BIOLOGICAL CONTROL OF MILE-A-MINUTE WEED, *PERSICARIA PERFOLIATA*, AND INTEGRATING WEED MANAGEMENT TECHNIQUES TO RESTORE INVADED SITES

by

Ellen C. Lake

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology and Wildlife Ecology

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ABSTRACT

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Polygonaceae), is an annual vine from Asia that has invaded the eastern U.S. where it can form dense monocultures and outcompete other vegetation in a variety of habitats. The host-specific Asian weevil *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) was first released in the U.S. in 2004 as part of a classical biological control program.

The weevil was intensively monitored in three release arrays over six years. Field cages at each site were used to determine the number of generations produced. The weevil established at all three sites and produced three to four generations before entering a reproductive diapause in late summer. Weevils dispersed at an average rate of 1.5 to 2.9 m wk^{-1} through the 50 m diameter arrays, which had fairly contiguous mile-a-minute cover. Weevils dispersing in the broader, more variable landscape located both large monocultures and small isolated patches of mile-a-minute 600 – 760 m from the release within 14 months. Weevil density ranged from fewer than 10 to nearly 200 weevils m^{-2} mile-a-minute weed. Six years post-release, mile-a-minute weed cover was reduced at two sites and mile-a-minute seed cluster production declined at all three sites. Although mile-a-minute cover appeared to rebound at all three sites in 2009, the weevils were able to suppress mile-a-minute growth and reproduction in 2010. The ability of the weevil to establish, produce multiple generations per season, disperse to new patches, and its

likelihood of having an impact on plants in the field suggests that *R. latipes* has the potential to be a successful biological control agent.

Efforts to suppress an invasive weed are often undertaken with the goal of facilitating the recovery of a diverse native plant community. In many cases, however, reduction in the abundance of the target weed results in an increase in other exotic weeds, the invasive treadmill effect. Six years post-release, only one of the three weevil release sites had a diverse plant community, while the abundance of exotic weeds appeared to be increasing at the other sites. Therefore an additional experiment was conducted to determine the effect of integrating biological control with other management strategies on mile-a-minute weed and the surrounding plant community.

At three additional sites invaded by mile-a-minute weed, biological control was integrated with different densities of native plantings. A pre-emergent herbicide was applied to one half of each planting treatment plot. The combination of biological control and pre-emergent herbicide decreased the abundance of mile-a-minute weed compared to the no-herbicide plots, but there were no differences by planting treatment. After 2.5 years, native plant cover was greater than 80% in the plots with plantings and pre-emergent herbicide at the two sites with the greatest pressure from exotic plants. In the planting treatments without herbicide, native cover was less than 30%. When mile-a-minute cover decreased at these two sites, these plots were dominated by another exotic weed, *Microstegium vimineum* (Trin.) A. Camus, Japanese stiltgrass. The combination of biocontrol, revegetation with natives and pre-emergent herbicide suppressed mile-a-minute weed, prevented invasion of Japanese stiltgrass, and increased the abundance of native plants. The results of this experiment suggest that the selection of the management strategies used to control mile-a-minute weed will determine the extent of recovery of the native plant community.

Chapter 1

ESTABLISHMENT AND DISPERSAL OF THE BIOLOGICAL CONTROL WEEVIL *RHINONCOMIMUS LATIPES* ON MILE-A-MINUTE WEED, *PERSICARIA PERFOLIATA*

INTRODUCTION

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Freeman and Reveal, 2005), is an invasive annual vine in the U.S. that germinates earlier in the spring than many native plants. Backward-projecting thorns on its leaves and stems enable mile-a-minute to climb over other vegetation and form dense mats (Moul, 1948). Although it prefers full sun, *P. perfoliata* can grow in partial shade and is a weed in the U.S. in a variety of settings including wetlands, stream banks, forest edges and clearcuts, meadows, rights-of-way and roadsides (Mountain, 1989; Hough-Goldstein et al., 2008a). Mile-a-minute weed's ability to outcompete native plants poses a risk to natural ecosystems (Oliver, 1996) and it reduces human access to natural areas.

The native range of *P. perfoliata* includes much of east Asia (Wu et al., 2002 and references therein). It established in the U.S. in the 1930s at the Gable Nursery in Stewartstown, York County, Pennsylvania, where it emerged with a planting of holly seeds that originated from Japan (Moul, 1948). Mile-a-minute has since invaded 12 states; its current range extends from Pennsylvania north to Massachusetts, west to Ohio and south to North Carolina (Hough-Goldstein et al., 2008a; EDDMapS, 2011).

Mile-a-minute weed can grow to a length of 6 m (Mountain, 1989) and its seeds (achenes) can persist for at least six years in the seedbank (Hough-Goldstein et al., 2008a). A single *P. perfoliata* plant in full sun can produce more than 2200 seeds (Hough-Goldstein et al., 2008b). Mile-a-minute seed is dispersed by water, birds, and mammals (Mountain, 1989; McCormick and Hartwig, 1995; Hough-Goldstein et al., 2008a).

