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Biology and Biological Control of Mile-a-Minute Weed

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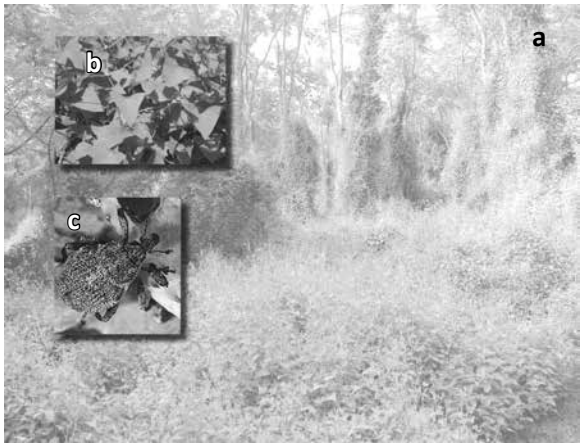

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b) Mile-a-minute weed, Judy Hough-Goldstein
c) Adult *Rhinoncomimus latipes*, Jon Gelhaus and Amy Diercks

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Introduction

Overview

Mile-a-minute weed (MAM), *Persicaria perfoliata* (L.) H. Gross (Fig. 1), is a member of the family Polygonaceae. It is an annual vine that can grow up to 6 meters long over the course of a season. It is widely distributed throughout east Asia, including Japan, China, Korea, India, Indonesia, Bangladesh, Siberia, Philippines, Malay Peninsula, Indochina Peninsula, Nepal, and Turkey (Wu et al. 2002). It was introduced to the northeastern United States in the mid-1930s from Japan, probably as seed unintentionally mixed in with holly seeds, and has since spread to thirteen states from New Hampshire to North Carolina, and the District of Columbia (Poindexter 2010, EDDMapS 2015, Fig. 2; note, MAM in New Hampshire has only been found near one nursery since 2011, and eradication efforts are continuing as of 2015; Douglas Cygan, personal communication).

Figure 1. Landscape infested with mile-a-minute weed. Inset, mile-a-minute terminal showing triangular leaf.

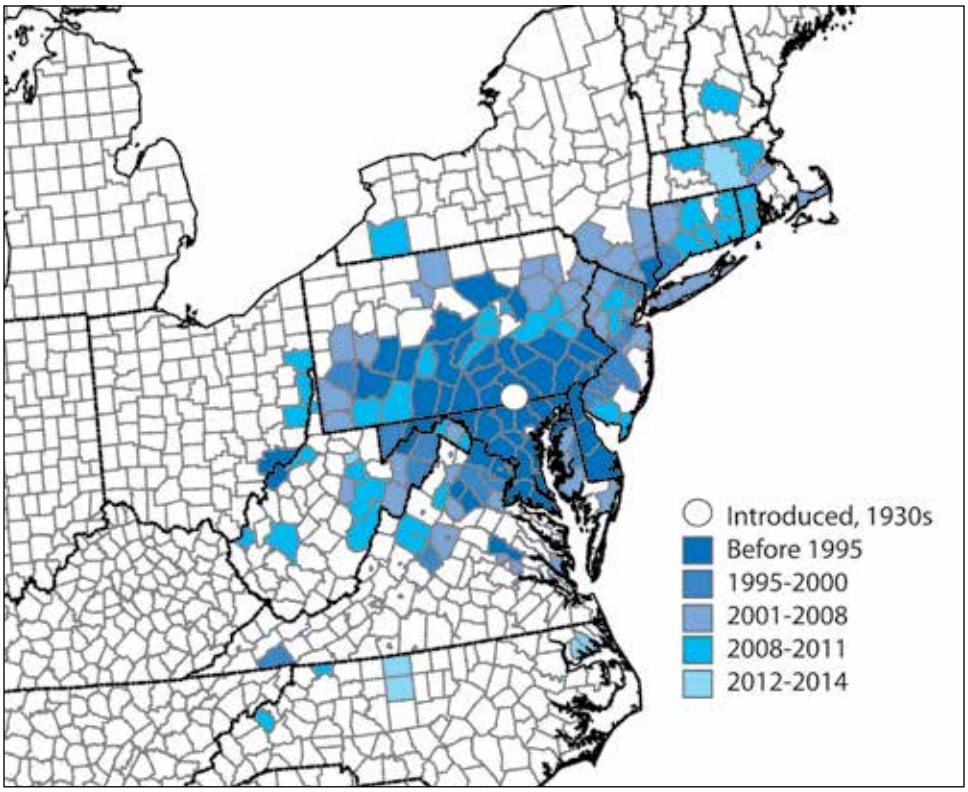

Figure 2. Counties in the United States in which mile-a-minute occurs.

Mile-a-minute invades disturbed areas, such as roadsides, stream banks, rights-of-way, openings in forested areas, and regeneration areas, and crowds out most native vegetation. At high densities it can create monocultures. In addition to the loss of native biodiversity, MAM is bothersome to people and their pets during outdoor activities because its stems and leaves are covered with recurved spines (Wu et al. 2002).

The seed remains viable in the seed bank in the soil for six years, so managing MAM successfully depends on yearly treatments. Herbicides and hand-pulling plants can be effective management methods, but these are difficult to accomplish on a landscape with intermittent MAM populations.

The biological control program for MAM began in 1996. That year, the United States Department of Agriculture (USDA) Forest Service, Forest Health Technology Enterprise Team (FHTET), together with the University of Delaware and the Chinese Academy of Agricultural Sciences, initiated surveys for natural enemies and host-range studies in China and the United States. In 2001, a colony of the weevil *Rhinoncomimus latipes* Korotyaev (initially misidentified as *Homorosoma chinensis* Wagner) was established in the USDA Agricultural

Research Service (ARS) quarantine facility in Newark, Delaware, to study its biology and life cycle. Host-range studies were initiated with input from the Technical Advisory Group for Biological Control Agents of Weeds (TAG), which represents the interests of a diverse group of Federal and other agencies. A petition for release in the U.S. was submitted to the USDA Animal and Plant Health Inspection Service (APHIS) in 2003, and approved in 2004. The New Jersey Department of Agriculture began mass rearing the weevil in 2004 and the first release was made in Delaware that same year. Subsequent releases have been made in New Jersey, Maryland, Pennsylvania, West Virginia, Connecticut, New York, Rhode Island, Virginia, Massachusetts, and North Carolina.

Biological Control of Weeds

Problems caused by exotic invasive plants have increased dramatically in recent decades. In the U.S., it is estimated that invasive plant species comprise 8-47% of the total flora of most states (Rejmánek and Randall 1994). Many possess characteristics that favor their population increases, and have no natural enemies in their invaded range. So, once they become established, they are not easily suppressed or eliminated.

Classical biological control involves reconnecting exotic plants with specialized natural enemies from their native ranges. This process begins with surveys in the target plant's area of origin to discover candidate natural enemies, progresses through studies of the candidate's biology and host specificity, and culminates with the release and evaluation of an agent's damage to the target plant. Damages may limit weed growth or reproduction or facilitate secondary infection by pathogens, which in turn will reduce the weed's ability to compete with other plants. In the eastern United States, projects have targeted aquatic, pasture, and forest weeds (Van Driesche et al. 2010).

Biological control agents cannot be retrieved once they are released; therefore, they must be carefully selected and extensively studied before being approved for release (Wilson et al. 2004). The question often arises as to what these specialized enemies will eat once they have reduced the target weed population. Specialist insects have evolved over thousands of years to deal with specific secondary plant chemicals in their hosts, and generally cannot expand their range to feed on other plant species. An extensive process of pre-release testing is required to ensure that the candidate biological control agent is host-specific to the target weed and does not pose a risk to related native species or plants of economic importance. This process can accurately predict the host range of potential biological control agents (Pemberton 2000). However, because even the most effective biological control agent will only reduce, not eradicate, the target weed species, the long-term goal of any release is for both plant and insect populations to persist, but at relatively low levels. Reducing the dominance of the invasive weed may facilitate recovery of the native plant community.

There are advantages and disadvantages to classical biological control of weeds:

Advantages

- It is selective against a specific weed or closely related group of weeds.
- It can provide long-term control.
- Agents can disperse to areas not accessible to humans or equipment for control, or into areas that are too sensitive to manage with traditional techniques.
- The biological control agents are self-perpetuating, so there are no recurring acquisition, rearing, and reintroduction costs.

Disadvantages

- There are high initial program costs.
- It is not certain that the agents will be effective, and even effective agents will not work in every habitat or under all environmental conditions.
- There is a risk of unintended, adverse impacts on other plant species (non-target effects).
- Impacts on the target weed may not be noticed for five to ten years.

The USDA-APHIS Plant Protection and Quarantine (PPQ) and the Canadian Food Inspection Agency (CFIA) are responsible for authorizing the importation of biological control agents into their respective countries. Federal laws and regulations in the United States 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 (TAG) mentioned on the previous page is an expert committee with representatives from regulatory agencies, federal land management and environmental protection agencies from the United States, Canada and Mexico. TAG is concerned with the safety and potential impacts of prospective biological control agents. To that end it reviews all petitions to import new agents into the United States and makes recommendations to USDA-APHIS. Weed biological control researchers work closely with USDA-APHIS-PPQ and TAG to assess the environmental safety of potential weed biological control agents and programs. The Canadian counterpart to TAG is the Biological Control Review Committee (BCRC) (Bourchier et al. 2006). 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 on mile-a-minute weed and the biological control insect *Rhinoncomimus latipes*, and provides guidelines for the use of biological control as either a stand-alone tactic or as a component in an integrated MAM management program. The contents are:

Chapter 1 provides a detailed description of MAM, including taxonomy, description of the leaves, stems, flowers, seeds, and habitat, life history, and occurrence in the United States.

Chapter 2 provides the results of surveys for natural enemies of MAM in the United States, Japan, and China. It describes the weevil *R. latipes*, its biology, and host range studies.

Chapter 3 describes the mass-rearing, releases and spread of *R. latipes*, and its behavior and impacts on MAM in the United States.

Chapter 4 includes different methods for managing MAM as well as biological control and integrated weed management.

Glossary defines technical terms essential in using and communicating about MAM biological control.

References provide critical literature on MAM biology, ecology, and biological control. Only publications cited directly in this manual are listed.

Appendices

A. Mile-a-Minute Weed Monitoring Protocol and Forms.

B. Control of Mile-a-Minute Weed with the Mile-a-Minute Weevil, *Rhinoncomimus latipes*: Fact Sheet and Frequently Asked Questions.

What's New in This Edition

The 2015 edition includes updated information on the spread of mile-a-minute weed and its weevil herbivore in North America, and summaries of studies undertaken in our laboratory and others between 2008 and 2015. These include results from monitored release and control sites; a trial showing that the mile-a-minute weevil has maintained its host specificity under open field conditions, experiments comparing laboratory-reared and field weevils; and studies showing that cool wet conditions favor the weed over the weevil, while warm dry conditions favor the weevil. The weevils' preferences for edge sites and sunny habitats are documented, and also the fact that immature (green) mile-a-minute seeds can be viable, especially later in the season. Finally, we summarize and discuss studies showing that restoration planting along with weevil release can help suppress mile-a-minute weed and prevent the "invasive species treadmill."

Chapter 1: Getting To Know Mile-a-Minute Weed

Description and Classification

Mile-a-minute weed is an herbaceous, annual vine with stems that grow up to 6 meters long in one growing season. It has triangular leaves, and its stems, petioles and leaf veins are covered with small, backward-projecting, recurved prickles. Leaves are alternate, simple, and 2.5 to 7.5 centimeters long and wide. Ocreae (fused stipules that surround the stem at each leaf node) are found in many species in the family Polygonaceae; in MAM they flare widely into a saucer shape (Fig. 3). Flower buds, and later flowers and fruits, develop at the terminal tips. Flowers are small, green, and generally inconspicuous. The flowers give way to clusters of green berry-like fruits, which turn an iridescent blue-purple when mature (Fig. 4). Each fruit encloses a single, hard, shiny, black, seed, or achene.

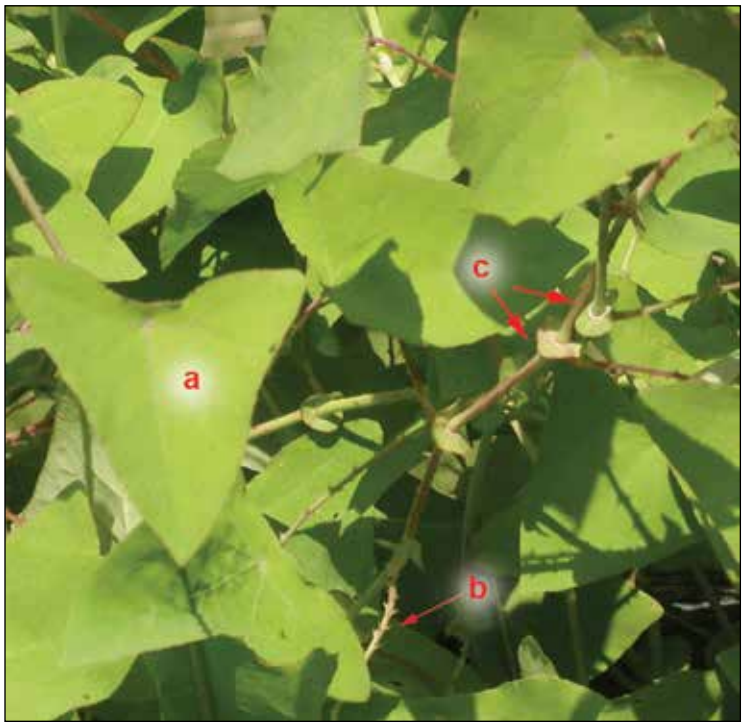

Figure 3. Mile-a-minute weed. Note triangular leaves (a), backward-projecting spines (b), and flared ocreae surrounding stems (c).

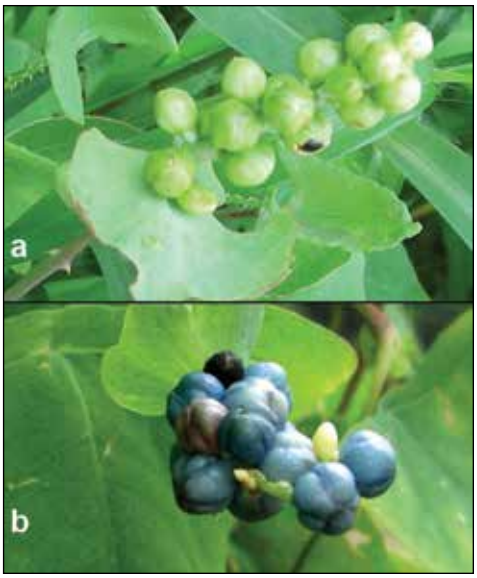

Figure 4. Immature (a) and mature (b) berry-like fruit clusters.

Mile-a-minute weed was long classified in the large genus *Polygonum*, as *P. perfoliatum* L. However, more recently most botanists in North America have agreed that this plant should be placed in the genus *Persicaria* (Hinds and Freeman 2005). Along with other "tearthumbs" (all of which have recurved prickles on their stems), this species is in the section Echinocaulon, and the scientific name is now *Persicaria perfoliata* (L.) H. Gross. The native North American species that are most closely related to *P. perfoliata* are *Persicaria sagittata* (L.) H. Gross (arrow-leaf tearthumb), and *Persicaria arifolia* (L.) Haraldson (halberd-leaf tearthumb). The smartweeds, which include both native and introduced species, are also now placed in the genus *Persicaria*.

Life History

In the mid-Atlantic United States, seeds germinate beginning in March or April (Fig. 5). Flowering begins in June or July and fruits may be produced beginning any time from June through August, probably depending on both site and weather conditions. Achenes are dispersed through human activities and by water, birds, deer and other mammals (see box: "Dispersal of Mile-a-Minute Seeds by Deer").

Ripe fruits not consumed by animals drop to the soil and many germinate under old plants the following spring. The seeds must go through a period of moist cold before they will germinate. Seeds can survive for multiple seasons and retain viability (see box: "Mile-a-Minute Seed Bank Persistence and Viability"). As an annual plant, the entire MAM plant dies with the first hard frost, generally in late October or early November in the Mid-Atlantic region.

Figure 5. Mile-a-minute seedlings in early spring.

Dispersal of Mile-a-Minute Seeds by Deer

Mile-a-minute (MAM) seed dispersal and germination can be facilitated by white-tailed deer, *Odocoileus virginianus* Zimm. Deer can consume large numbers of a wide variety of seeds while they forage (Myers et al. 2004, Vellend 2002). They may travel substantial distances before defecating, thus transporting seeds hundreds to thousands of meters, and even farther during seasonal migration. In one study, 64% of the plant species that germinated from seeds present in deer pellet samples were from non-native species (Myers et al. 2004).

As MAM plants mature, seeds ripen and plant stems get woodier near the terminals. Plants in the field appear to hold the seed clusters up and out over the mat of vegetation (personal observation, E. Lake). Often, these terminal fruit clusters are missing (Fig. 6), with only an ocrea and part of the stem left behind. Erica Dale and Ann Herzig (Bryn Mawr College, unpublished data) collected deer scat and searched the samples for MAM seed. Although large numbers of MAM seed fragments were found, many seeds passed through the gut intact. In 18 deer pellet groups collected in the fall of 1997 and 1998, an average of 17.6 intact MAM seeds were found per pellet group (range 1–111). A germination experiment demonstrated that 40% of MAM seed scarified via passage through deer gut was viable.

Figure 6. Animal browse on mile-a-minute terminal.

Mile-a-Minute Seed Bank Persistence and Viability

Judith A. Okay, former Riparian Specialist, Virginia Department of Forestry
and Chesapeake Bay Program, Annapolis, Maryland

To assess *P. perfoliata* seed bank longevity and persistence, two experiments were conducted using achenes collected in the 1997 growing season. The first was a temperature-controlled experiment using refrigeration to induce germination, and the second involved achenes buried in soil. Both tests ran from September 1997 through July 2003.

Temperature-controlled Test

A total of 264 achenes were placed on moist sponges in petri dishes in groups of about ten per dish. They were kept in an incubator without lights, and exposed to temperatures that simulated seasonal temperature changes, i.e., 1.7-2.8 °C (35 to 37 °F) through fall and winter (October through April), and 18.3-20.0 °C (65 to 68 °F) through spring and summer (May through September). Sponges were kept moist, and achenes were checked weekly for germination, defined as the protrusion of the radicle through the seed coat.

The majority of the seeds germinated during the period when they were exposed to cold temperatures, and most germinated during years one and two (Fig. 7). However, a small number of seeds continued to germinate each year through year six. By the end of year six, more than 99% of the seeds had germinated.

Mile-a-Minute Seed Bank Persistence and Viability (continued)

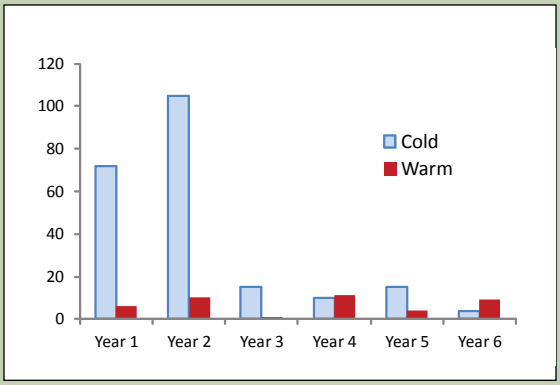

Figure 7. Numbers of mile-a-minute seeds germinating when kept cold (35-37 °F, Oct.-April) and when kept warm (65-68 °F, May-Sept.) from a single batch of 264 mile-a-minute weed achenes collected in 1997 (Year 1 = Oct. 1997-Sept. 1998).

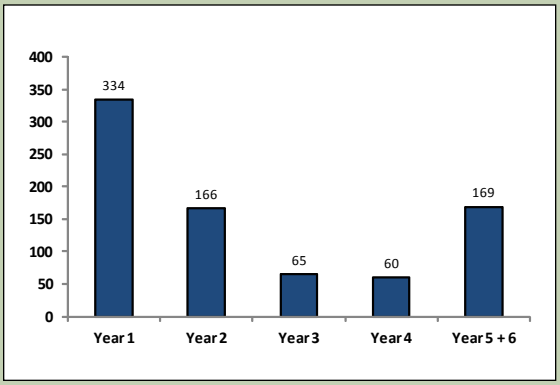

Figure 8. Number of seeds that had germinated under natural conditions, buried in mesh bags and exhumed each year (not checked in year 5).

