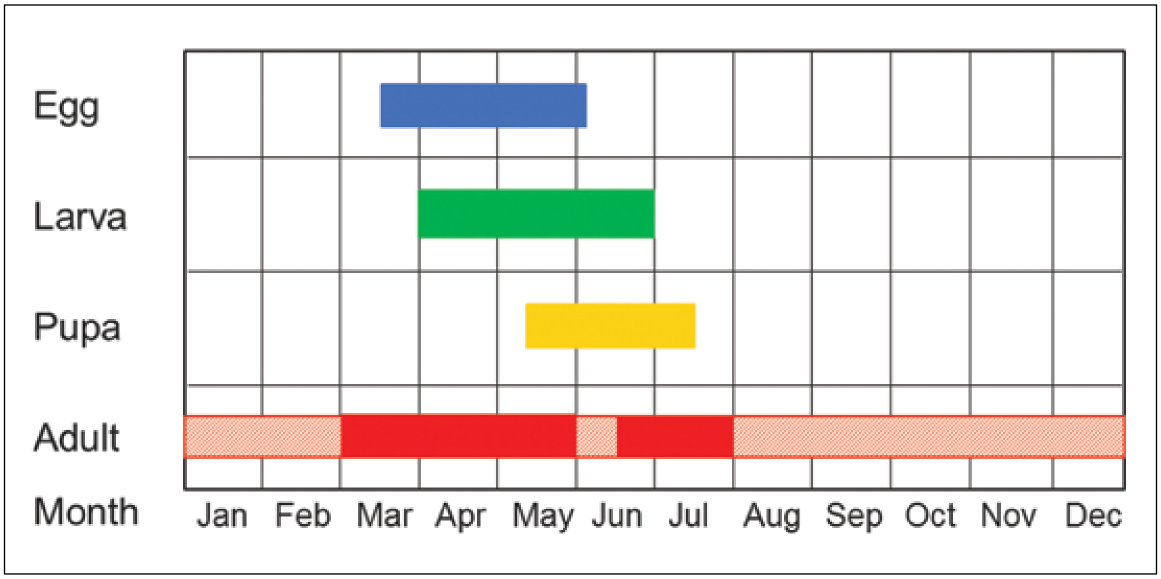

Figure 3-13. Life cycle of *Ceutorhynchus alliariae* and *C. roberti*. Bars indicate the approximate length for each life stage. Patterned bar for adults indicates periods without activity. (Esther Gerber, CABI)

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

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

Feeding Stage and Host Impact

Adult weevils feed on garlic mustard foliage. The most damaging stage is however the larval stage. In manipulative experiments, attack by *C. alliariae* caused a decrease in plant height and a reduction in seed output per plant. Larvae of the two stem-mining species, *C. alliariae* and *C. roberti*, cannot be distinguished morphologically and for this reason, attack from the range where both co-occur (sympatric range) almost certainly comprise both species. In field sites in the sympatric range, up to 30 larvae can be recorded in a single shoot. Plants with such high attack levels show clear signs of damage: attacked shoots desiccated and do not produce any seeds (Figure 3-15). In some cases, the whole plant can die. Up to 100 percent of shoots can be attacked by the shoot miners at a site.

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

Host Specificity

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

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

Comments

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

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

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

Ceutorhynchus roberti Gyllenhal

Order: Coleoptera

Family: Curculionidae

Native Distribution

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

Original Source

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

Description

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

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

Life History

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

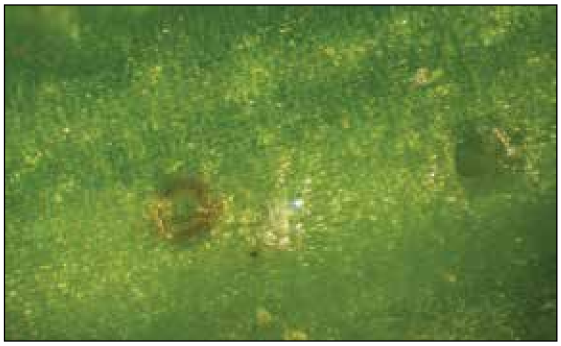

Figure 3-17. Feeding hole (right) and oviposition hole covered with secretion (left). (Tim Haye, CABI)

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

Feeding Stage andHost Impact

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

Host Specificity

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

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

Comments

See information given for *C. alliariae*.

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

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

Introduction to Integrated Weed Management (IWM)

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

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

Integrating the Biology of Garlic Mustard into Control Strategies

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

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

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

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

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

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

Integrating Biological Control Methods

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

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

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

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

Weed Control Methods Used to Manage Garlic Mustard

Education, Prevention and Early Detection, and Rapid Response

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

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

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

Biological Control

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

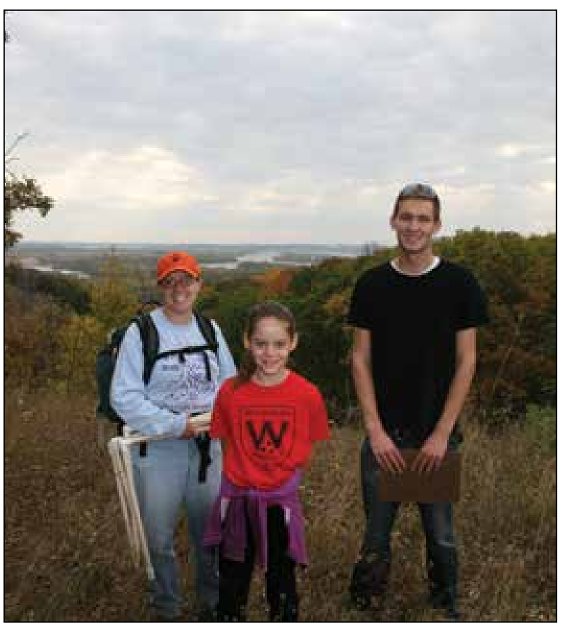

Figure 4-6. Monitoring crews can bring communities together and build support for control efforts. (Roger Becker, University of Minnesota)