Buried-seed Test

In October of 1997, 800 achenes were placed in four mesh bags (200 per bag), marked with orange survey flagging and buried side by side in a 3' x 3' plot at a depth of 5 to 6 inches in natural loamy-clay soil. The achenes were not watered or tended, but were left in the soil under natural conditions until the following spring. The mesh bags were exhumed each spring in late May or early June after a flush of *P. perfoliata* seedlings had emerged in the area of the test plot, indicating most germination had ceased. This was done each year from 1998 through 2001, and again in 2003. Undamaged achenes that had not germinated were counted, returned to the mesh bags, and reburied.

Of the 800 achenes buried in fall of 1997, over 40% germinated the following spring (Year 1, Fig. 8), and an additional 21% germinated the second year after burial. Most of the remaining seeds germinated at a lower rate over the next four years. By 2003 (Year 6), 99.3% of the buried seeds had germinated.

Conclusions

The seasonal dormancy observed in both the temperature-controlled test and the buried-seed test is a common response for summer annuals, which produce seeds that generally go dormant in response to the high temperatures of late summer and early fall, and germinate only during the cooler conditions of early spring. The need for a period of cold-wet stratification to break seed dormancy in *P. perfoliata* has been shown by others (summarized by Colpetzer and Hough-Goldstein 2004), but this is the first test to show this pattern continuing over multiple years with a single batch of seeds exposed sequentially to a 7-month cold period and a 5-month warm period.

In both experiments, *P. perfoliata* seed persisted and remained viable in the seed bank for 6 years following collection, although most of the seed germinated during the first and second year. Van Clef and Stiles (2001) reported 32.6% viability of MAM seed that had been buried for three years, but did not test longer periods. The results presented here suggest natural resource managers attempting to control *P. perfoliata* should plan to continue control efforts for a minimum of six growing seasons, because viable seed is likely to persist in the seed bank for at least that long.

Distribution

Mile-a-minute weed is indigenous to, and widely distributed in, Asia. It was first reported in the United States near Portland, Oregon, in the 1890s, but apparently did not establish west of the Rocky Mountains. The plant was introduced into the eastern United States in the mid-1930s at the Gable Nursery in Stewartstown, Pennsylvania, probably with holly seeds from Japan (Moul 1948). Analysis of random amplified polymorphic DNA (RAPD) profiles of MAM populations from China, Japan, Korea and the eastern United States support the suspected single introduction and Japanese origin of the eastern U.S. population (Shuppert 2001). No genetic variation was detected among populations in North America, suggesting an effectively clonal population. Specimens from the U.S. sites more closely resembled MAM from Japan than those collected in China and Korea, further supporting the likely Japanese provenance of the U.S. population.

Before 1980, MAM was limited to five counties in Pennsylvania and parts of Maryland. By 1995 it had been reported in 51 counties in seven states plus the District of Columbia (Fig. 2). An additional 19 counties, some in two new states, Connecticut and New Jersey, were added by 2000, and another 41 counties and one new state, Massachusetts, were added between 2001 and 2008. By the end of 2014, MAM had been found in three additional states, Rhode Island, North Carolina, and New Hampshire (although, as noted above, the population in New Hampshire may still be eradicated), and in additional counties in other states (Fig. 2). Other states in plant hardiness zones 6 and 7 are thought to be vulnerable to invasion by MAM in areas where adequate moisture is available (Okay 1997). It is not likely that the eastern U.S. population of MAM will progress into more tropical climates because those zones lack the cold vernalization period needed to break achene dormancy and stimulate germination.

In the United States, MAM is a weed of parks, preserves, conservation easements, nursery crops, orchards, roadsides, drainage ditches and rights-of-way. Although it prefers low wet ground and full sun, it will tolerate semi-shade. Mile-a-minute appears to be more restricted to moist flood plains in Japan and China than in the United States.

Chapter 2: Mile-a-Minute Weed Biological Control Agents

Basic Insect Biology

Insects are a very large, diverse class of animals. Knowing basic insect anatomy and biology can help land managers recognize and identify biological control insects in the field. Adult insects have several unique characteristics: 1) an exoskeleton (external skeleton), 2) a segmented body comprising three distinct regions: head, thorax, and abdomen, and 3) three pairs of legs (Fig. 9). The biological control agent for mile-a-minute has a life cycle with four distinct stages: egg, larva, pupa, and adult (Fig. 10). This form of development is called complete metamorphosis.

Immature insects also have an external skeleton that they must shed in order to grow. The process of shedding the exoskeleton is called molting. The stage of the insect between successive molts is called an instar. As larvae, insects generally complete three to five molts. The mature larva then molts into a pupa, the non-feeding stage when the insect changes from a larva to an adult.

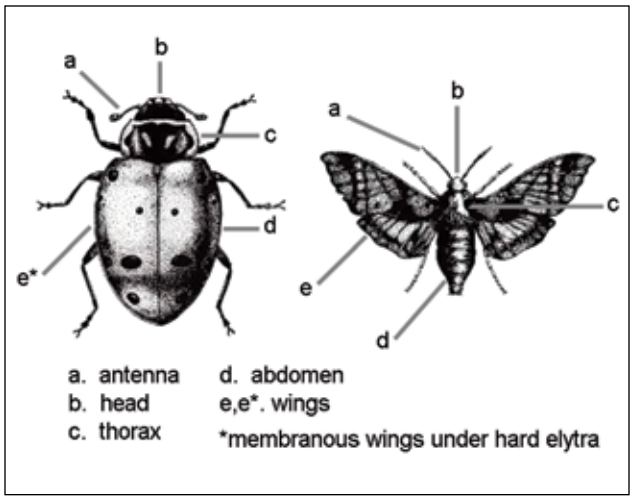

Figure 9. Generalized adult insect anatomy.

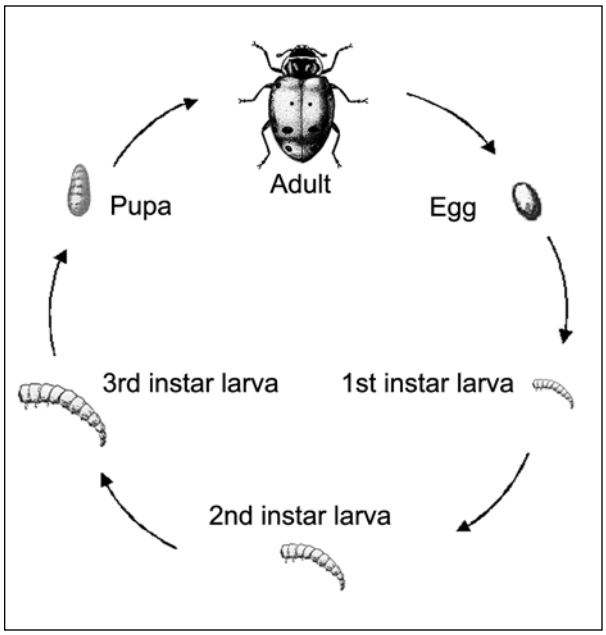

Figure 10. Complete metamorphosis.

Insects Found on Mile-a-Minute Weed in the United States

One of the earliest surveys for natural enemies associated with mile-a-minute weed was conducted by Wheeler and Mengel (1984) in south central Pennsylvania in 1981 through 1983. They recovered more than 30 insect species (five orders, 15 families) that developed on MAM and 12 species that appeared to use the plant only for adult feeding. Feeding by these 30 species caused only minor damage. There were no leafminers, stem borers, internal fruit feeders, or gall makers. Three relatively oligophagous species were identified: *Lithacodia* [now *Pseudeustrotia*] *carneola* Guenee (Lepidoptera: Noctuidae), *Calothysanis amaturaria* Walker (Lepidoptera: Geometridae), and *Ametastegia* sp. (Hymenoptera: Tenthridinidae). All three species were rare.

Two surveys were initiated in the late 1990s in the eastern United States in an effort to identify native natural enemies of MAM and their relative effectiveness prior to the release of exotic species of natural enemies. The first was conducted by Jim Fredericks, M.S. student at the University of Delaware. Fredericks surveyed MAM populations in White Clay Creek State Park in New Castle County, Delaware; Elk Neck State Park in Cecil County, Maryland; Eastern Neck Island in Kent County, Maryland; and Pennypack Park in Philadelphia County, Pennsylvania in 1997 (Fredericks 2001). He collected insects associated with MAM once a week from June through October. He collected a total of 35 insect species, 21 of which were not previously reported to be associated with the plant. No internal stem or seed feeders were identified, supporting the observations of Wheeler and Mengel (1984). Fredericks (2001) did not recover the three oligophagous species recovered by Wheeler and Mengel (1984). Fredericks attempted to rear *C. amaturaria* on MAM, but the larvae failed to feed and died.

The second was a broader survey of various habitats that documented the accumulation of natural enemy species and their associated damage on MAM and evaluated their potential as biological control agents. This effort was conducted in Pennsylvania, Maryland, Delaware, and Virginia, from 1997 through 2000. The results of this broader survey are reported here.

Materials and Methods

In 1997, 37 sites of various sizes and habitats containing MAM were located by the State Departments of Agriculture or Forestry in Delaware (2 sites), Maryland (16 sites), Pennsylvania (8 sites) and Virginia (11 sites). The center of each site was marked with a 6-foot metal stake, a photo was taken to represent the density of MAM, and GPS coordinates were recorded. Additional data for each site, including abundance of MAM, habitat type, and other plant species growing in association with *P. perfoliata*, were recorded. Each site was visited once every two weeks from June through September and insects were either hand-picked or aspirated from MAM plants, or collected by shaking plants over a white sheet. Most of the insects were collected on the leaves; other parts of the plants were also examined in an attempt to recover root borers, stem borers, and internal fruit feeders. Type and severity of damage and the plant parts affected were also

recorded. Attempts were made to keep immature Lepidoptera alive and rear them to maturity; adult Lepidoptera were placed in a kill jar, and all other insect specimens were placed in 70% ethyl alcohol (ethanol). Field collectors provided initial taxonomic identification to family prior to submitting the completed forms and insect specimens to research associates in the Entomology Department at West Virginia University, who, in turn and when possible, provided the initial identification to genus and species. Identifications to genus and species were then confirmed by taxonomic specialists, including Drs. Linda Butler (Lepidoptera), John Strazanac (Orthoptera), Dave Smith (Symphyta), Shawn Clark (Coleoptera), and Charles Bartlett (Hemiptera, suborder Auchenorrhyncha). Portions of the sample areas were monitored again in 1998 (24 sites), 1999 (19 sites) and 2000 (13 sites).

Results

During the four-year study, more than 2,000 specimens were recovered from *P. perfoliata*, representing seven orders and 110 families. However, many of these were known to be non-herbivores. Abundantly recovered phytophagous species were the oriental beetle, *Anomala orientalis* Waterhouse (Coleoptera: Scarabaeidae); Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae); and meadow grasshoppers, *Conocephalus* spp. (Orthoptera: Tettigoniidae). Table 1 shows insects recovered from *P. perfoliata* that were likely to be phytophagous on MAM, including polyphagous insects that are known to feed on *P. perfoliata*, polyphagous insects that are known to feed on *Persicaria* or *Polygonum* species, and very polyphagous insects that might feed on species in these genera or related plants. Seventeen species of insects were common to this survey and the one conducted by Wheeler and Mengel (1984). Larvae of the fall webworm, *Hyphantria cunea* (Drury), were recovered in both surveys in the United States and this was the only species also recovered from MAM in China and in Japan (Miura et al. 2008).

Numerous insect species were observed on, or collected from, MAM, although most were not actually observed either feeding on or causing damage to *P. perfoliata*. Those few insect species that were observed damaging MAM plants were polyphagous species that either might or are known to feed on Polygonaceae or related plants. Of these species, the most abundantly recovered phytophagous species was the Japanese beetle, *P. japonica*, followed by, in decreasing order of abundance:

- tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) [Hemiptera, suborder Heteroptera: Miridae]
- potato aphid, *Macrosiphum euphorbiae* (Thomas) [Hemiptera, suborder Sternorrhyncha: Aphididae]
- locust leafminer, *Odontota dorsalis* (Thunberg) [Coleoptera: Hispinae]
- a small stink bug, *Mormidea lugens* (F.) [Hemiptera, suborder Heteroptera: Pentatomidae]

Table 1. Herbivorous insects collected from *P. perfoliata* in DE, MD, PA, and VA, 1997-2000.

Order / Family	Species	Rel. Freq.[a]	Part sun		Full sun		Other surveys[b]
			Wet	Dry	Wet	Dry	
Polyphagous species known to feed on mile-a-minute weed							
Coleoptera							
Chrysomelidae	*Odontota dorsalis* (Thunberg)	R	X	X			W
Scarabaeidae	*Anomala orientalis* Waterhouse	C			X		
	Popillia japonica Newman	C	X	X	X	X	W
Hemiptera, suborder Heteroptera							
Miridae	*Halticus bractatus* (Say)	R	X	X			
	Lygus lineolaris (Palisot de Beauvois)	O	X	X	X	X	W
Pentatomidae	*Acrosternum hilare* (Say)	R	X	X	X	X	W
	Euschistus servus (Say)	R		X	X	X	
	Euschistus tristigmus (Say)	R	X	X		X	W
Hemiptera, suborder Auchenorrhyncha							
Acanaloniidae	*Acanalonia bivittata* (Say)	R	X	X		X	W
Cicadellidae	*Graphocephala coccinea* (Forster)	R	X	X	X	X	W
	Graphocephala versuta (Say)	O		X	X	X	W
Flatidae	*Metcalfa pruinosa* (Say)	R	X	X	X	X	W
Hemiptera, suborder Sternorrhyncha							
Aphididae	*Macrosiphum euphorbiae* (Thomas)	R	X	X	X	X	W
Lepidoptera							
Arctiidae	*Estigmene acrea* (Drury)	R		X			W
	Spilosoma virginica (F.)	R		X		X	W
Geometridae	*Calothysanis amaturaria* (Walker)	R			X		W
Noctuidae	*Palthis asopialis* (Guenée)	O	X	X		X	
Orthoptera Acrididae	*Melanoplus differentialis* (Thomas)	R	X	X		X	W
Tettigoniidae	*Amblycorypha oblongifolia* (DeGeer)	R	X	X		X	W
	Amblycorypha rotundifolia (Scudder)	R					
	Atlanticus sp.	R	X	X		X	
	Conocephalus brevipennis (Scudder)	R					
	Conocephalus sp.	C	X	X	X	X	
	Scudderia furcata Brunner	R	X	X		X	W
Polyphagous species known to feed on Polygonaceae							
Coleoptera							
Chrysomelidae	*Diabrotica undecimpunctata* Mannerheim	R	X	X			W
	Diachus auratus (F.)	R	X	X		X	
	Disonycha glabrata (F.)	R			X		
	Luperaltica senilis (Say)	R	X	X	X	X	

[a]Relative frequency: R, rare, taken at one or two sites in one state, usually in small numbers; O, occasionally collected at 2 or more sites in one or two states; C, common, taken at most sites in more than two states.

[b]Other surveys: D, also listed as associated with MAM in China (Ding et al. 2004); W, also listed as associated with MAM in Pennsylvania (Wheeler and Mengel 1984).

Table 1 (continued)**.** Herbivorous insects collected from *P. perfoliata* in DE, MD, PA, and VA, 1997-2000.

Order / Family	Species	Rel. Freq.[a]	Part sun		Full sun		Other surveys[b]
			Wet	Dry	Wet	Dry	
Hemiptera, suborder Heteroptera							
Miridae	*Halticus* sp.	R	X	X		X	
Thyreocoridae	*Corimelaena* sp.	R	X	X			
Lepidoptera Arctiidae	*Pyrrharctia isabella* (Smith)	R	X				
Geometridae	*Prochoerodes tranversata* (Drury)	R		X			
Tortricidae	*Sparganothis sulfureana* (Clemens)	R				X	
Orthoptera Acrididae	*Melanoplus sanguinipes* (F.)	R	X	X		X	
Tettigoniidae	*Microcentrum* sp.	R		X			
Very polyphagous species that might feed on Polygonaceae							
Coleoptera							
Chrysomelidae	*Epitrix fuscula* Crotch	R				X	
	Oulema sayi (Crotch)	R	X	X		X	
Curculionidae	*Mylloceru hilleri* Faust	R	X	X		X	
	Otiorhynchus ovatus (L.)	R	X	X	X	X	
Hemiptera, suborder Heteroptera							
Berytidae	*Jalysus* sp.	R	X	X		X	
	Neides muticus (Say)	R	X	X		X	
Coreidae	*Leptoglossus* sp.	R	X		X	X	
Cydnidae	*Sehirus cinctus* (Palisot de Beauvois)	R	X	X			
Miridae	*Adelphocoris* sp.	R	X		X	X	
	Stenodema trispinosa Reuter	R	X		X	X	
	Stenodema vicinum (Provancher)	R	X		X	X	
Pentatomidae	*Holcostethus limbolarius* (Stal)	R				X	
	Menecles sp.	R	X	X		X	
	Mormidea lugens (F.)	R	X	X	X	X	
	Nezara sp.	R	X	X	X	X	
Rhopalidae	*Arhyssus* sp.	R			X		
Hemiptera, suborder Auchenorrhyncha							
Cercopidae	*Philaenus spumarius* (L.)	R	X	X	X	X	
Cicadellidae	*Draeculacephala mollipes* (Say)	O	X	X	X	X	
	Oncometopia orbona (F.)	O		X			
	Paraulacizes irrorata (F.)	R	X	X		X	
	Tylozygus bifidus (Say)	R	X	X		X	
Membracidae	*Entylia carinata* (Forster)	C				X	
Hemiptera, suborder Sternorrhyncha							
Aphididae	*Aulacorthum solani* (Kaltenbach)	R		X			
Lepidoptera Arctiidae	*Hyphantria cunea* (Drury)	R		X			D, W

[a]Relative frequency: R, rare, taken at one or two sites in one state, usually in small numbers; O, occasionally collected at 2 or more sites in one or two states; C, common, taken at most sites in more than two states.

[b]Other surveys: D, also listed as associated with MAM in China (Ding et al. 2004); W, also listed as associated with MAM in Pennsylvania (Wheeler and Mengel 1984).

During August, *P. japonica* adults were especially abundant on all of the sites in each of the four states. In some areas, the adults defoliated 80 to 100% of the MAM plants in a localized area as well as individual plants. The defoliated plants recovered and continued to grow and produce quantities of viable seed.

The habitat of each site was recorded as being either partly sunny dry, partly sunny wet, full sun dry, or full sun wet. In its native range, *P. perfoliata* seems to persistently occupy wet sites (e.g., edges of creeks and rivers) whereas in this survey it occupied both wet sites and drier upland sites (e.g., roadsides, forest edges) (Table 1). In the upland sites, organic matter (leaves, plant material, etc.) may be required to enhance seed germination and/or to keep the shallow root system moist and cool (Mountain 1989). Many of the other plant species associated with MAM are also considered invasive weeds, including Japanese stiltgrass (*Microstegium vimineum* (Trin) A. Camus), multiflora rose (*Rosa multiflora* Thunb. ex Murr.), crownvetch (*Coronilla varia* L. (Fabaceae)), Canada thistle (*Cirsium arvense* (L.) Scop.), and garlic mustard (*Allaria petiolata* (Bieb.) Cavara & Grande).

Discussion

In this survey there was no evidence of seed or root feeders even though adults of several families of Coleoptera (e.g., Elateridae, Scarabaeidae) were recovered, and their immatures are associated with polyphagous root feeding. Many taxa were recovered from MAM foliage but few were associated with herbivory. Aphids were recovered on leaves and stems of many plants but the damage was minimal (less than 1%) on individual plants. Obviously, there has been an accumulation of taxa on MAM but at least 90% are transient or highly polyphagous.

Persicaria perfoliata appeared to be equally abundant in moist and dry sites in this survey, although the quantity of organic matter might be a critical factor on the drier sites. In its native range, MAM is associated with moist sites, where populations may be regulated by seasonal flooding as well as natural enemies (Hyatt and Araki 2006).

Under optimal conditions plants can compensate for the negative effects of herbivory; therefore, both the timing and duration of defoliation are important factors in regulating the host. *Popillia japonica*, Japanese beetle, was the most abundant defoliator of MAM but had minimal impact on the survival and seed production of individual plants.

Insects Found on Mile-a-Minute Weed in Asia

China

In 1996, a collaborative project was initiated between USDA Forest Service, Forest Health Technology Enterprise Team (FHTET) and the Chinese Academy of Agricultural Sciences Institute of Biological Control (now Institute of Environment and Sustainable Development in Agriculture) to survey and screen biological control agents of MAM in China for possible release in the eastern United States. Surveys for phytophagous insects were conducted from 1996 to 2001 in 23 provinces including some in northeastern China, where the climate is similar to that of the eastern United States, and southwest China, which is considered the center of origin of the family Polygonaceae (Ding et al. 2004).

A total of 111 phytophagous species from six orders and 29 families were associated with MAM in China. Although most were leaf feeders, several stem borers, fruit feeders, and seed feeders were found. No insects were recovered from the roots. Eleven of the species were regarded as important because either they cause severe damage on MAM or have a narrow host range (Ding et al. 2004). Included among the species collected were:

- the weevil *Rhinoncomimus latipes* Korotyaev (Curculionidae)
- three oligophagous leaf beetles, *Smaragdina nigrifrons* (Hope), *Gallerucida bifasciata* Motschulsky, and *Galerucella placida* Baly (all Chrysomelidae)
- a moth, *Timandra griseata* Peterson (Geometridae)
- a hemipteran, *Cletus schmidti* Kiritschenko (Coreidae)
- the sawfly, *Allantus nigrocaeruleus* (Smith) (Tenthredinidae)

Japan

In 2004 and 2005, Dr. Kenji Fujisaki at Kyoto University initiated a survey for herbivorous insect fauna of MAM. Parts of Japan are in the native range of MAM (Ohwi 1965) and many of the survey sites are a good climatic match to the northeastern United States (Miura et al. 2008). Fujisaki conducted surveys at 15 sites from Kagoshima in the south to Sapporo in the north. They consisted of timed visual surveys (15 minutes per sample, two to six samples per site on a given sample date) with only one or two visits per year to most of the sites. A total of 50 herbivorous insect species were recovered on MAM:

- 26 Hemiptera (52%)
- 11 Lepidoptera (22%)
- 9 Coleoptera (18%)
- 3 Orthoptera (6%)
- 1 Hymenoptera (2%)

Six species appeared to be potential Polygonaceae specialists:

- 2 Hemiptera, the bug *Coptosoma parvipictum* Montandon (Pataspidae) and aphid *Trichosiphonaphis ishimikawae* (Shinji) (Aphididae)
- 2 Lepidoptera, *Timandra apicirosea* (Prout) (Geometridae) and *Oligonyx vulnerata* (Butler) (Noctuidae)
- 1 sawfly, *Allantus luctifer* Smith (Tenthredinidae)
- 1 beetle, *Rhinoncomimus latipes* Korotyaev (Curculionidae)

Of the six specialist herbivores, *R. latipes* appeared to be the most promising natural enemy. This observation supports results from surveys conducted in China, as well as host-range testing, and the release of *R. latipes* in the United States (Miura et al. 2008).

In 2006 and 2007, additional surveys for natural enemies of MAM were conducted by Dr. Naoto Kamata at the University of Tokyo. Twelve habitats with sites established along rivers or streams in the suburbs of the Tokyo Metropolitan area were monitored. Mile-a-minute weed appeared above ground in mid-May, began to decline in early October, and disappeared by mid-November. These sites were scouted for insects once or twice a week from the middle of May to the end of November. During each scouting session, at least 400 stems of MAM were inspected for 2 to 3 hours. In total, eight species of herbivorous insects were recovered on MAM in 2006: a sawfly, *Allantus luctifer* (Smith); five moth species, *Hyphantria cunea* Drury, *Timandra apicirosea* (Prout), *Cifuna locuples confusa* (Bremer), *Orgyia thyellina* Butler, *Helicoverpa armigera* (Hübner); and two weevils, *Apoderus erythrogaster* Vollenhoven and *Rhinoncomimus latipes*. Another moth, *Spodoptera litura* F., was recovered in 2007. The weevil *R. latipes* was the most common herbivorous insect. As was reported in the Fujisaki surveys in 2004 and 2005, the moth *Timandra apicirosea* was recovered in fairly abundant numbers in 2006 and 2007. It was considered less promising because its congener, *T. griseata*, was not host-specific (Price et al. 2003, Miura et al. 2008).

Insects Tested in the United States and China for Host Specificity

Timandra griseata Peterson (Lepidoptera: Geometridae)

In August 1999, Ding Jianqing, with the Institute of Environment and Sustainable Development in Agriculture (formerly the Chinese Academy of Agricultural Sciences Institute of Biological Control) in Beijing, collected larvae and pupae of *Timandra griseata* from the field in Henan and Hubei provinces and sent them to the USDA-ARS Beneficial Insects Introduction Research (BIIR) quarantine facility in Newark, Delaware. *T. griseata* defoliated potted MAM, developing from egg to adult in approximately 26 days. However, its host range was considered to be too broad for it to be released in the United States, because it also fed and developed on common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*F. tartaricum* Gaertn), and accepted these species and MAM equally in choice tests (Price et al. 2003).

Rhinoncomimus latipes Korotyaev (Coleoptera: Curculionidae)

Adults of *R. latipes* (initially misidentified as *Homorosoma chinensis* Wagner) were collected in Changsha, Henan province in China by Ding Jianqing and sent to the BIIR quarantine laboratory in July of 1999 and 2000 (Fig. 11). These weevils were found to have a relatively high reproductive rate and short generation time on potted MAM. Adults lay eggs on MAM leaves, stems, or buds. Eggs hatch in about 3 days (Price et al. 2003). Larvae quickly bore into stems at nodes, and feed internally (Fig. 12). Once fully grown, they crawl or drop to the soil where they pupate. The new adults emerge from the soil, crawl up nearby MAM plants, feed, mate, and begin laying eggs.

In tests in China, *R. latipes* did not feed on 28 species of plants in 18 families outside of the Polygonaceae (see box: "Host Specificity Testing"). In quarantine in Newark, Delaware, *R. latipes* did not oviposit or complete larval development on two crop plants, buckwheat and rhubarb, within the family Polygonaceae (Price et al. 2003). Subsequent tests were conducted on representatives from all of the Sections within the genus *Polygonum* sensu lato and on representatives of genera other than *Polygonum* within the family Polygonaceae, especially genera that contain threatened and endangered species. Also included were representatives of families thought to have chemical affinities with the Polygonaceae. Adult weevils in these tests fed and survived on a few species, but did not lay any eggs on plants other than MAM. In choice tests adults almost exclusively ate MAM, and newly hatched larvae placed on other plant species did not survive (Colpetzer et al. 2004). Based on these results, a release permit was granted by USDA-APHIS in July of 2004.

Figure 11. Adult *Rhinoncomimus latipes*.

Figure 12. Larva feeding in stem.

Host Specificity Testing

Matthew J. Frye, Ph.D., University of Delaware

Host specificity testing of potential weed biological control agents is an essential step in determining the safety and efficacy of the insect or pathogen under evaluation. The primary objective of these tests is to determine the physiological host range of the agent, i.e. in addition to the target weed, which plant species from the introduced range are suitable for insect feeding, development, and reproduction. The process can be visualized as a ‘‘filter of safety” (Fig. 13), a series of tests used to accumulate information on the biology and host specificity of natural enemies. Each ‘‘sieve” in the filter is an opportunity to sift and remove unsafe organisms capable of causing non-target damage.

The first step in host specificity testing is to develop a list of plants that may be at risk of damage from an imported phytophagous insect.

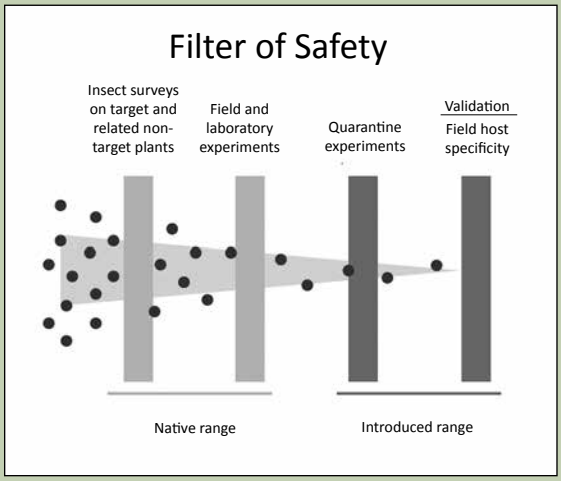

Figure 13. “Filter of safety” used in modern biological control programs.

This list must be reviewed by the Technical Advisory Group (TAG), an independent committee that reports to the USDA Animal and Plant Health Inspection Service (APHIS). Test-plant species are selected based on their phylogenetic (evolutionary) relationship to the target weed, focusing primarily on species closely related to the target. The list of test plants may also include host plant species compiled from historical accounts of a potential agent, host plants of insects closely related to a potential agent, plant species that share morphological and biochemical traits or habitat requirements with the target weed, and crop and ornamental plants of economic value.

After a potential biological control agent has been selected from field surveys and preliminary tests in the native range of the target weed, the insect should be sent to a quarantine facility in the country where it is to be introduced for further evaluation. Included in the evaluation are no-choice tests in which insects are presented with a single, non-target, test-plant species at a time. Feeding, development, and survival rates are recorded and compared to those for insects on the target weed. No-choice oviposition tests are conducted to assess whether a female will oviposit (lay eggs) when confined to a single test plant. Tests used to determine the insect’s host specificity may include choice tests, in which insects are presented with a combination of test-plant species along with the target weed, and their oviposition or feeding is recorded. Choice tests may include all plant species used by adults for oviposition as well as plant species from the no-choice tests fed upon by insects in any life stage.

In the field in China, adults of *R. latipes* were collected from the upper petiole and the upper surface of the lamina, particularly on the first or second youngest leaves of MAM plants (Ding Jianqing, personal communication). Adults fed externally by scraping the epidermal layer and underlying cells, usually penetrating through to the other side of the leaf to form a characteristic feeding hole. Newly hatched larvae bored into and tunneled inside the stem. The combination of heavy defoliation by adult weevils and larval stem boring caused leaves to desiccate and curl until young shoots gradually withered away (Ding et al. 2004).

There are at least two generations of weevils per year in China. They overwinter as adults and emerge in early to mid-May when MAM vines are 12 to 15 inches long (Ding Jianqing, personal communication). High adult weevil populations have been observed in July, when they can be collected easily from MAM, often as mating pairs. Typically, three or four weevils per plant are found at this time, but in an exceptional year there could be as many as six to ten weevils per plant.

In culture in China, females began to oviposit 2 to 8 days after copulation, and continued to oviposit for 80 to 100 days. Tests with 25 pairs of adults showed that mean egg production was about 180 per female (Ding, unpublished data). No parasites were found in weevils collected as adults or in laboratory cultures. No insect pathogens were observed in the field or laboratory.

In quarantine in Newark, Delaware, the total development time (egg to adult) averaged 26 days, and egg production averaged about 130 eggs per female (Price et al. 2003). Adults can live up to 1 year in the laboratory. Adult *R. latipes* are black upon emergence, but turn orange-brown soon after feeding on MAM (Fig. 14).

Figure 14. Black (a) and orange (b) weevils.

A field host-specificity test was conducted in 2009 in Newark, Delaware with MAM and 11 closely related species, including two native species that often occur in the same sites as MAM, *Persicaria sagittata* and *P. arifolia*. Open-field tests permit insects to use their full range of host-finding behaviors, some of which may be limited by tests conducted in cages. The test plants were planted in a field in six randomized complete blocks. Ten weevils were released at the base of each plant; weevils placed on MAM were coated with yellow fluorescent dust and weevils released on non-target plants were coated with red fluorescent dust. Weevils that were released on MAM were never found on non-target plants. In contrast, weevils released on non-target plants rapidly colonized the MAM plants, and after 44 hours, 97% of the weevils that remained in the plots were found on MAM. The MAM plants were then killed, and the weevils rapidly dispersed from the test plots. No weevils fed or laid eggs on non-target plants during the course of this study (Frye et al. 2010).

Chapter 3. *Rhinoncomimus latipes* in the United States

Mass Rearing

Dan Palmer,
Amy Diercks,
Caryl Ott, and
Cyndi Detweiler

In August, 2004, mass rearing of *R. latipes* was initiated at the New Jersey Department of Agriculture's Phillip Alampi Beneficial Insect Laboratory (PABIL), in West Trenton, New Jersey. The lab was specifically designed for mass rearing insects. Rearing is ongoing, with continual improvement in methods and efficiency.

It is well known that the quality of host material is paramount to successful insect rearing. Seeds collected from the field were initially used to propagate the plant. The seed-scarification process required for germination proved to be very time consuming, so vegetative reproduction was tried. It was successful, and the use of cuttings from "mother plants" replaced the seed method of propagation. Standard growing procedures in the greenhouse were investigated to determine the needs of the plant. Good conditions include:

- fertilizer (Scotts™ General Purpose/Peter's Professional® ammonium nitrate fertilizer, 20-20-20), strength of 200 ppm
- day and night greenhouse temperatures of 75 °F and 65 °F respectively
- care in frequency of watering
- Fafard® mix for soil medium
- 6-inch plastic pots
- a 7-week-old plant

A healthy mother plant provides thick stems to use for cuttings. Replacing these plants approximately every 5 weeks ensures the supply of good cutting material and avoids greenhouse pests. The two lower nodes of the cutting (trimmed 0.25-inch below the lowest node and with the leaf cut off of both lower nodes) are soaked in rooting hormone for 10 seconds and then placed in plastic containers with individual compartments filled with a moist mixture of vermiculite and milled sphagnum moss (Fig. 15). The cuttings are placed in a shallow bin under fluorescent lights at 82 °F and 100% humidity for 2 weeks. After a 3-day graduated venting process, the cuttings are transplanted into pots, watered, misted, and covered loosely with plastic for 1 day. The plants are placed in the greenhouse four days after transplant. Watering is carefully monitored to avoid over-watering.

Figure 15. Mile-a-minute weed (*Persicaria perfoliata*) cuttings.

A precision pruning technique was developed to keep the plants at a manageable size while still providing the terminals (growing tips) needed for weevil reproduction. Female *R. latipes* lay most of their eggs on plant terminals; newly hatched larvae only burrow into the very young leaf nodes on a terminal. A plant with a sturdy base and five to eight thick-stemmed terminals is best suited for insect production (Fig. 16).

Figure 16. "Ideal" pruned mile-a-minute plant for weevil rearing.

Plants are kept under grow lights through all insect rearing stages to optimize plant quality, leading to increased weevil production. Room temperatures are kept at 80 °F with 16L: 8D lighting conditions. Tek-5 grow lights are used and they maintain the temperature inside the containers at about 82 °F. The humidity inside both the egg-laying and the development containers is between 95 and 100%. For egg laying, mating pairs of *R. latipes* are placed on seven-week-old MAM plants inside the containers (Fig. 17). Eggs start hatching on day three or four. Every 2 to 3 days, the plants are moved to development containers (large plastic bins) and new plants are added to the egg-laying containers (Fig. 18).

Figure 17. Egg-laying container.

Figure 18. Development containers.

Figure 19. *Rhinoncomimus latipes* pre-pupa.

As the eggs hatch, the larvae burrow into the youngest leaf nodes and develop through all larval instars within the nodes and stems. Grow lights are needed over the development bins because many of the leaf nodes occupied by the larvae are new growth that began at about the time the eggs were laid. Seven or 8 days after egg hatch, the mature larvae chew their way out of the nodes and either crawl or drop to the moist soil medium below, where they pupate (Fig. 19). At this point the foliage inside the bin is replaced with a "trap plant." The pre-pupa builds a capsule around itself with the soil medium attached to the outside and pupates inside the capsule. After spending the pre-pupal and pupal stages in the soil, the adults emerge between day 17 and 20 and crawl up the trap plant to feed, where they can be collected using an aspirator. The adults are either stored in a cage with an abundance of MAM plants or, if to be shipped soon, placed in a release cup with a honey-water sponge and kept at 55 °F until shipped. Two methods are being evaluated for storing adults during the winter:

- Some adults are put in cages with large MAM plants and left outdoors through fall and winter to have the weevil go through a natural winter season.
- Some are put in a cage indoors with an abundance of MAM plants, kept at 55 °F, and brought out to feed at 74 °F three times a week.

An organization could use similar procedures to rear *R. latipes* in its own facilities. The insects should be reared in a room kept at 80 °F (or 82 °F inside the container). The egg-laying containers can be clear plastic display boxes with a "no-see-um" netting covering a 1.5-inch hole in the top. The development containers can be polycarbonate clear plastic bins with three 2-inch holes on each side covered with netting, and lids with three 2-inch holes covered with netting. The bins will keep the environment humid, so humidity inside the room may not be a concern. Grow lights over both oviposition and development containers are very important. The maximum rearing temperature inside the containers is around 88 °F. If temperatures inside the containers drop below 78 °F, the insects will have a longer life cycle.

Multiple generations of *R. latipes* have been mass-reared at PABIL since 2004 with no addition of new genetic material. Hough-Goldstein et al. (2014) compared life history traits between these lab insects and weevils collected from a field site in Delaware that was inoculated with the same genetic stock as the PABIL colony in 2004. Additional tests were conducted with weevils collected from the native range in China. Laboratory weevils produced more eggs but had lower survival and reduced response to cues that induce diapause. Hough-Goldstein et al. (2014) concluded that there was no need to add new genetic material to the PABIL colony.

Release of *Rhinoncomimus latipes* in New Jersey

Mark Mayer

Monitored Sites

In addition to mass rearing *R. latipes*, PABIL personnel have released the weevils at numerous sites in New Jersey, several of which have been monitored using the "Mile-a-Minute Monitoring Protocol" developed by Dr. Judy Hough-Goldstein (Appendix A). The results of the first four years of monitoring at these and other sites were published by Hough-Goldstein et al. (2009) and are summarized below. Monitoring has continued at the New Jersey sites. Four field sites were set up for monitoring, three in southern New Jersey (two at Floodgate Road in Greenwich, Gloucester County, and one at Department of Defense [DOD] Ponds Wildlife Management Area [WMA] in Pilesgrove, Salem County), and one in Central New Jersey at Pinelands Water and Wastewater Company in Vincentown, Burlington County. Weevils were released on two sites, and two were monitored as control sites. The control sites did not receive weevils, but MAM populations were monitored for comparison with release sites.

In spring, 2005, PABIL field personnel established two new sites in Salem County and dropped the Vincentown and the DOD Ponds sites, because these sites had been disturbed frequently by the public and there was a possibility that chemical control measures had been implemented. The new 2005 release site was at the Abbotts Meadow Wildlife Management Area in Elsinboro Township; the control site was located in the Supawna Meadows National Wildlife Refuge in Pennsville Township. One of the two Floodgate Road sites was retained as a release site and the other as a control site. However, the Floodgate control site was not monitored after the first two years because it was overrun by weevils and therefore no longer served as a control.

For all releases, weevils were brought to the field in 16-oz. wax-lined, hot-beverage Sweetheart® cups with holes cut into each end (Fig. 20). Nylon mesh was secured over the holes and a Pioneer plastics® Petri dish containing a sponge moistened with honey and water was taped to the bottom of the cup. Excelsior was placed in the cup to give the weevils more resting sites. Upon release, the excelsior and any weevils on it were removed from the cup and placed gently on the MAM. The cup was placed in the MAM to allow the rest of the weevils to walk out on their own.