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

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

Hand-pulling or Cutting and Mowing to Control Garlic Mustard

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

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

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

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

Prescribed Fire

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

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

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

Cultural Control

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

Herbicidal Control

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

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

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

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

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

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

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

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

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

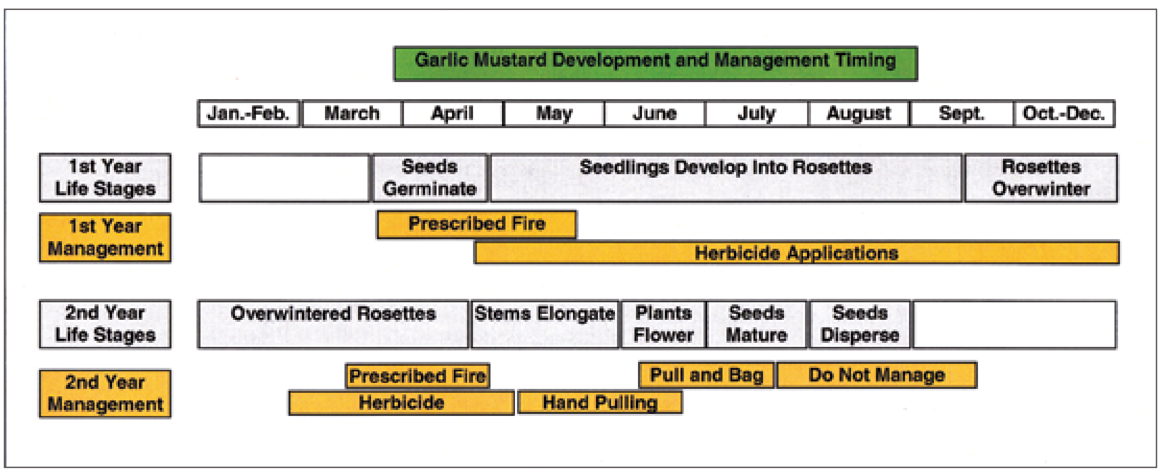

Figure 4-14. Schematic showing growth and development of garlic mustard and windows of opportunity for management. Note that many sites have predominately one life-cycle form present in a given year, and a few have both first and second year life-cycles present at the same time. Though best applied during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

Conclusion

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

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APPENDIX: MONITORING GARLIC MUSTARD BIOCONTROL AGENTS

Monitoring Garlic Mustard Biocontrol Agents

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

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

Garlic Mustard Monitoring Protocol

Garlic Mustard Monitoring Protocol
June 2003

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Contents:

Introduction

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

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

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

Site Selection and Quadrat Setup

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

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

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

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

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

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

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

Data Collection

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

Form 1: Garlic Mustard Biocontrol Monitoring (Site Location)

FORM 1: GARLIC MUSTARD biocontrol monitoring (site location)

Site Name:____________________________ State:________________ GPS: N _______º_____________’

Town: ____________________________ County: ______________ W _______º_____________’

Date: _________ __________ __________
year month day

CONTACT PERSON:

Name: ____________________________
Address ____________________________
City: ____________________________
State: ____________________________
Phone: _____ - ______ - __________
e-mail: ____________________________

LEGAL LANDOWNER:

Name: ____________________________
Address ____________________________
City: ____________________________
State: ____________________________
Phone: _____ - ______ - __________
e-mail: ____________________________

SITE CHARACTERISTICS:
Habitat type: ___Upland forest ___Floodplain forest ___Field ___Roadside ___ Other _____________

Road Map to Site

N

Site and Vegetation Map
Site

N

INSECT RELEASE HISTORY:

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

Form 1: Site Location, Background Information

Site Location:

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

Contact Person and Legal Landowner:

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

Site Characteristics:

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

Road Map:

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

Site and Vegetation Map:

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

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

Insect Release History:

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

Form 2a: Garlic Mustard Biocontrol Monitoring (Summer)

FORM 2a:

GARLIC MUSTARD BIOCONTROL MONITORING (Summer)

SITE: ______________________ STATE: __________

DATE: ________ ________ _____ GPS: N _____°_________'

year *month* *day* W _____°_________'

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

Investigators: Last name *First name*

Chart A: Percent cover & Leaf Attack

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

Chart B: Estimated Density

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

Notes: 0.5 m^2 quadrat

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

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

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

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

SITE: ____________________ STATE: __________

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

Notes:

Investigators: Last name *First name*

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

QUADRAT #

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

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

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

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

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

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

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

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

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

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

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

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

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

Form 2a:

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

Form 2b:

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

Form 3: Garlic Mustard Biocontrol Monitoring (Fall)

FORM 3:
GARLIC MUSTARD BIOCONTROL MONITORING (Fall)

SITE: ____________________ STATE: __________

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

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

Investigators: Last name *First name*

Chart A: Percent cover & Leaf Attack

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

Chart B: Estimated Density

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

Notes: 0.5 m^2 quadrat

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

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

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

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

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

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

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

Form 3: Fall Monitoring Quick Reference Guide

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

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