Figure 20. Field release of *Rhinoncomimus latipes.*

Weevil counts can be misleading, because they tend to drop, undetected, from the plant when disturbed during the survey process. Often, weevils can be found by first looking for feeding damage near the release site (Fig. 21), and then by searching for them on nearby leaves and terminals. Another sign of weevil activity in the field is the presence of damaged nodes (Fig. 22), indicating areas where larvae have bored into or out of stems. Although they are very tiny, the presence of weevil eggs (Fig. 23), with their characteristic peanut shape and thin covering of frass strips (insect fecal material), is another definitive sign of weevil activity. Foliage damage alone is not always adequate proof of weevil presence, because other organisms can also feed on MAM, notably Japanese beetles, which can be found on the plants in July and August (Fig. 24). Although not definitive proof of infestation, where weevils occur, feeding holes on MAM often make the plant stand out among other plant species (including other closely related plant species), especially in early spring (Fig. 25).

Figure 21. Adult weevil feeding damage in early spring.

Figure 22. Damaged nodes, indicating larval feeding.

Figure 23. *Rhinoncomimus latipes* egg.

Figure 24. Japanese beetles feeding on mile-a-minute.

Figure 25. Early spring damage to mile-a-minute weed (note that the closely related *Persicaria sagittata* is untouched).

The Floodgate Road release site in Gloucester County received 200 weevils in July 2004 and 3,297 in 2005. The weevils established and the population grew so rapidly that the MAM was completely defoliated by October 2006 (Figs. 26, 27). There were so many weevils present at that site that more than 200 were collected in less than a minute simply by putting a clipboard under the defoliated stems and gently tapping the plants (Fig. 28). The large numbers of weevils present in October 2006 indicated there was potential to establish field insectaries. To that end, in September of 2007, 200 weevils were collected from defoliated plants and redistributed on a site in Hunterdon County.

Figure 26. Mile-a-minute at Floodgate Road July 2004 (left), in October 2006 (middle) and October 2007 (right) after *R. latipes* feeding. Note the *Prunus* sp. bush in the foreground (middle) was not visible prior to weevil release in 2004 (left), because it was covered by mile-a-minute. The *Prunus* sp. grew once mile-a-minute was reduced.

Figure 27. Defoliation at Floodgate Road, October 2006.

Figure 28. Weevils on clipboard, October 2006.

Following release of nearly 7,000 weevils between April and September, 2005, both the spring seedling counts and percent of MAM cover at Abbott's Meadow were dramatically reduced (Figs. 29 and 30). In contrast, both seedling counts and percent cover remained high at the control site. In 2006, a large number of *R. latipes* adults emerged after overwintering at Abbott's Meadow. Heavy feeding by these adults depleted the available MAM and apparently triggered weevil dispersal. In 2006 *R. latipes* was recovered 4 kilometers (2.5 miles) from the original release site and by the end of the 2007 season, *R. latipes* was recovered from, and had caused feeding damage to, MAM 5.6 kilometers (3.5 miles) from the release site.

Other New Jersey Releases

Between 2004 and 2014, PABIL released a total of more than 190,000 *R. latipes* adults in New Jersey (Fig. 31, Table 2). Adult *R. latipes* and/or their feeding damage were observed at all 78 release sites (100%) as well as at more than 200 non-release sites where the weevils dispersed on their own. Two of the release sites in New Jersey have been subjected to flooding. One of the 2005 sites (Washington Crossing) was located along the Delaware River and experienced a "100-year flood" in spring of 2006. The high waterline was two feet above the release site and no weevils were expected to survive; nevertheless, *R. latipes* adults were recovered at the release site in late May. Weevils were recovered from another site, along the Delaware River on the DOD Ponds Wildlife Management Areas in Salem County, even though it was periodically flooded by tides.

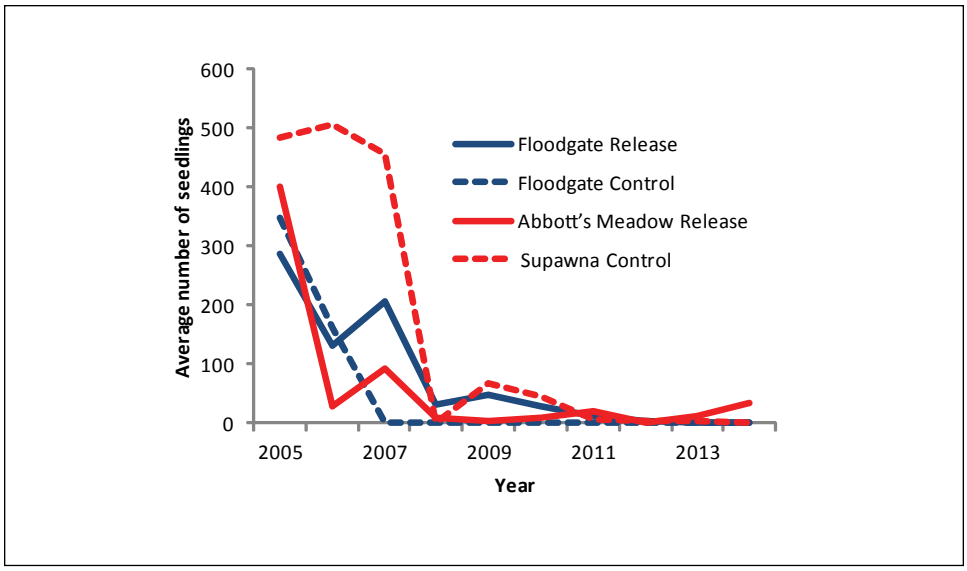

Figure 29. Average number of mile-a-minute seedlings at release and control sites in New Jersey. Note: Floodgate Control monitored in 2005 and 2006 only.

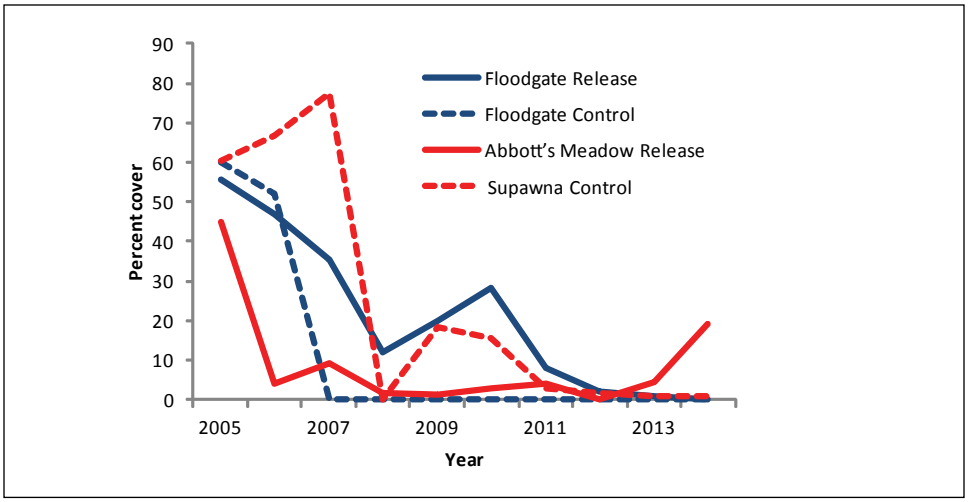

Figure 30. Average percent cover of mile-a-minute at release and control sites in New Jersey. Note: Floodgate Control monitored in 2005 and 2006 only.

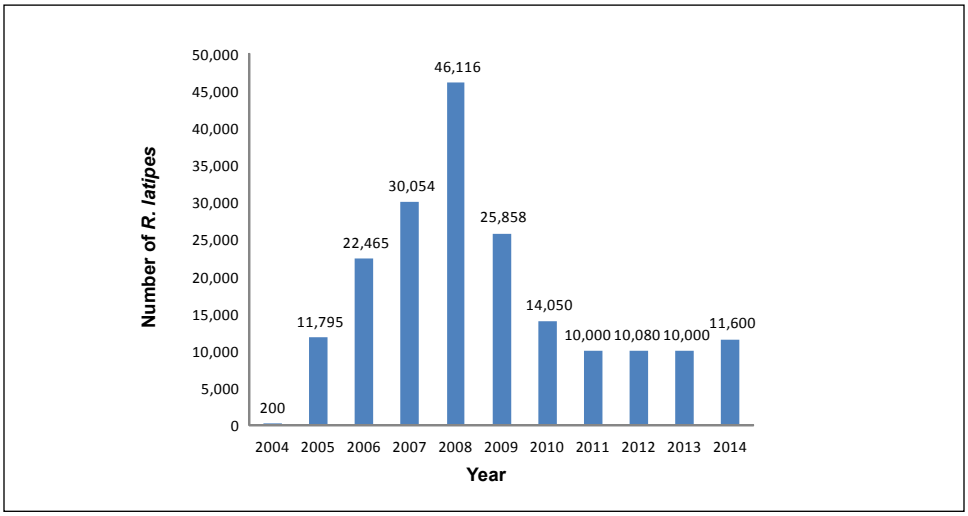

Figure 31. Number of *Rhinoncomimus latipes* released in New Jersey.

Table 2. Shipments of *Rhinoncomimus latipes* from PABIL to different states, 2004-2011.

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	State Total
NJ	200	11,795	22,465	30,054	46,116	25,858	14,050	10,000	10,080	10,000	11,600	192,218
DE	--	--	1,850	3,174	8,253	12,886	6,850	6,000	6,100	1,350	--	46,463
PA	--	310	2,400	3,057	900	4,800	7,000	20,000	15,500	24,700	11,000	89,667
WV	--	404	1,000	--	2,450	10,000	11,500	7,000	5,000	6,510	5,700	49,564
MD	--	--	500	1,100	1,800	6,000	7,000	4,000	2,000	2,000	5,000	29,400
CT	--	--	--	--	--	7,100	6,200	5,050	5,350	5,000	5,000	33,700
NY	--	--	--	--	--	6,500	8,000	13,500	17,250	19,000	12,550	76,800
RI	--	--	--	--	--	2,000	6,000	3,200	600	--	5500	17,300
VA	--	--	--	--	--	2,000	3,500	5,500	5,000	9,000	6,000	31,000
MA	--	--	--	--	--	--	6,000	5,500	5,000	5,000	5,000	26,500
NC	--	--	--	--	--	--	--	2,000	4,800	3,500	5,000	15,300
Year Total	**200**	**12,509**	**28,215**	**37,385**	**59,519**	**77,144**	**76,100**	**81,750**	**76,680**	**86,060**	**72,350**	**607,912**

Adult *R. latipes* have dispersed from release sites in New Jersey, and some have migrated across the Delaware River to Amico Island. This ability to disperse is important because, despite weevil releases and other control activities, MAM continues to be a problem in New Jersey. For example, in the aftermath of hurricane Sandy (October 2012), MAM rapidly developed large populations in new openings in the forest canopy where trees had fallen.

In recent years, fewer weevils have been released in New Jersey (Fig. 31, Table 2). This is primarily because since 2010, every site with mile-a-minute weed that has been scouted for new weevil release sites has been found to already have weevils. Therefore PABIL has concentrated primarily on sending weevils to other states where they have not already colonized on their own.

Releases in Other States

Between 2004 and 2014, more than 600,000 weevils were shipped from the Phillip Alampi laboratory to 11 states for release (Table 2).

Impact of *Rhinoncomimus latipes* on Mile-a-Minute Weed

Impact in Field and Greenhouse Cages

The impact of *R. latipes* feeding on *P. perfoliata* was studied in field cages over a 2-year period (Hough-Goldstein et al. 2008; Fig. 32). In 2006, 20 weevils introduced into cages with single plants in May (when weevils first emerge from overwintering) suppressed seed production for about 9 weeks, whereas weevils introduced in June (when the first summer generation of adults emerge) did not affect seed phenology. Plants in all cages produced substantial numbers of seeds late in the year, but the average individual seed (achene) weight was reduced for plants with 20 weevils per plant introduced in May.

Figure 32. Mile-a-minute weed with heavy weevil damage in field cage.

In 2007, plants grown within field cages, but with some competition from other plants, showed substantial mortality. By mid-August, 63% of plants with 10 or 20 weevils, and 75% of plants with 40 weevils per plant were dead, compared with 12.5% mortality for control plants (Hough-Goldstein et al. 2008; Fig. 33). Reproduction was delayed by more than a month in surviving plants with 10 or

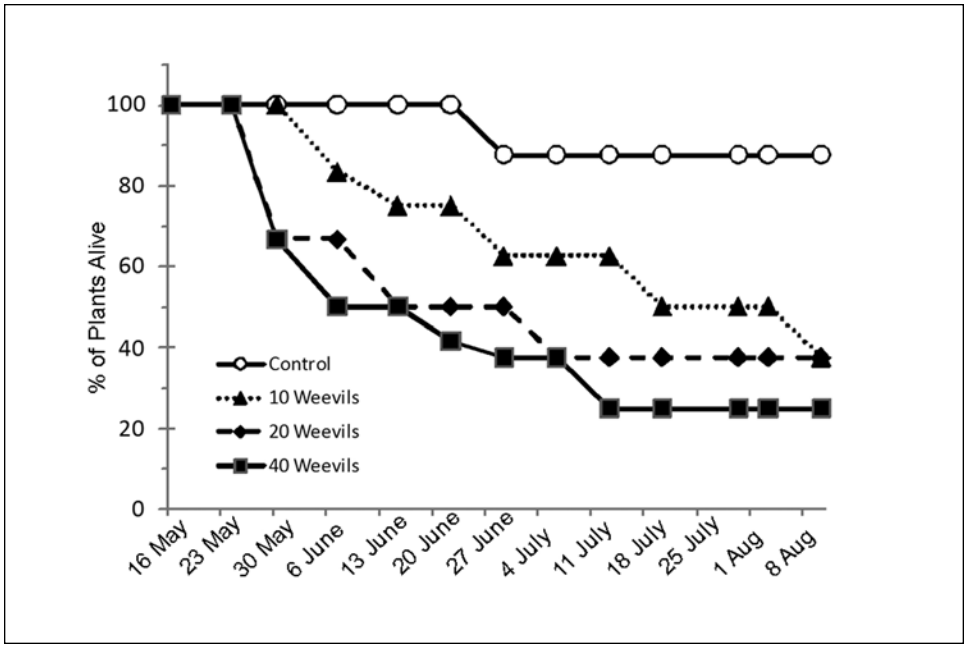

Figure 33. Survival of individual mile-a-minute plants exposed to 0 (Control), 10, 20, or 40 weevils in field cages in 2007.

20 weevils, and by more than 2 months in the few survivors with 40 weevils. Surviving plants with 40 weevils per plant showed loss of apical dominance, which can allow plants to compensate for herbivore damage; however, in the case of a light-adapted vine such as *P. perfoliata*, this may prevent the plants from achieving needed sun exposure. These results suggest that *R. latipes* feeding on *P. perfoliata* can impact plant growth and reproduction, and may put affected plants at a substantial competitive disadvantage.

Subsequent studies in the greenhouse and in the field have confirmed and extended knowledge of the weevil impact on MAM seed production (Smith and Hough-Goldstein 2014). In greenhouse cages, addition of weevils delayed the production of immature and mature seed clusters by 7 weeks compared to plants without weevils, and reduced the total number of seeds produced by about a third. Weevil-infested plants also had an average of only 7 seeds per cluster, compared to 11 in uninfested plants. Where weevils were confined in mesh bags on developing seed clusters in the field, seed weight and viability were reduced by direct weevil feeding (Smith and Hough-Goldstein 2014).

Monitored Release and Control Sites, 2004-2008

Standardized monitoring of fixed quadrats was conducted in paired release and control sites at eight locations in the mid-Atlantic U.S. from 2004 through 2008 (Hough-Goldstein et al. 2009; monitoring protocol in Appendix A). Significant differences in mile-a-minute weed populations in the presence and absence of

weevils were found at three locations, with reduction in spring densities to 25% or less of what they had been at the start within 2-3 years at release sites, while weed densities at control sites were largely unchanged. Mile-a-minute weed populations at a fourth site were similarly reduced at the release site, but without control data for comparison due to rapid colonization of the paired control site. At the other four locations, all on islands, mile-a-minute weed populations were reduced at both release and control sites without large weevil populations developing, apparently due to environmental conditions such as late frost and severe drought. Weevils dispersed from monitored release sites at a rate of 4.3 kilometers (2.7 miles) per year in the broader landscape 1-3 years after release (Hough-Goldstein et al. 2009).

Replicated Release Arrays in Pennsylvania, 2005-2010

Weevil dispersal, population growth, and impact in the field were studied in three replicated release arrays in Chester County, Pennsylvania (Lake 2007, Lake 2011, Lake et al. 2011). One array was located at the Brandywine Conservancy's Laurels Preserve and the other two at the Brandywine Valley Association (BVA) Myrick Conservation Center (BVA CREP and BVA Wetland sites). Each array consisted of a central release point surrounded by a total of 76 monitoring points: 60 points placed on concentric circles spaced 5 meters apart to a maximum distance of 25 meters (Fig. 34); eight points 1 meter from the release; and eight points approximately 2.5 meters from the release. On June 9, 2005, 450 weevils were released in the center of each array.

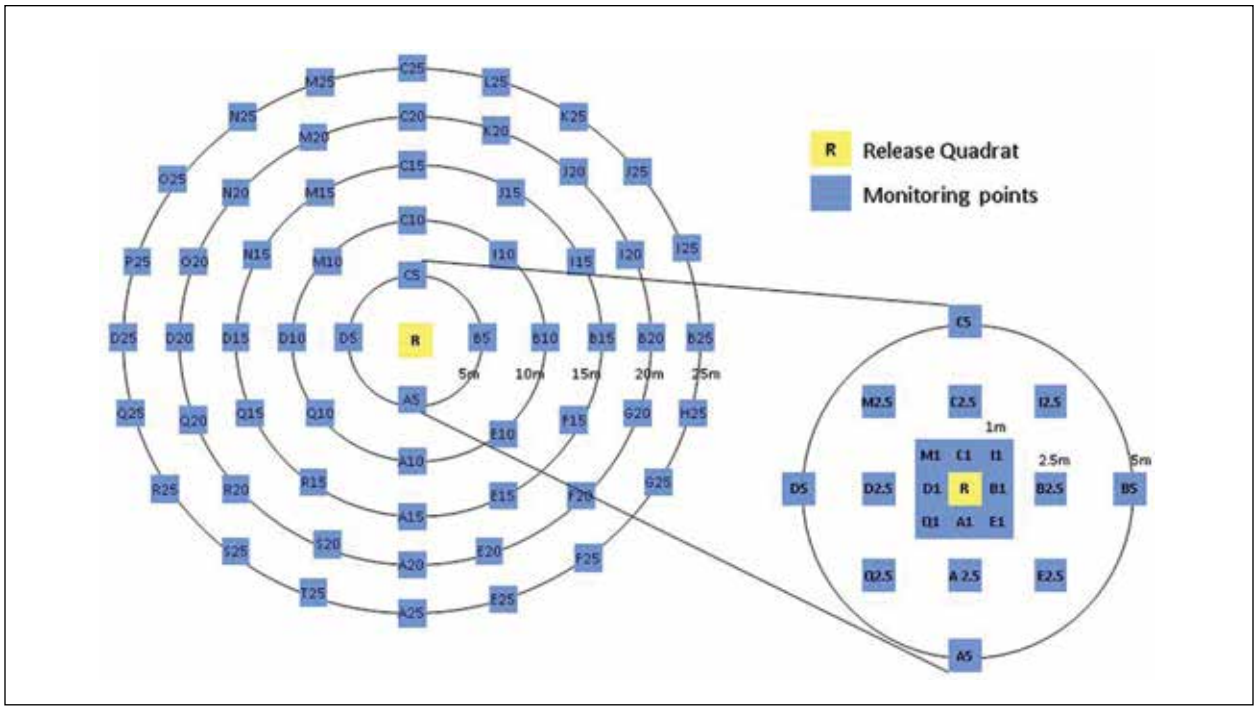

Figure 34. Generalized release and monitoring array for replicated releases.

Dispersal

Weevils dispersed at a rate of 1.5-2.9 meters per week (15 to 25 meters) within the arrays during the first four months following release (Lake et al. 2011). Long-distance dispersers were found up to 200 meters (0.12 miles) away four months post-release. Within 14 months, weevils were found in MAM patches nearly 800 meters (0.5 miles) from the release sites. Dispersing weevils located both large MAM populations and small isolated patches (Lake 2007). In 2007, approximately 27 months post-release, weevils were found at several sites within 5.6 kilometers (3.5 miles) of the release points and at one site approximately 8 kilometers (5 miles) away. By June 2008, three years post-release, weevils were observed on numerous MAM weed patches 11.3 kilometers (7 miles) from the original release sites. These patches ranged in size from small isolated vines to large infestations. As of July, 2008, the farthest-removed weevil dispersal was observed 29 kilometers (18 miles) from the nearest release sites.

Population growth and impact

Weevils were active in the field from early spring through fall and completed three or four generations before MAM was killed by a hard frost. In 2005, 2006, and 2007, the proportion of monitored MAM weed quadrats that contained eggs decreased from 60% in late August to zero in early October (Fig. 35). This decrease occurred before a substantial temperature drop, but coincident with a decrease in day length. Declining plant quality in late summer and early fall may also be a factor cueing the decrease in egg production.

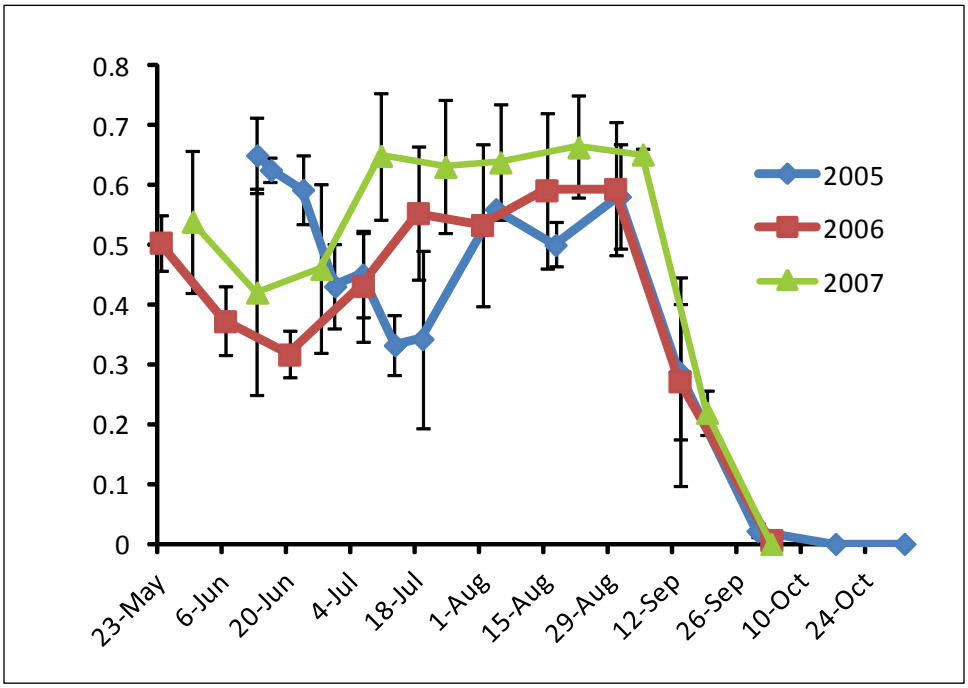

Figure 35. Weevil egg production (proportion of sampled quadrats with eggs, average (± SEM) of all three sites each year).

The percent cover of MAM varied greatly among monitoring points in the release arrays. In order to evaluate the weevil population in the context of different MAM cover, the number of weevils per monitoring quadrat was divided by the percent cover of MAM in that quadrat to generate the number of weevils per m^2 of MAM. For each site and year, the area under the curve for quadrats within 5 meters of the release was calculated based on the number of weevils per m^2 of MAM.

At the Laurels—the array with the largest monoculture of MAM, as well as the largest weevil population—weevil density increased significantly from 2005 to 2007 and then declined in 2008, when MAM cover was greatly reduced. At the same time, the percent cover of mile-a-minute at the Laurels declined from 2005 through 2008 (Fig. 36). The spring of 2009 was very cool and wet and

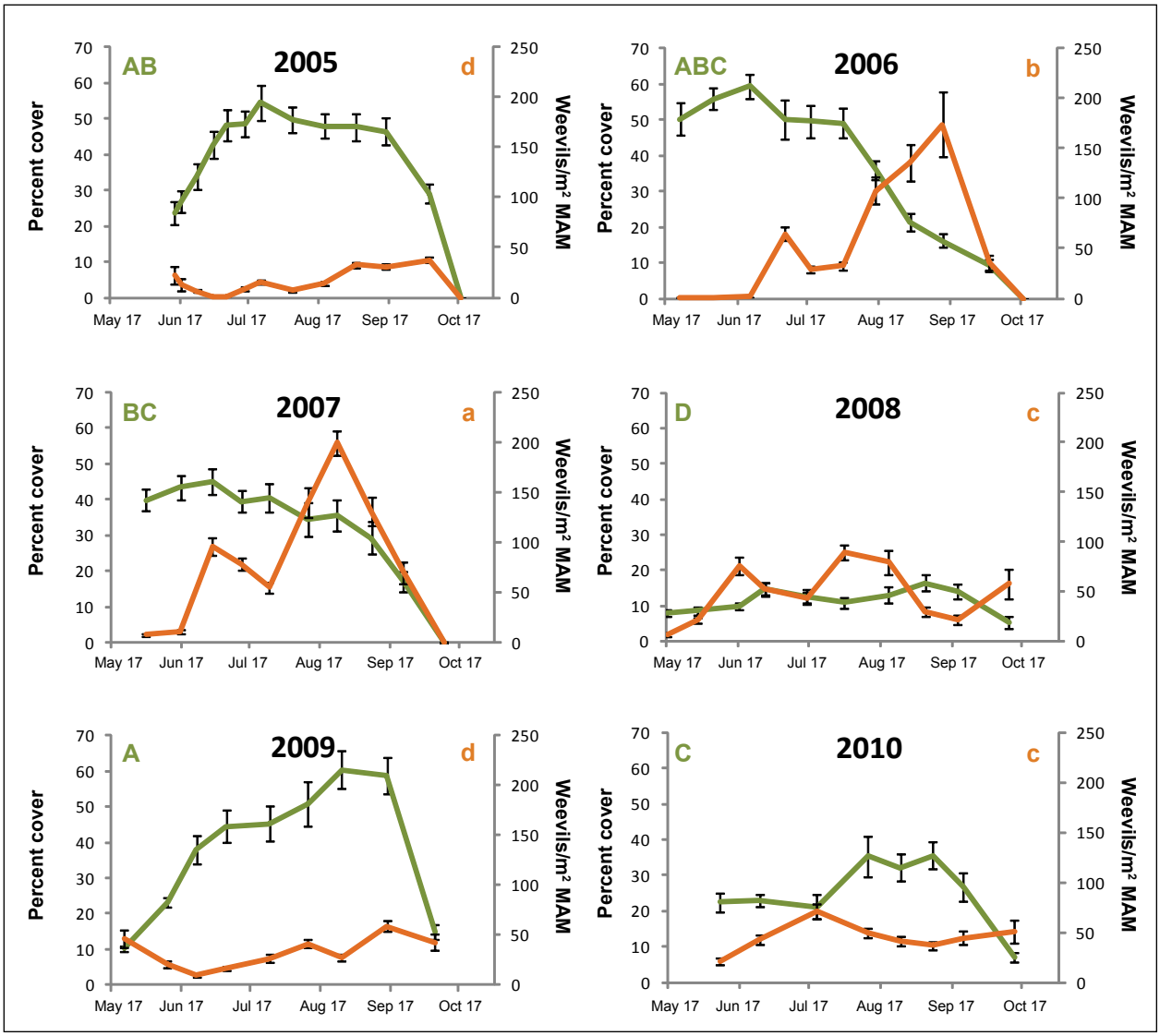

Figure 36. Average (± SEM) percent cover of mile-a-minute and weevils per m^2 of mile-a-minute at the Laurels. Years marked with different letters (capital letters for cover and lower case letters for weevils) were significantly different.

MAM grew vigorously. The cool weather slowed the development of the weevil population and reduced the number of new weevils that could be produced (see moisture and temperature effects; Fig. 36, E. L. and J. H.-G., unpublished data). In 2010, when weather conditions were more favorable for weevil population growth, the percent cover of mile-a-minute was lower at all three sites compared to 2009.

The number of MAM seedlings per 0.5 m^2 declined significantly between 2006 and 2008 at two of the three release arrays (Fig. 37). At the Laurels, the number of seedlings declined from more than 100 seedlings per 0.5 m^2 in 2006 to fewer than 20 in 2008. The number of seedlings increased between 2008 and 2010 at the Laurels and BVA Wetland sites, likely because of increased MAM cover and reduced weevil population growth in 2009 due to the cool, wet spring.

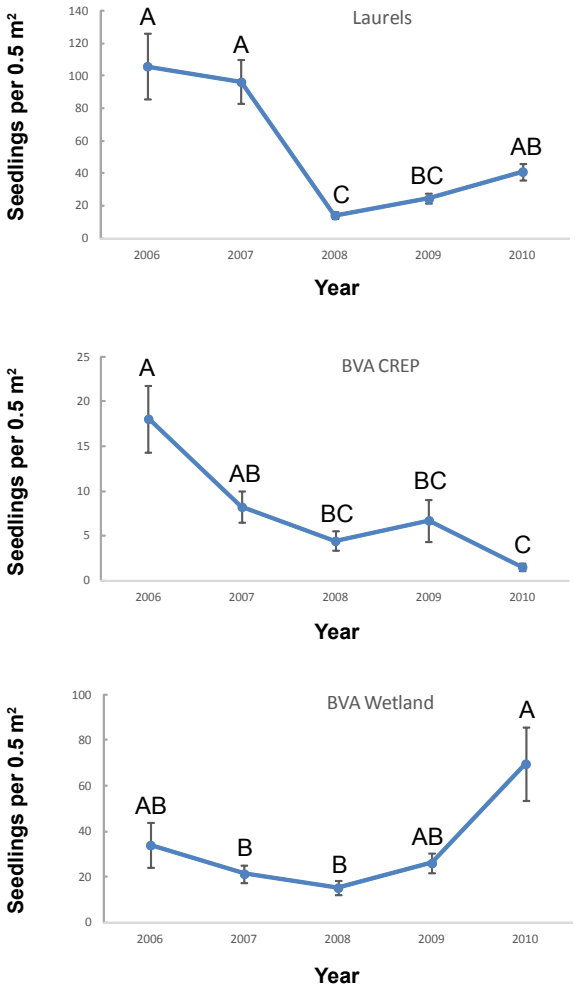

Figure 37. Average (± SEM) number of mile-a-minute seedlings at three release sites. Years at each site marked with different letters were significantly different.

Figure 38. "Stacked" nodes on weevil-damaged mile-a-minute plants.

In addition to experiencing significant loss of photosynthate, damaged plants typically had stem nodes that were very close together ("stacked" nodes) (Fig. 38), apparently due to loss of apical dominance (Hough-Goldstein et al. 2008). This reduction in apical dominance may limit the ability of MAM to climb and compete with other plants.

Six years post-release, MAM cover was significantly reduced at the Laurels and BVA CREP site and the number of MAM seed clusters (Lake 2011) declined at all three sites. Although it was not directly measured, the native plant community at the BVA CREP site appeared more diverse than the other two sites prior to weevil release. The combination of plant competition and herbivory by the weevils may have contributed to the overall decline in MAM at this site (see integrated weed management in Chapter 4).

Effects of Different Environmental Conditions on Weevil Behavior and Impact on MAM

Orientation to Forest Edge

In an experiment assessing dispersal behavior, weevils were more likely to colonize potted mile-a-minute plants located closest to the point of release, and those found on forest edges, than plants located in an open field, in the forest, or further away from the point of release (Fig. 39, Hough-Goldstein et al. 2012). The preference for MAM located along forest edges may be adaptive behavior because the plant is primarily found in riparian areas in its native range (Hyatt and Araki 2006). The very low colonization of plants located in forests suggests that the weevil may not locate MAM patches that develop in canopy gaps in forests, and therefore other management techniques should be used to control these patches. Avoidance of host plants located in shade was also observed in other studies (Hough-Goldstein and LaCoss 2012, Smith and Hough-Goldstein 2013; see below).

Figure 39. Experimental array where weevils were released (white bucket) and preferentially colonized potted mile-a-minute plants along the forest edge.

Sun Versus Shade

In a study conducted in 2005, MAM plants isolated in the field that were growing in full sun were more than 10 times larger and produced more than 6 times as many seeds as plants growing in the shade (Hough-Goldstein 2008). A subsequent study tracked weevil numbers and plant growth in field plots where shade was artificially applied to half of the plots and the others were left in full sun. A second experiment was similar, except that half of the sun and shade plots had their weevil populations eliminated through the use of a systemic insecticide (Fig. 40; Hough-Goldstein and LaCoss 2012). Weevil density and plant damage was generally higher in the sun than in the shade in the first experiment. In the second experiment plant biomass was reduced by about half by the presence of weevils in the sun, while biomass in the shade was low both with and without weevils. Plant nodes were thicker in sun-grown plants, which may provide a better habitat for the stem-boring larvae than the thinner shade-grown plant stems (Hough-Goldstein and LaCoss 2012). In field cages, Hough-Goldstein et al. (2014) found that many more weevils were produced from plants in full sun than from plants in the shade.

Figure 40. Experimental set-up to determine weevil and mile-a-minute response to artificially applied shade in the field.

A study of weevil behavior in the greenhouse (Smith and Hough-Goldstein 2013) showed the importance of both response to sunlight and response to host-plant cues. In this study, weevils placed in a tube between two cages, one in full sun and one in shade (Fig. 41) always preferred the sun cage, even when only the shaded cage had a host plant. However, when both cages were in the sun but only one had a host plant, weevils were attracted to the host plant. This experiment simulates natural conditions where weevils have no suitable host plant, either due to excessive feeding or environmental factors such as drought. Under these conditions, dispersal is primarily controlled by response to light, and secondarily by response to host plant cues. A second set of experiments had weevils placed on host plants in the cages themselves, so that they would have to travel through the connecting tube in order to reach the other cage. Weevils placed in a shaded cage were found to leave that cage and travel to the cage in the sun if the host plant in the sun was of equal or better quality than the one they were on; but if the shaded cage had a high-quality plant and the sun cage did not, the weevils were more likely to stay where they had been placed (Smith and Hough-Goldstein 2013). Thus in the field, weevils that emerge near host plants in the shade are likely to move to sunny areas if there are better-quality mile-a-minute plants present there.

Figure 41. Shaded and unshaded cages connected by a mesh tube, used to determine weevil response to light and host plant cues.

Moisture and Temperature Effects

Field observations suggested that under conditions of high spring rainfall and cool temperatures during the spring and summer, MAM was able to outgrow the weevils, while dry conditions and warm temperatures favored the weevil and allowed it to suppress the weed (see Replicated Release Arrays in Pennsylvania, above). Therefore a series of experiments were conducted to test the role of moisture and temperature on weevil population growth and efficacy.

The combined effects of herbivory and water stress on growth and reproduction of MAM were investigated in greenhouse trials over two years, with well-watered or water-limited plants either exposed or not exposed to herbivory by *R. latipes* (Berg et al. 2015). The first year, the experiment was conducted during August through October, with limited opportunity for the weevils to reproduce, while the second year's experiment was conducted from May through October, and weevils reproduced well in the cages. Both years, MAM responded strongly to presence or absence of adequate water, suggesting that wet years favor growth of the weed. Plant biomass and seed production was considerably lower in the low-water treatments. Herbivory also affected MAM biomass and seed production, especially in the full-season experiment where weevil numbers increased over the course of the season (Fig. 42).

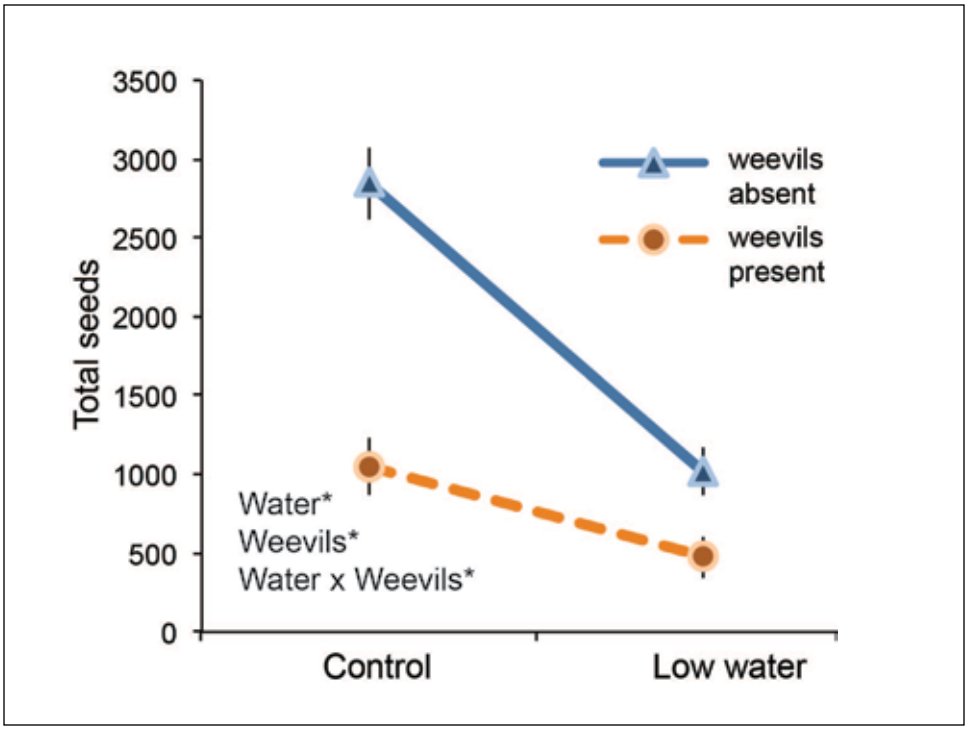

Figure 42. Average (± SEM) number of mile-a-minute seeds produced by plants that were watered three times per week (control) or once per week (low water), and kept with or without weevils.

As with all insects, temperature directly affects weevil development time. In environmental chambers set at different temperatures, the lower developmental threshold for the weevil was estimated at 10.2 °C (50.4 °F), and average time of development from egg to adult varied from 19 days at 30 °C (86 °F) to 39 days at 20 °C (68 °F; J. H.-G., unpublished data). Each generation increases its population exponentially, and thus during warm summers a larger weevil population will develop, allowing for greater MAM suppression.

As noted above, weevils stop laying eggs at the end of August, which is adaptive since any eggs produced after early September will probably not have enough time to develop into adults and prepare for overwintering before the first frost. The last successful date for egg-laying in several typical years in the mid-Atlantic region, based on the estimated number of degree days required for development from egg to adult, varied from September 4 to 8 (J. H.-G., unpublished data).

Chapter 4. Biological Control as a Component of an Integrated Mile-a-Minute Weed Management Program

Integrated Weed Management

Integrated weed management (IWM), an extension of the concept of integrated pest management that was applied first to insect pests and subsequently to plant disease pests, is rapidly gaining acceptance among weed scientists (Buhler 2002). Among the key elements of integrated pest management are the use of multiple control tactics and the integration of a thorough knowledge of pest biology into the management system. Elements of IWM systems may include:

- Education and prevention
- Physical or mechanical control
- Cultural methods
- Herbicides
- Biological control

The ultimate goal of an effective weed management program in a natural area is to replace undesirable plants that cause resource, economic, habitat, or aesthetic losses with a plant or plants that are beneficial to the environment. The short-term objective is to implement the most effective combination of control methods available for the target weed. Concurrently, landowners and managers should develop a long-term plan for managing undesirable plants and maintaining desirable vegetation.

Weed Control Methods Used to Manage MAM

Education and Prevention

Because mile-a-minute weed is still expanding its range (Fig. 2, page 2), and is patchily distributed even in areas where it is well entrenched, efforts to increase public awareness of this noxious weed are important to the success of any area-wide integrated management program. Mile-a-minute weed can grow to unmanageable proportions within a fairly short time of establishing itself in a new area. For example, the plant was first noticed in very small patches in 2001 in the heronry on Pea Patch Island, Delaware. The extent of infestation was mapped in 2002, when the population was still small, and in 2003 and 2004, when populations exploded (Fig. 43). Although not mapped, populations on the

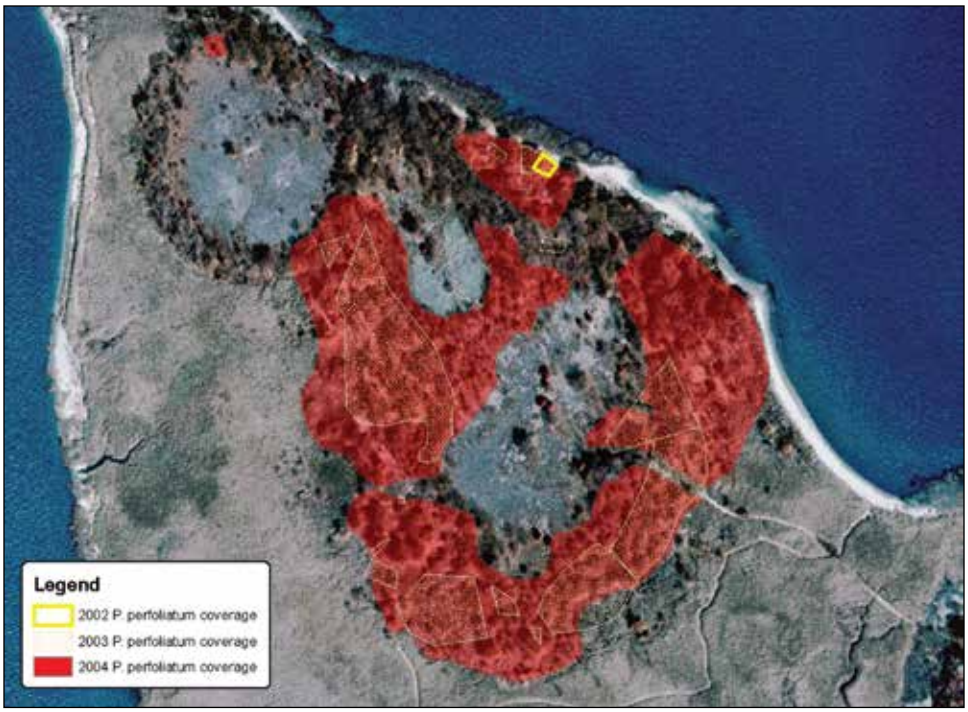

Figure 43. Mile-a-minute distribution in heronry, Pea Patch Island, Delaware.

remainder of the island outside of the heronry increased in a similar pattern (Fig. 44). Populations of MAM remained extremely high from 2005 through 2008. Weevils were released in 2007 and 2008, and MAM was much suppressed after several years (J. H.-G., unpublished data).

Figure 44. Mile-a-minute on Pea Patch Island, Delaware, August 2003.

MAM can respond rapidly to disturbance. For example, in 2003 MAM was probably present on a Chester County, Pennsylvania, site slated for development, but the weed was not a problem at that time. In the spring of 2004, soil disturbance occurred during testing to determine septic feasibility. By the spring of 2008, the site was a virtual monoculture of MAM, with an average of 86 seedlings per square meter (E.L., unpublished data). A similar response was noted above in 2012 in New Jersey, where disturbance was caused by trees felled by hurricane Sandy.

In areas where MAM is present, land managers must anticipate the potential for it to colonize and/or dominate disturbed sites. It can also dominate land cleared for restoration projects. For example, a preserve in Chester County, Pennsylvania, decided to convert a site with a mixture of woody and herbaceous invasives, including MAM, to a native meadow. Heavy equipment and herbicides were used to prepare the site in the fall of 2007, and a mixture of native grasses and wildflowers was seeded. The following spring, the site was a monoculture of MAM with little to none of the desirable vegetation visible.

Eradication of MAM may be possible where a population is still small. For example, a nursery in Kingston, Rhode Island, has successfully controlled a small infestation through hand pulling and mowing, though an occasional plant still recurs and is removed (R. Casagrande, Univ. Rhode Island, personal communication).

Physical or Mechanical Control Methods

Mile-a-minute weed has a relatively weak root system, and small plants can be hand-pulled easily. Gloves should be worn to protect the skin from the plant's sharp spines. Longer vines can be pulled out using a garden rake, as has been done in parts of Little Paint Branch Park, near Beltsville, Maryland (Marc Imlay, personal communication). Regardless of the method used, MAM should be pulled before it sets seed to avoid spreading seed to new locations. Even green seed can germinate (see box: "Germination of Mature and Immature Seed"); therefore, if any seed clusters are present, plants should be removed from the area and the seed destroyed.

Adequate methods of destroying MAM seed have not been confirmed through research, but experiments on other types of weed seeds suggest possible methods. For example, high temperatures in an active compost pile can destroy weed seed, but internal temperatures need to reach at least 60 °C (140 °F) for 7 days to kill weed seeds (Rynk 1992). It is very difficult to reach these temperatures near the surface of compost piles, so only weed seeds in the interior of the pile are killed (Gordon et al. 2001). Therefore, to ensure all seeds are exposed to the internal temperatures, compost piles need to be turned or mixed periodically. Burning can kill seeds, but the fire must be very hot. Work in Australia suggests that

Germination of Mature and Immature Seed

A common reaction of land managers to the appearance of seed clusters on an uncontrolled MAM infestation is to attempt to remove the vines or apply post-emergent herbicides (personal observation). It was not known whether green seed present at the time these management techniques were implemented was viable, and if this management strategy could further the spread of MAM and increase the seed bank.

In a recent study (Smith et al. 2014) full-sized immature (green) and mature (blue) fruits were collected from five field sites every 2 weeks over a 3-month period, and seed viability was assessed. At the onset of seed production in mid-August, 35% of seeds from immature fruits were viable. This percentage increased steadily, peaking at 84% in late September before declining at some sites around the time of the first frost. In contrast nearly all seeds with mature fruits (96%) were viable at all collection dates (Fig. 45). Thus land managers who apply physical or chemical control methods for mile-a-minute weed should do so before the onset of any seed production and not simply before fruit maturation. If it is necessary to apply control methods after fruit set, it should be done as early in the season as possible.

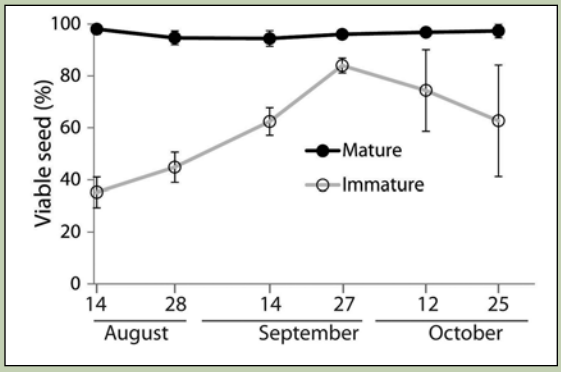

Figure 45. Average (± SEM) percentage of viable seeds from mature (blue) and immature (green) mile-a-minute fruits.

400 °C (750 °F) for 20 to 30 seconds may be required for hard-coated seeds (Walsh and Newman 2006), so simply putting vines in a trash barrel and burning them may not be adequate. Ultimately, until more effective seed-killing methods are determined, land managers should make every effort to control the plant *before seed clusters develop*.

Where practical, MAM can be mowed. However, while low mowing may kill plants, leaving too much of the plant above ground can release apical dominance (Fig. 46) and cause re-growth of sturdy bushy plants, possibly with more terminals and a consequent increase in the potential to produce more seed clusters.

As noted earlier, MAM seed can persist and remain viable for 6 years in the seed bank. Even if plants are removed or killed by physical means, control efforts must continue for several years to exhaust any remaining seed bank.

Cultural Methods

Observations and experiments suggest that MAM does not thrive in the shade (Hough-Goldstein and LaCoss 2012). Therefore, one important component for

Figure 46. Side terminals produced by mile-a-minute plants that have been mowed.

controlling it long-term is to add shade trees wherever practical and desirable. Competition with other plants is also key in determining whether MAM can dominate a site. Fostering desirable plants, whether by planting or relying on natural populations, should be part of a management plan (Fig. 47). See the integrated weed management section below for examples of how to incorporate plant competition.

Figure 47. Damaged mile-a-minute plant, with competing native vegetation.

Herbicides

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and Art Gover, Research Support Associate, Dept. of Horticulture, Penn State University, College Park, PA

Mile-a-minute weed is quite susceptible to a number of herbicides, including both pre- and post-emergent products. Pre-emergent herbicides control plants before they emerge from the ground by injuring the plant as the seed germinates. They can be applied to soil and will enter the plant through the roots or shoots emerging from the germinating seed. Post-emergent herbicides act on plants after they have emerged from the ground, entering the plant through the foliage or stems. Some herbicides work both ways.

Effective control using herbicides is easiest early in the season, before seed set and before MAM begins to climb onto neighboring plants. Depending on weather, seasonal conditions, and the herbicide you choose, pre-emergent herbicides should be applied between early March and early April in the mid-Atlantic United States. An advantage of pre-emergent applications is that most of the existing perennial species will not be affected, particularly if you apply prior to bud break.

Post-emergent herbicides may be selective or non-selective. Glyphosate is a widely used non-selective herbicide. When used on very dense stands or monocultures of MAM, it will provide effective control with minimal damage to non-target plants. However, when MAM is growing among desirable plants, it may be very difficult or tedious to avoid contacting the non-target plants. If most of the desirable plants are grasses or grass-like, a selective herbicide such as triclopyr (Garlon® 3A) can be useful. Triclopyr will control MAM without injuring grasses, but will injure on contact other broadleaf forbs, shrubs, and trees. Other effective post-emergent products include Journey®, Plateau®, Overdrive®, Escort®, and Milestone® VM.

Adding a surfactant is recommended for better control of MAM with post-emergent sprays. Surfactants improve herbicide effectiveness by increasing the spray's adherence to the leaf surface, reducing the surface tension of the mixture so that it spreads over more of the leaf, and aiding penetration of the waxy outer cuticle of the leaf, all of which promotes better uptake of herbicide into the treated leaf.

Several herbicide products are readily available for both consumer and commercial applicators. Generally, consumer products are less concentrated, and come in smaller containers than commercial products. Any of the commercial products listed in Table 3 could be purchased for home use: none of them are "Restricted Use" meaning the purchaser need not be a state-certified pesticide applicator. However, if you buy a commercial product for residential use you will likely end up purchasing much more material than you will need. Although

the unit cost of commercial products is lower, the larger container size can make them too expensive for small-scale use. Plus, you must safely store or dispose of the surplus product.

Commonly used pre-emergent (PRE) and post-emergent (POST) herbicides are listed in Table 3. Pre-emergent herbicides often used to control MAM are not readily available as consumer products. The herbicide pendimethalin is available in some crabgrass-prevention products, but often includes fertilizer and is intended for use on established turfgrass.

Prior to applying any herbicide, please check your equipment thoroughly and consult the product label for proper application rates and precautions.

Table 3. Commonly used herbicides for mile-a-minute control

Herbicide	PRE or POST	Homeowner product examples[a] (concentration)	Commercial product examples[a] (concentration)
pendimethalin	PRE	Halts® Crabgrass Preventer (1.7%)	Pendulum® Aquacap™ (39%) Pendulum® 3.3 EC (37%) Pendulum® 2G (2%)
imazapic	Both	None	Plateau® (gov't only) (24%) Journey® (plus *glyphosate*) (8%)
sulfometuron	Both	None	Oust® XP (60%)
glyphosate	POST	Many (1%) Many (18%) Many (41%)	Roundup® Pro (41%) Rodeo® (54%)
triclopyr	POST	Roundup® Poison Ivy & Tough Brush Killer (8%)	Garlon® 3A (44%) Garlon® 4 (62%)

[a] Brand names are listed for example only. All herbicides listed are available in other products as well. *Glyphosate* is so widely available that homeowner product examples are listed by common concentrations rather than brand names.

There are many factors to consider when selecting an herbicide, including time of year, surrounding vegetation, rate of infestation, herbicide volatility and translocation. Factors that can cause variation in results include rainfall during or immediately after application, and drought. Drought-stressed plants are usually less responsive to herbicide applications than actively growing plants.

The ability of a restoration site to recover from weed competition once the weeds have been removed will determine short- and long-term management decisions. Complete control may not be feasible. The most efficient and effective strategy results from a thorough understanding of the environmental forces in the area and a management goal that works with and not against these forces. There are many techniques for controlling MAM. Usually, the control on a site will require a combination of two or more methods. What will be common to every site is that, owing to the prolific nature of MAM and the persistence of the seed bank, periodic monitoring over many years will be required to prevent a disruption to the aesthetic and ecology of a site.

The Biological Control Component

Planning your Program

In areas where the MAM population is a massive monoculture that must be controlled quickly, such as where trees have been planted and are in danger of being overrun, it is probably wise to plan multiple modes of attack. Such a plan would include the application of pre-emergent herbicide in areas where other valued annual plants are not likely to be harmed; fostering or planting desirable plant species as competitors; and releasing weevils, which over time should increase their populations to the point where they will permanently suppress the target plant and help promote a healthy, diverse ecosystem.

Selecting Weevil Release Sites

Rhinoncomimus latipes are present and abundant on MAM throughout China, from north to south, so there is no obvious reason why weevil populations should not establish and develop throughout the current and future MAM range in North America. So far this has been the case. Weevils have established populations at nearly all sites where they have been released from Massachusetts to North Carolina. In the mid-Atlantic region they can develop three to four overlapping generations over a single season (Lake et al. 2011). Because of the cooler temperatures in more northerly regions, we would expect fewer generations (see Moisture and Temperature Effects, Chapter 3), and therefore it may take more time for large populations to develop further north.

After release and while the insect populations are developing, at least a portion of the selected release site should remain undisturbed by other methods of control, e.g., herbicides, mechanical methods, etc. The selected “weevil nursery” site should be one where the MAM population can be tolerated for several years.

Obtaining Weevils for Biological Control

R. latipes are currently commercially available from the Phillip Alampi Beneficial Insect Laboratory (PABIL), New Jersey Department of Agriculture. Weevils can be reared at other sites if resources are available (see Mass Rearing, Chapter 3); however, they can only be shipped or transported across state lines if a USDA-APHIS-PPQ 526 permit is obtained in advance. The form for requesting this permit, along with other relevant information, is available online, at http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml.

The weevils can also be collected from a previously released/established site within the same state. Look for adult feeding "shot holes" in leaves and larval emergence holes at plant nodes near where the saucer-shaped ocrea encircles stems or where stems diverge. The adult weevils, although very small, can be observed directly in the field especially at the ends of terminals. Recommended weather for weevil collection are sunny, warm days. Adult weevils can be collected using a large funnel placed in a narrow necked plastic container. Shake foliage with weevils into the funnel and they will drop into the container.

If weevils cannot be released on the day they are collected (or received in shipments) they should be stored at room temperature, not in a refrigerator, for release as soon as possible (Hough-Goldstein et al. 2014).

Timing of Weevil Releases, and Number to Release

The weevils have been successfully released from April to mid-October. However, early release will help the weevil build up a good population in the current year. A typical release consists of about 200 weevils. Although people often ask what the "release rate" should be, i.e., how many weevils should be released per acre, this concept is based on a pesticide paradigm (M. Mayer, personal communication), and does not work well in a biological control situation because both plant and insect (populations) are growing. As noted earlier, each female weevil has the potential to lay some 130-180 eggs. If each egg laid survives to produce an adult, the population will grow more than 100-fold during the first generation following release, and can increase by many thousand-fold later in the summer. At the same time, plant growth can be substantial, especially under wet conditions (see Moisture and Temperature Effects, Chapter 3).

Monitoring Weevils

Although wide-scale coordinated monitoring (as in Hough-Goldstein et al. 2009) is no longer being conducted, you may wish to document effects of biological control on the target plant population in a new release. Ideally, to make sure that any observed changes are not due simply to varying or seasonal conditions, or that they would have occurred with or without the introduced insect, one would

keep track of several weed populations that are exposed to the insect along with other similar weed populations not exposed to the insect. That said, if an introduced insect is a successful biological control agent, sooner or later its population is likely to increase to the point that any control site will be invaded by the insect, at which time the site will cease to function as a control.

The initial MAM monitoring protocol was based on monitoring protocols for purple loosestrife and garlic mustard developed by Bernd Blossey, Victoria Nuzzo, and coworkers (http://www.invasiveplants.net/). The 2008 version of the MAM monitoring protocol (used by Hough-Goldstein et al. 2009, and by PABIL in ongoing monitoring in New Jersey) is included here as Appendix A.

The MAM monitoring protocol is designed to track the population of the weevil and the MAM population over time. Ten permanent 0.5- by 1.0-meter quadrats, numbered 1-10, are established in a heavily infested MAM patch where weevils are to be released, and ten quadrats are established in a similar control site at least 500 meters away. Experience with the protocol showed that weevils will reach control sites within 1-2 years at distances of 200-500 m, and even a control site 10.6 km from the release site was invaded within 3 years (Hough-Goldstein et al. 2009). Thus the control site will inevitably be converted into a release site in time.

Weevils are released in quadrat #5 of the first (release) array. The full monitoring protocol calls for a spring sample, where MAM seedlings and weevils are counted within a quadrat frame (Fig. 48). Once a month following the spring sample, weevils observed within quadrats are counted, the percentage of leaf

Figure 48. Frame used for monitoring mile-a-minute and weevils.

area removed by insects is estimated, and presence or absence of node damage indicating weevil reproduction is noted. Later in the season the number of mature and immature fruiting terminals is counted. The percentage of MAM cover in each quadrat is estimated during each survey. The expectation is that as the weevil population increases, the percentage of MAM cover will be reduced in the quadrats.

Combining Biological Control with Other Methods

Some MAM control methods are compatible with the use of weevils. Although herbicides are not likely to have a direct detrimental effect on adult weevils, death of the plant will likely kill any developing larvae in the stems, and cause adults to disperse. High mowing of sites with weevils may make the site more conducive to weevil population growth by causing the plants to produce more of the tender terminals favored by the weevils.

As noted, competition with other plants is key in determining whether MAM can dominate a site. Weevil damage can cause MAM plants to become poorer competitors by reducing the number and size of seeds, reducing seed viability, shifting phenology of seed production to later in the year, and suppressing plant growth (Hough-Goldstein et al. 2008; Smith and Hough-Goldstein 2014; Berg et al. 2015).

An important aspect of developing an integrated weed program is to assess the other vegetation that is present at a site dominated by MAM. Not much is gained if biological control agents suppress the target weed, only to have the target weed replaced by other nonnative invasive plant species (the invasive treadmill effect; Thomas & Reid 2007). In some cases, control of MAM by whatever means should be followed or accompanied by planting of desirable vegetation.

Two experiments have successfully integrated biological control with other management techniques to restore sites invaded by mile-a-minute weed (Cutting and Hough-Goldstein 2013; Lake et al. 2014). Although these experiments were not conducted at the same scale at which a land manager may undertake a restoration effort, both demonstrate how integrating management strategies for mile-a-minute weed improved the native plant community.

Integrating biological control and native seeding

A 3-year study (Fig. 49; Cutting and Hough-Goldstein 2013) tested the effects of weevils and seeding, separately and together, using insecticide to eliminate weevils. This experiment was conducted at a site in Kennett Square, Pennsylvania, that was dominated by mile-a-minute weed. Biological control was integrated with a native seed mix that consisted of Canada wildrye (*Elymus canadensis* L.), big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.), sweet smooth oxeye (*Heliopsis helianthoides* [L.]), and

Figure 49. Plots used to test effects of biological control integrated with restoration planting using a native seed mix.

blackeyed Susan (*Rudbeckia hirta* L.). Weevils were present at the site in low densities and supplemental releases were conducted; the number of weevils added to each plot was dependent on the percent cover of mile-a-minute. A randomized complete block design was applied with four treatments: no weevils and no seeds, no weevils with seeds, weevils but no seeds, and weevils plus seeds. The weevils were excluded from the no weevil treatments by applying the systemic insecticide dinotefuron. The experiment was installed in the spring of 2009 and the weevil population, mile-a-minute weed cover, and the response of the plant community was monitored for three years.

Mile-a-minute cover was generally lower in all plots in 2011 compared to the first year of the experiment. In 2011, the seed plus weevil treatment plots produced 57% less mile-a-minute biomass than the no weevil and no seed treatment. By July 2011, the weevil plus seed treatment contained twice as many native plant species as the no weevil and no seed treatment. This included species from the seed mixture plus colonization of the plots by other native species. In contrast, the no weevil and no seed treatment had twice as many introduced plant species as the weevil plus seed treatment (Fig. 50; Cutting and Hough-Goldstein 2013).

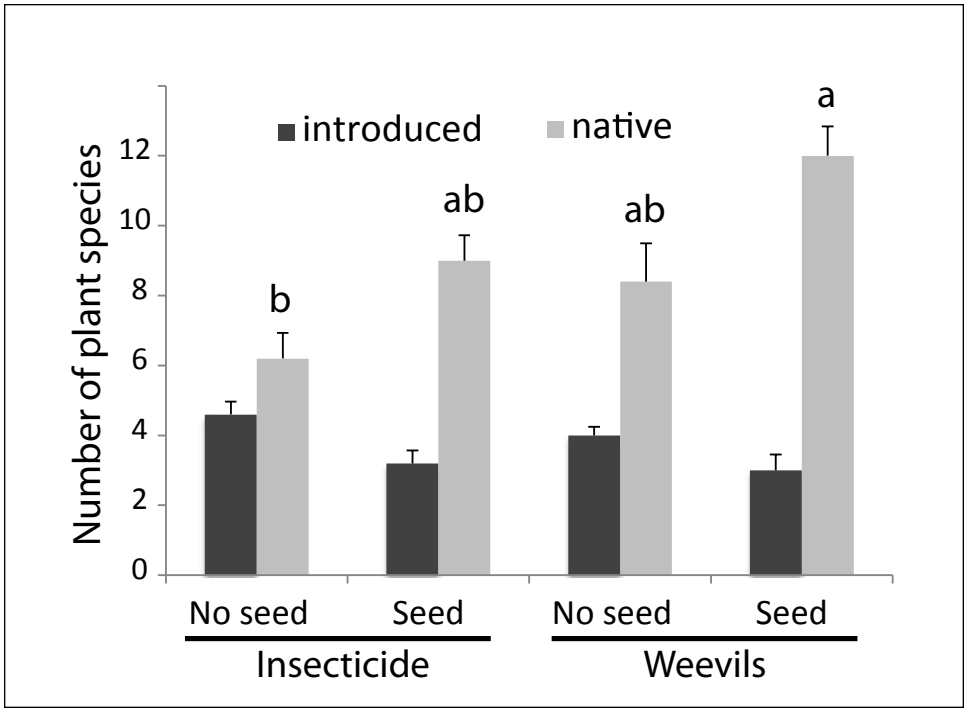


Figure 50. Average (± SEM) numbers of native and introduced plant species, July 2011. Means with different letters are significantly different.

Integrating biological control, pre-emergent herbicide, and native plantings

Three sites in southeastern Pennsylvania where mile-a-minute weed had been present for at least five years were selected for this experiment. In the fall of 2008, four 6.1 x 6.1 meter plots were established within each site, and all plots were treated with the post-emergent herbicides triclopyr and glyphosate. This killed all vegetation in the plots, which were then raked in preparation for planting. Two native plants were used to provide plant competition: the perennial *Euthamia graminifolia* (L.) Nutt., flat-top goldentop, and seedlings of American elm trees, *Ulmus americana* L. The elm was selected because of its fast growth and *E. graminifolia* is a vigorous native that was already present at all sites; neither plantings were affected by the subsequent application of pre-emergent herbicide. The plots were randomly assigned one of four treatments: control, low-density *E. graminifolia*, high-density *E. graminifolia*, and low-density *E. graminifolia* plus elm trees (Fig. 51). The plantings were installed in the fall of 2008. The plots were split in half and one side was randomly selected for a one-time application of the pre-emergent herbicide prodiamine on 1 April 2009. Mile-a-minute weevils were already present in low densities at all sites; 500 additional weevils were released in each plot in June 2009.

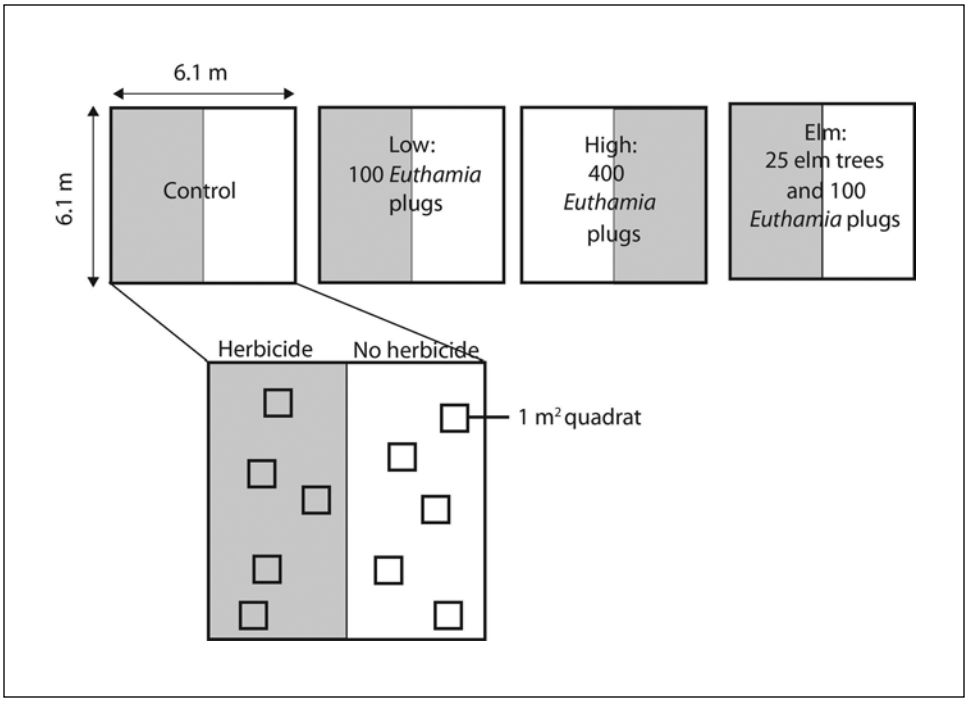


Figure 51. Experimental design for restoration planting experiment using *Euthamia graminifolia* and elms, with and without pre-emergent herbicide.

Mile-a-minute seedling counts were significantly lower in the herbicide than the no-herbicide plots in 2009, 2010, and 2011, even though the pre-emergent herbicide was only applied in 2009. In September 2010, the cover of native plants was higher in the herbicide than the no-herbicide plots. However, the cover of unplanted *E. graminifolia* at one field site was so high that it masked differences between the planting treatments. When that site was excluded from the analysis the cover of native plants and *E. graminifolia* was significantly higher in the herbicide than the no-herbicide plots. In contrast, the cover of *Microstegium vimineum*, Japanese stiltgrass, an invasive grass that often occurs with MAM, was significantly higher in the no-herbicide than the herbicide plots. In both of these sites, within the herbicide and no-herbicide plots, the control plots had less native plant cover and more Japanese stiltgrass cover than the planting treatments considered as a group (Figs. 52 and 53). The elm trees were taller in the herbicide plots. Integrating the biological control weevil with pre-emergent herbicide and native plantings reduced mile-a-minute weed seedlings and cover, prevented the invasive species treadmill effect with Japanese stiltgrass, and increased the abundance of native plants (Lake et al. 2014).

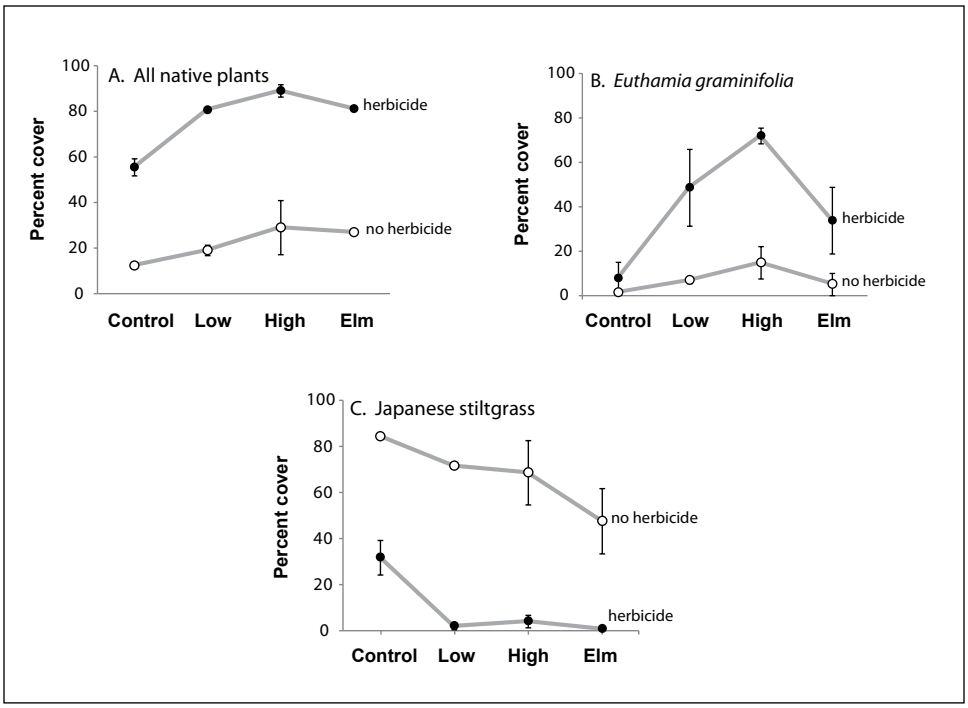


Figure 52. Average (± SEM) percent cover of all native plants (A.), *Euthamia graminifolia* (B.), and Japanese stiltgrass (C.) in restoration planting experiment with and without herbicide, September 2010.

Figure 53. Difference in cover where pre-emergent herbicide had been used (right side of red dashed line), and where it had not (left side of red dashed line), July 2009.

Conclusion

The biological control program for *P. perfoliata* in the eastern United States was initiated in 1996, and a permit for release of the host-specific weevil, *R. latipes*, was obtained eight years later, in 2004. Our first 11 years of experience with this agent in the field suggest that this weevil will be very successful overall in suppressing the target weed. The weevil shows all the characteristics of a desirable biological control agent, including: a high reproductive rate, with three to four overlapping generations occurring each season; extreme host specificity; excellent dispersal capabilities; and the ability to suppress the target weed. Time will tell the extent to which it will control mile-a-minute weed in a variety of environments throughout the introduced range.

Appendix A: Mile-a-Minute Weed Monitoring Protocol (revised March 2008)

Judy Hough-Goldstein, Department of Entomology & Wildlife Ecology, University of Delaware (jhough@udel.edu)

Introduction

Mile-a-minute weed (*Persicaria perfoliata*) is an annual Asian vine that invades a variety of habitats in the northeastern and mid-Atlantic United States, including forested floodplains, streamside herbaceous wetlands, and upland forests. A biological control program targeting mile-a-minute weed (MAM) was initiated by the USDA Forest Service in 1996, with field surveys and laboratory host-specificity tests conducted in China and subsequent testing under quarantine conditions in Delaware. A stem-boring weevil, *Rhinoncomimus latipes* Korotyaev, has been determined to be host-specific to MAM and a permit application for field release was approved in July 2004. The following guidelines are intended to help monitor the abundance of both MAM and the weevil, and assess the long-term impact of biological control. Ideally, monitoring should be initiated one or more years before biological control organisms are released, so that changes can be tracked pre- and post-release.

Mile-a-minute weed is a prickly, branching, viney annual plant that germinates in early spring, usually in April in the mid-Atlantic region. Vines grow rapidly, climbing over other plants, and attain lengths of 6 m or more. Flowers are inconspicuous, and iridescent blue berry-like achenes are produced, beginning in mid-summer and continuing until the plants are killed by frost in the fall. Seeds require a cold period before germinating. Many will germinate underneath established patches the following year, while others are spread by birds, mammals, water, and in the treads of shoes and tires. Mile-a-minute seeds can survive for up to 6 years in the seed bank.

Adult *R. latipes* are about 2 mm long, and are black, but once they start feeding they may be covered by an orange film derived from plant exudates. Adult weevils eat small holes in young leaves of *P. perfoliata* and lay eggs on leaves and stems. After hatching, larvae bore into the stem where they complete development, then exit the stem and drop to the soil for pupation. Development from egg to adult takes about 26 days under laboratory conditions. Weevils are very small, but can be observed directly in the field, especially at the ends of terminals (Fig. 54a). The pale yellow eggs have a characteristic peanut shape and are covered by a thin strip of fecal material (Fig. 54b); however, they are difficult

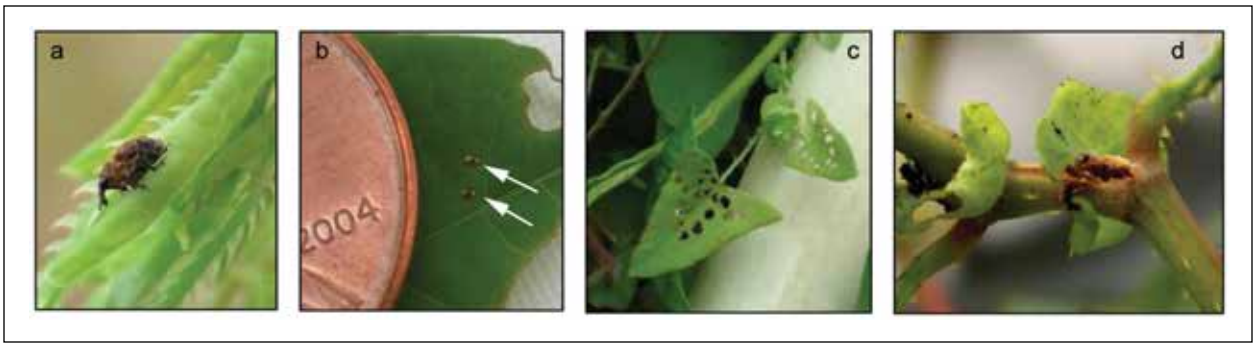


Figure 54. Adult weevil (a); eggs with penny (b); adult feeding damage (c); larval node damage (d).

to spot in the field due to their very small size. Characteristic adult feeding holes ("shot holes" in leaves) are relatively easy to see (Fig. 54c). Larval emergence holes at plant nodes (near where ocreae encircle stems or where stems diverge) can sometimes be seen in the field (Fig. 54d).

Because of MAM's rampant summer growth, and the fact that all reproduction is by seed (because plants die off, roots and all, following one or more hard frosts in the fall), we believe that plant populations are best assessed in the spring, when individual small plants can be counted in measured quadrats. Counts of overwintered weevils can be done at this time, too. Summer/fall assessments of weevil abundance and damage should be conducted, too, because populations will increase and spread during the summer. Summer and fall counts of seed production will help assess impacts on the plant population.

Site Selection and Quadrat Placement

Selection

Select a weevil release/monitoring site that will be protected from other uses that could jeopardize insect establishment and continued monitoring, i.e., a site where the landowner will not attempt to control vegetation through mowing, herbicide use, etc. The study site should contain an ample population of MAM; however, ideally, native vegetation should be present so that control of MAM will result in the establishment of a more desirable plant community.

Quadrat Placement

Materials needed:

One 0.5 m² quadrat frame (see "Constructing a Quadrat Frame"), 80 pieces of 0.5-inch or 0.75-inch plastic conduit pipe ~1 m long (to mark corners of 20 quadrats in each site), hammer, permanent marker, 50 or 100-m tape measure, GPS unit (if available), camera, work gloves

Within a single monitoring area (e.g., a state park), establish two 100-m-long transects, with similar habitat, vegetation, and mile-a-minute populations, but

located approximately 500 m away from each other. Randomly assign one of these transects as the "release" transect, where weevils will eventually be released. The other transect will serve as a non-release control site, at least until the weevils disperse into it. Along each transect, locate 10 quadrats, approximately 10 m apart. Permanently mark the position of each quadrat by placing the quadrat frame (Fig. 55) on the ground and hammering a 1-m long piece of conduit pipe into the ground at each inside corner. Using a permanent marker, write the quadrat number (R-1 through R-10 for the release transect and C-1 through C-10 for the control transect) on each corner pipe. Remove the frame but leave the pipes in the ground as markers for future reference. Move 10 meters along the transect and repeat the process until you have ten sets of quadrat markers in the ground. If 100-m long patches of MAM are not available, the 10 quadrats can be placed at random within the MAM infestation. Quadrat #5 will serve as the release point and should be located near the center. Make note of the approximate distance between quadrats on a sketch or map and attach it to Form 1, along with GPS coordinates and/or landmarks to help to find the quadrats, later. A brightly colored flag placed in one of the corner pipes will also help when locating a quadrat. Identify permanent photo-points and take photographs of the study site, including one or more set of markers. Leave the markers in place until you have completed the study. Note: be sure to remove all the markers when you complete the study.

Constructing a Quadrat Frame

Materials

One 10-foot length of 0.5- or 0.75-inch-diameter PVC or CPVC conduit pipe; four right-angle elbows of the same diameter; PVC or CPVC glue; hacksaw or pipe cutter; permanent marker; measuring stick or tape measure.

Assembly

The inside dimensions of the finished frame should measure 1 m by 0.5 m.

Using the hacksaw or pipe cutter, cut the pipe into four pieces; two pieces 1 m long, and two pieces 0.5 m long.

Glue an elbow to each end of one of the long pieces (a), taking care that the elbows are perfectly aligned with each other (share the same right-angle plane). Set this assembled piece aside; it will be the fourth side of the frame. (b) Glue the elbows on the remaining long piece and then glue a short piece into each elbow so as to form an open U-shaped frame. Using a permanent marker, mark 10-cm intervals on each side to assist in estimating percent cover and seedling numbers.

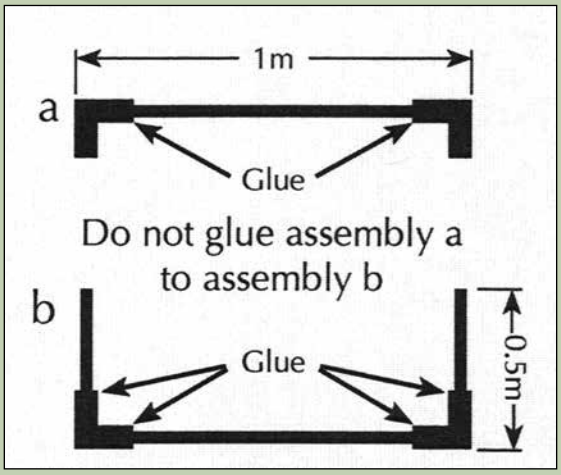


Figure 55. Diagram of a quadrat frame.

Establish the quadrats initially during the period of MAM germination and seedling emergence, making sure each quadrat has a MAM population. (Note, if other tearthumbs are present you may need to wait until plants have developed characteristic ocreae, encircling stems, before establishing quadrats).

Weevil Release

Release approximately 200 or more adult weevils within quadrat #R-5. Carefully document all releases, including the date, numbers released and exact site of release. If weevils are shipped overnight and cannot be released on the day they are received they should be stored at room temperature (not in a refrigerator) for release the next day (Hough-Goldstein et al. 2014).

Spring Mile-a-Minute Survey (Form 1)

Materials needed:

Ruler, Form 1 (see page 62; make copies as needed), clipboard, pencils, camera, maps, work gloves, GPS unit, hand tally.

Choose a date in spring after the main flush of mile-a-minute germination is complete (probably between April 15 and May 15), but before vines have become too dense to count. Ideally, MAM stems should be 15-30 cm (6”-12”) tall. Measure the height of an average stem (or a range of heights if there is much variation) and note it on the survey form. Slide the quadrat frame in place around the four corners, and survey each quadrat for the following:

- Number of weevils (if released in previous years, or where weevils may have spread on their own). Adult weevils tend to drop from plants when disturbed, so approach each quadrat site carefully. First count and record all adult weevils that can be seen on plants within the approximate confines of the quadrat. Weevils will generally be on MAM terminals or foliage, often near characteristic “shot hole” feeding damage.
- Total number of mile-a-minute seedlings present in the quadrat. Use a tally counter for accuracy. If too many are present to count, you may count the number in half or a quarter of the quadrat and multiply by 2 or 4 to arrive at a reasonable estimate within the entire quadrat; however, if you do this, to avoid errors note it on Form 1 under “Comments” (last column).
- Number of stems of other plant species. Identify as many other species as possible, especially those that are most abundant, and note these on the form under “Comments.”
- Percent cover. Standing over the frame, look straight down and estimate how much of the quadrat is covered by mile-a-minute foliage and vines, and how much is covered by all other vegetation (these estimates may total >100%, due to layering).
- Note presence or absence of “shot holes”, the characteristic damage of feeding weevils.

Summer/fall Assessment of Weevil Abundance, Plant Damage, and Seed Production (Form 2)

Materials needed:

Form 2 (see page 63; make copies as needed), clipboard, pencils, work gloves, (optional: GPS unit, hand tally).

Once each month following the seedling counts (or weevil release) until plants senesce, return to each quadrat site and survey for the following within each quadrat and record your findings on Form 2. (Note: if substantial mile-a-minute growth has occurred, it may be necessary to search out, locate, and cut a path to, each quadrat the week before the samples are taken.)

- Number of weevils. Carefully approach each quadrat site and first count and record all adult weevils that can be seen on plants within the approximate confines of the quadrat.
- Percent defoliation. Scan the foliage for "shot holes" in leaves, the characteristic damage caused by feeding weevils, and assess the percentage of leaf area removed from mile-a-minute foliage within the approximate confines of the quadrat (see Fig. 56). If insects other than the weevil are present, e.g., Japanese beetles, and appear to be damaging the foliage note this under "Comments" on Form 2.
- Node damage (yes or no). Look closely at stems where adult weevil feeding damage is evident, and note presence or absence of node damage, where larvae have fed in stems or emerged for pupation.
- Percent mile-a-minute cover. Standing over the frame, look straight down and estimate how much of the quadrat is covered by green (not senescent) mile-a-minute foliage and vines.
- Number of fruiting terminals. Once seed clusters have formed, count the number of mature (containing at least one blue or purple seed) and immature (all green) seed clusters within each quadrat.

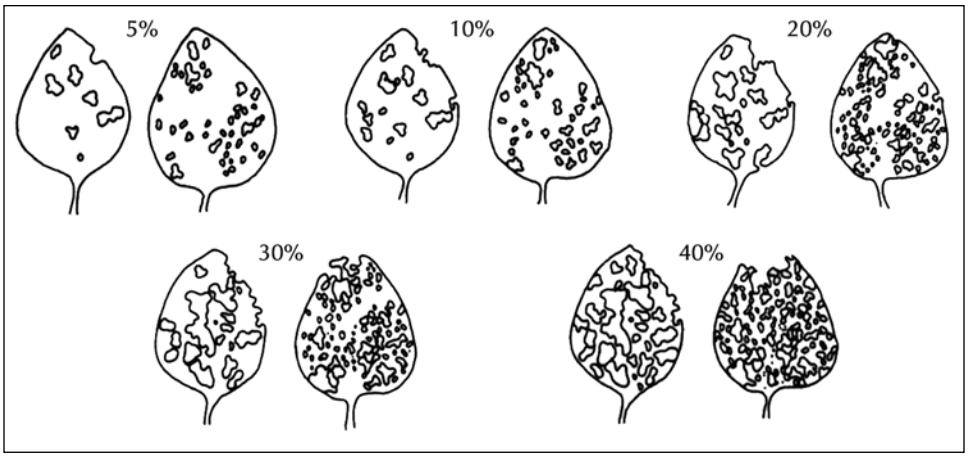


Figure 56. Percent of defoliation. Note, examples of damage up to 40% are shown, but damage up to 100% can be estimated.

Form 1. Spring mile-a-minute survey.
To be conducted after flush of MAM germination, probably between April 15 and May 15, but before vines become too dense, when stems are between 15 and 30 cm (6 to 12″) long.
Site _____
Date _____
Approximate length of MAM stems _____
Person(s) conducting survey _____

Quadrat number	Number of weevils (count first)	Number of stems originating in quadrat		Percent cover in quadrat		Weevil feeding damage		Comments (Other plants present in the quadrat, etc.)
		MAM	Other	MAM	Other	Yes	No	
R-1								
R-2								
R-3								
R-4								
R-5								
R-6								
R-7								
R-8								
R-9								
R10								
C-1								
C-2								
C-3								
C-4								
C-5								
C-6								
C-7								
C-8								
C-9								
C-10								

Form 2. Summer/fall weevil and plant monitoring.
To be conducted monthly.
Site _____
Date _____
Person(s) conducting survey _____

Quadrat number	Number of weevils (count first)	Percent of leaf area removed by insects	Node damage		Percent MAM cover in quadrat	Number of fruiting terminals*		Comments (Other plants present in the quadrat, etc.)
			Yes	No		Mature	Immat.	
R-1								
R-2								
R-3								
R-4								
R-5								
R-6								
R-7								
R-8								
R-9								
R10								
C-1								
C-2								
C-3								
C-4								
C-5								
C-6								
C-7								
C-8								
C-9								
C-10								

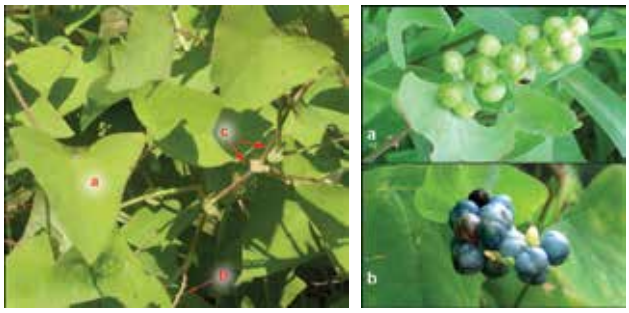
*Mature fruiting terminals have at least one blue/purple seed; immature terminals have buds or seeds that are all green.

Appendix B: Control of Mile-a-Minute Weed with the Mile-a-Minute Weevil, *Rhinoncomimus latipes*: Fact Sheet and Frequently Asked Questions

Control of Mile-a-Minute Weed with the Mile-a-Minute Weevil, *Rhinoncomimus latipes*

Basic Information: the plant

Mile-a-minute weed (*Persicaria perfoliata*) is an aggressive annual vine that was accidentally brought from Asia to the mid-Atlantic region of the US in the 1930s. It grows rapidly during the summer, and can produce a near-monoculture, out-competing other plants and preventing regeneration of trees by overtopping saplings. It is covered with small spines, which help it to climb over other plants and also make it a painful nuisance to humans. Berry-like seeds are produced in clusters during the summer and fall, turning from green to blue as they mature. Some of the full-sized green seeds are viable, ranging from 35% in mid-August to more than 80% in late September. Vines die in the fall, and seeds germinate the following spring under old vines, or in places where they have been spread by wildlife or water. Seeds can remain viable in the soil for 6 years.



MAM (left) with triangular leaves (a), backward projecting spines (b), and flared ocreae (c). MAM berry-like fruit clusters (right): immature (a) and mature (b).

Basic information: the weevil

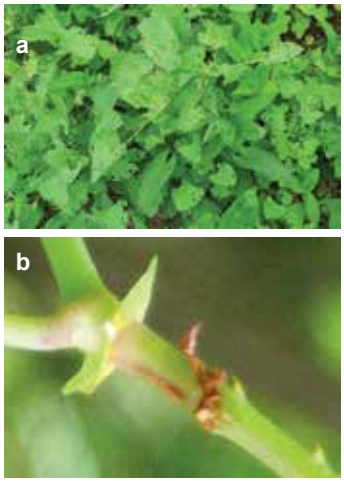
The mile-a-minute weevil (*Rhinoncomimus latipes*) was imported into the US from China in 2004, following extensive testing showing that it feeds and reproduces only on mile-a-minute weed and is not likely to have any negative direct or indirect effects in North America. Adults feed on mile-a-minute leaves, and lay eggs on the plant. Newly emerged larvae bore into the plant stems at nodes, feeding internally until they are full-grown, when they leave the plant and pupate in the soil. Adults emerge, mate, and lay eggs. The entire life cycle from egg to adult takes about 25 days (shorter under warm conditions and longer when it is cool). About 3 or 4 generations are produced during the summer. In late August the weevils stop laying eggs, and adults spend the winter in the soil or leaf litter.

Adult weevils are black (a) upon emergence, but turn brown (b) soon after feeding on MAM.

Impact of weevils on mile-a-minute weed

Feeding damage by weevils can delay and reduce seed production, decrease vine elongation, and reduce the overall growth of mile-a-minute. Various environmental conditions can affect this interaction:

- Sun versus shade. Both the weed and the weevil do better in the sun than in the shade. Weevils are attracted to sunny areas and thrive on sun-grown plants. Mile-a-minute may "hang on" in the shade in sites where it has been suppressed by weevils in the sun, but plants in the shade will not be as vigorous, and will produce fewer seeds.
- High versus low moisture conditions. Mile-a-minute is a moisture-loving plant, and can grow very rapidly when rain is plentiful. Conversely, with its shallow root system, the plant may die under dry conditions, especially if it has been damaged by weevils.
- Surrounding plant community. Mile-a-minute grows well when the surrounding plant community has been disturbed – for example, where trees have fallen or been harvested, where power line rights-of-way have been mowed, or where a site has been cleared in anticipation of future construction (but seeds are present in the soil). Mile-a-minute is not likely to invade a well-established plant community. Before releasing weevils, you should assess the plant community present with the mile-a-minute. If it consists primarily of other invasive non-native plants (such as Japanese stiltgrass) then you should consider replanting the area with desirable native plants in conjunction with weevil release.



Adult weevil feeding damage (a); damaged nodes from larval feeding (b).

Frequently Asked Questions:

How many weevils should I release? When should I release weevils?

Even small releases (20-50 weevils) any time from mid-May through August are likely to become established, but the more you release and the earlier in the season weevils are released, the more rapidly they will build up their populations and begin impacting the weed. A typical release is about 250 to 500 weevils depending upon the mile-a-minute infestation.

How soon will I see results?

This depends on site and weather conditions (see over) and on the number of weevils and timing of release. However, you should not expect anything like the immediate impact of an herbicide application. The impact of weevil feeding will be gradual, and may take several years to become noticeable. Take photos before release to remind yourself how bad the problem was before weevils were present!

What will the weevils eat after they kill off the mile-a-minute weed?

The weevils are co-evolved with their host plant, and therefore it would not be to their advantage to kill it off. We rarely see death of plants directly due to weevil feeding. The more common scenario is suppressed plants that languish in competition with other plants. If the mile-a-minute does die or deteriorate substantially, the weevils will leave the area, flying off in search of another mile-a-minute patch. The weevil will not eradicate mile-a-minute weed, but it will help to suppress it and reduce its competitive advantage over native plants.

Even though they currently feed only on mile-a-minute, couldn't the weevils evolve the ability to eat something else?

It is possible, but not likely. Some insects are generalists, feeding on a broad variety of unrelated plants. This includes the Japanese beetle, which you may see feeding on your mile-a-minute weed in addition to many other plants. The monarch butterfly is an example of a specialist insect that responds to the unique chemical cues from milkweed and whose caterpillars can only develop on milkweed. Like the monarch, the mile-a-minute weevil specializes on feeding on mile-a-minute weed, responding to its unique plant chemistry.

If I release weevils, can I also control mile-a-minute using herbicides or mechanical means?

Yes, but we recommend that you set aside a "weevil nursery" area, preferably in a sunny but out-of-the way place, where the weevils can be left to reproduce and build up their population to the point where they will impact plants and disperse to other patches. Mile-a-minute can be controlled using herbicides, mowing, or pulling in areas of high traffic or high ecological importance, leaving these nursery areas undisturbed. All management techniques are most effective when applied early in the summer before mile-a-minute starts to produce seeds.

If I spray or pull mile-a-minute plants that have weevils on them, will the weevils die?

Adult weevils will fly off as their host plant deteriorates, but any eggs or larvae present on the plants will die.

Can I move weevils around on my property or give some to my neighbors as the population increases?

Yes, as long as you are moving them within a state (you need a permit to move them between states). Adult weevils can be collected using a large funnel placed in a narrow-necked plastic container. Shake foliage with weevils into the funnel and they will drop into the container.

For more information, see http://canr.udel.edu/biocontrol/mile-a-minute-weed/

Glossary

abdomen	The last of the three insect body regions; usually contains the digestive and reproductive organs.
abiotic	Non-living, e.g. environmental factors such as temperature and humidity.
achene	A small, dry, indehiscent fruit with a single seed.
alternate	Leaves that are arranged singly at each node along a stem.
annual	A plant that flowers and dies within a period of one year from germination.
apical dominance	Influence exerted by a terminal bud in suppressing the growth of lateral buds.
aspirator	An apparatus used to suck insects into a collection container.
beetle	A member of the very large and variable insect order Coleoptera; adults have hardened or leathery forewings (elytra) while larvae may be grub-like or mobile; beetles exhibit complete metamorphosis.
biological control	The reduction in the abundance of a pest through intentional use of its natural enemies (predators, parasitoids, and pathogens); also called "biocontrol."
biological control agent	A natural enemy of a target pest used in biological control efforts.
chemical weed control	Weed control strategies employing herbicides.
choice test	A test of host specificity in which the potential biological control agents are presented with a combination of test-plant species along with the target weed, and their oviposition or feeding is recorded.
classical biological control	A biological control strategy employing the release of a pest's natural enemies imported from another region; typically directed against exotic pests, it uses natural enemies from areas where the pest is native.
cold stratification	A period of moist cold required for some seeds before they will germinate.
competition	Negative interactions between individuals of the same or different species that utilize the same resource(s); if the resource is in short supply, one individual or species may survive and increase in number at the expense of the other(s).

complete metamorphosis	A type of insect development characterized by immature stages (larvae and pupae) that look quite different from the adults, and typically live in different habitats, eat different foods, and exhibit different behaviors than do the adults.
community	A naturally occurring group of different species of organisms that live together and interact as a more or less self-contained "unit."
cover	The portion of the vegetative canopy in a fixed area attributable to an individual or a single plant species.
cultural methods	Weed control methods that modify the plant's environment, such as adding or removing shade or fostering competition with other plants.
defoliation	The loss of foliage, often due to insect feeding.
defoliator	An organism, usually an insect, that consumes plant foliage.
density	Number of individuals per unit area.
diapause	The physiological condition of overwintering insects.
dispersal	The spread of animals and plants from any point; the redistribution of plant seeds, fungal spores, or insect eggs, larvae, and adults.
dormant	In a state of temporarily reduced metabolic activity.
emergence	Act of adult insect leaving the pupal case or reappearing after overwintering.
eradicate	Total elimination of an organism from an area.
excelsior	Long, thin wood shavings used for packing.
exoskeleton	A hard outer structure, such as the shell of an insect or crustacean, that provides protection or support for the organism.
exotic	Not native.
FHTET	Forest Health Technology Enterprise Team, a division of the USDA Forest Service.
field insectary	An area where host plants or animals are abundant and biological control agents are released and propagated with or without additional human manipulation.
forb	A herbaceous plant that is not a grass nor grass-like in form.
frass	The excrement produced by insects, containing feces and undigested plant material.
genus (pl. genera)	A taxonomic category ranking below a family and above a species and generally consisting of a group of species exhibiting similar characteristics. In taxonomic nomenclature the genus name is used, either alone or followed by a Latin adjective or epithet, to form the name of a species.
herbicide	A chemical substance used to destroy or inhibit the growth of plants, especially weeds.

herbivory	Feeding on plants.
host	The plant or animal on which an organism feeds; the organism utilized by a parasitoid; a plant or animal susceptible to attack by a pathogen.
host range	The different host species that may be utilized by a plant- or animal-feeding organism.
host specificity	The dietary restriction of an organism to a single or limited food (for herbivores: the number of plant species accepted as food; the highly-evolved, often obligatory association between an insect and its host(s); the degree to which an organism is restricted to a particular number of plant or animal hosts).
insect	A small arthropod animal that, as an adult, normally has six legs, three distinct body regions, one pair of antennae, and one or two pairs of wings.
instar	Period or stage between successive molts in an insect larva.
integrated weed management	A system for planning and implementing a program to contain or control an undesirable plant species or group of species, using all available methods and a thorough knowledge of pest biology.
invasive plant	An aggressive and dominant plant, likely to colonize and become established in new habitats; usually refers to non-native weeds.
invasive species treadmill	Where one invasive species is controlled, only to be replaced by another invasive species.
lamina	The expanded portion, or blade, of a leaf or petal.
larva (pl. larvae)	Immature insect stage between the egg and pupa.
mass rearing	The mass production of a natural enemy.
mechanical weed control	Mechanical methods that employ physical means to remove or control weeds, including activities such as hand pulling, hoeing, tilling, mulching, burning, and mowing.
metamorphosis	The change from one life stage to another in insects, such as from larva to pupa.
molt	The process by which insects and other arthropods shed their exoskeleton (“skin”) as they grow and develop; among insects, molting is typically restricted to larval or nymphal stages.
monoculture	An area vegetated by a single plant species.
natural enemies	The parasites, predators, pathogens, and other antagonists associated with a species of animal or plant that cause debility or mortality.
no-choice test	A test of host-specificity in which the potential biological control agent is presented with a single, non-target test-plant species at a time. Feeding, development, survival rate and/or oviposition rates are recorded and compared to those on the target weed.

node	The position on a stem where leaves or branches orignate; also known as a "joint."
non-target	Not being the target of a control method, e.g., not a desired host or food source for a biological control agent.
ocrea (pl. ocreae)	Fused stipules that surround the stem at each leaf node; in mile-a-minute weed the ocreae flare widely into a saucer shape.
oligophagous	Feeding on a few (usually related) different types of plants or prey.
phenology	Related to seasonality.
oviposit	To lay or deposit eggs.
PABIL	Phillip Alampi Beneficial Insect Laboratory, a New Jersey Department of Agriculture facility for rearing biocontrol agents, located in West Trenton, NJ.
perennial	A plant living more than two years.
petiole	A leaf stalk.
physiological host range	All plant species that support feeding, development, and reproduction of a particular insect species, when tested under laboratory conditions.
phytophagous insect	An insect that feeds on plants.
polyphagous insect	An insect that feeds on many types of plants or prey.
post-emergent herbicide	An herbicide that controls plants via uptake of chemical through the plant foliage or stems.
pre-emergent herbicide	An herbicide that controls plants before they emerge from the ground by injuring the plant as the seed germinates.
pupa (pl. pupae) (v. pupate)	Non-feeding, inactive stage between the larva and adult in insects with complete metamorphosis.
phylogenetic	Relating to or based on evolutionary development or history.
quadrat	A specific area used to sample vegetation (e.g., 1 square meter, or 1 m^2).
radicle	The first part of a seedling (a growing plant embryo) to emerge from the seed during the process of germination; the embryonic root of the plant.
scarification	Cutting or softening the hard wall of a seed to break seed dormancy.
seed bank	An accumulation in the soil of long-lived seeds, which can potentially germinate many years after they were produced.
species	A fundamental category of taxonomic classification, ranking below a genus or subgenus and consisting of related organisms capable of interbreeding.
stipule	Outgrowths of the base of a leaf stalk (petiole).
surfactant	A compound that increases the effectiveness of an herbicide by increasing the adherence of the herbicide mixture to the leaf surface, reducing the surface tension of the mixture so it spreads over more of the leaf and aiding penetration of the waxy outer cuticle of the leaf.

TAG	Technical Advisory Group for Biological Control Agents of Weeds.
taxonomy	The classification of organisms in an ordered system that indicates natural relationships. The science, laws, or principles of classification; systematics.
terminal	The growing tip of a mile-a-minute weed vine or vine branch; may eventually develop into a flower cluster.
thorax	Body region of an insect, behind the head, bearing the legs and wings.
transect	A straight line or path through an area.
USDA-APHIS-PPQ	United States Department of Agriculture-Animal and Plant Health Inspection Service-Plant Protection and Quarantine.
vegetative reproduction	Reproduction in plants other than by seeds, such as from rhizomes, stolons, and from nodes on lateral, often creeping, roots.
viability	The proportion of propagules (e.g., seeds) that are alive and can germinate.
weevil	A type of plant-eating beetle, the adults having distinct snouts of variable lengths.

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Chapter 4: Biological Control as a Component of an Integrated Mile-a-Minute Weed Management Program

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