Two surveys in the mid-Atlantic U.S. failed to identify any insect species causing extensive enough damage to potentially control mile-a-minute weed (Wheeler and Mengel, 1984; Fredericks, 2001). The USDA Forest Service started a classical biological control program in 1996 (Wu et al., 2002). Ding et al. (2004) identified 111 insect species from a variety of feeding guilds on mile-a-minute in China between 1996 and 2001. Based on its density, distribution, host range, and the apparent damage it caused to mile-a-minute, the weevil *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) was subjected to host range testing in China and the U.S. (Wu et al., 2002; Ding et al., 2004). This testing indicated that *R. latipes* was extremely host specific to *P. perfoliata* (Price et al., 2003; Colpetzer et al., 2004a). These results were later validated via field host specificity testing with closely related members of the Polygonaceae (Frye et al., 2010).

The USDA issued a release permit for *R. latipes* in July 2004. The New Jersey Department of Agriculture Phillip Alampi Beneficial Insect Laboratory in Trenton, NJ began mass rearing the mile-a-minute weevil in 2004 (Hough-Goldstein et al., 2008a).

Weevils have since been released in ten states (J.H-G., unpublished data; Hough-Goldstein et al., 2009).

The native range of *R. latipes* extends south of the Russian Far East and through continental China, Korea and Japan (Ding et al., 2004; Korotyaev, 2006; Miura et al., 2008). Adult *R. latipes* are 2.0-2.5 mm long, are black upon emergence and turn orange after feeding on mile-a-minute weed, apparently due to chemicals found in mile-a-minute sap. Adult weevils feed on the capitula, leaves, and ocreae, and oviposit on the capitula, leaves, and stems. Larvae bore into the mile-a-minute stem at unoccupied nodes and feed within the stem, then exit the stem and drop to the soil to pupate. Under laboratory conditions, development from egg to adult takes approximately 26 days (Colpetzer et al., 2004b).

Existing North American biological control programs have placed greater emphasis on the search for, screening and release of agents than monitoring their impact post-release (McEvoy and Coombs, 1999). The lack of adequate post-release data is a common criticism of biological control (McClay, 1995; Blossey and Skinner, 2000) and protocols to improve post-release monitoring have been suggested (Blossey and Skinner, 2000; Blossey, 2004; Denslow and D'Antonio, 2005; Carson et al., 2008; Morin et al., 2009).

Information about the ability of *R. latipes* to disperse and reproduce, and its potential long-term impact on mile-a-minute weed will facilitate the design of protocols for future releases and help to increase the immediate effectiveness of the weevil as a biocontrol agent. In this study, the first to intensively evaluate *R. latipes* in the field in

North America, three weevil releases were conducted in arrays in order to track weevil dispersal within the release arrays and to surrounding areas. In addition, during the first year, field cages were supplied with adult weevils and potted *P. perfoliata* to track the development of new generations during the season. The objectives of this study were to: determine the life history of *R. latipes* in North America including establishment and population dynamics; track weevil dispersal from a central release point; and follow the change in *R. latipes* and mile-a-minute weed populations over time during the first six years following release. The relationship between weevil populations and *P. perfoliata* seed production over time was also assessed, as a partial measure of weevil impact on the weed.

MATERIALS AND METHODS

Site history and array set-up

Three weevil releases were conducted, two at the Brandywine Valley Association's (BVA) Myrick Conservation Center, and one at the Brandywine Conservancy's Laurels Preserve, all located in Chester County, Pennsylvania. Control plots were also established at each site, between 40 and 150 m from the release sites, but weevils dispersed to these plots within four months of the release, and therefore monitoring was discontinued after 2005.

Mile-a-minute weed has been present at the BVA Wetland release site (39º55'06.66"N, 75º40'41.38"W) since the early 1990s, and is now found in large dense patches throughout the wetland (personal communication, Kevin Fryberger, former BVA Land Manager). The other BVA release site (39º54’42.35”N, 75º40’33.30”W) was located 800 m away and experienced significant disturbance in preparation for installation of Conservation Reserve Enhancement Program (CREP) tree plantings in 2002. Mile-a-minute weed established at the BVA CREP site following this disturbance; it is less common and found in smaller patches in this site than the wetland. The third release site was at the Laurels (39º55’48.30”N, 75º47’23.30”W), approximately 9.5 km from the BVA releases. Mile-a-minute weed established at this site in the early 1990s (personal communication, Kevin Fryberger, Brandywine Conservancy Land Manager), and there was more of a monoculture of mile-a-minute at the Laurels than the BVA sites, as quantified in this study.

During May of 2005, a mile-a-minute weed patch as close to 50 m in diameter as possible was located for each release site. At each site the monitoring array was centered with the release point in a dense, sunny patch of mile-a-minute. A maximum of 76 1-m^2 monitoring points located on concentric circles between 1 m and 25 m from the release point were established at each release site (Fig. 1.1). The center of each monitoring quadrat was marked with a bamboo pole labeled with a combination of a number denoting the distance from the release and a letter corresponding to its position within the array. In order to compensate for a decreased likelihood of observing weevils at the greater distances within the array, the number of monitoring points on each concentric circle increased with distance from the release (Turchin, 1998; Fig. 1.1). A portion of the 20 m and 25 m circles at all three sites were located in hedgerows or other areas with trees. The majority of, but not all, monitoring quadrats contained *P. perfoliata* when the arrays were established in 2005.

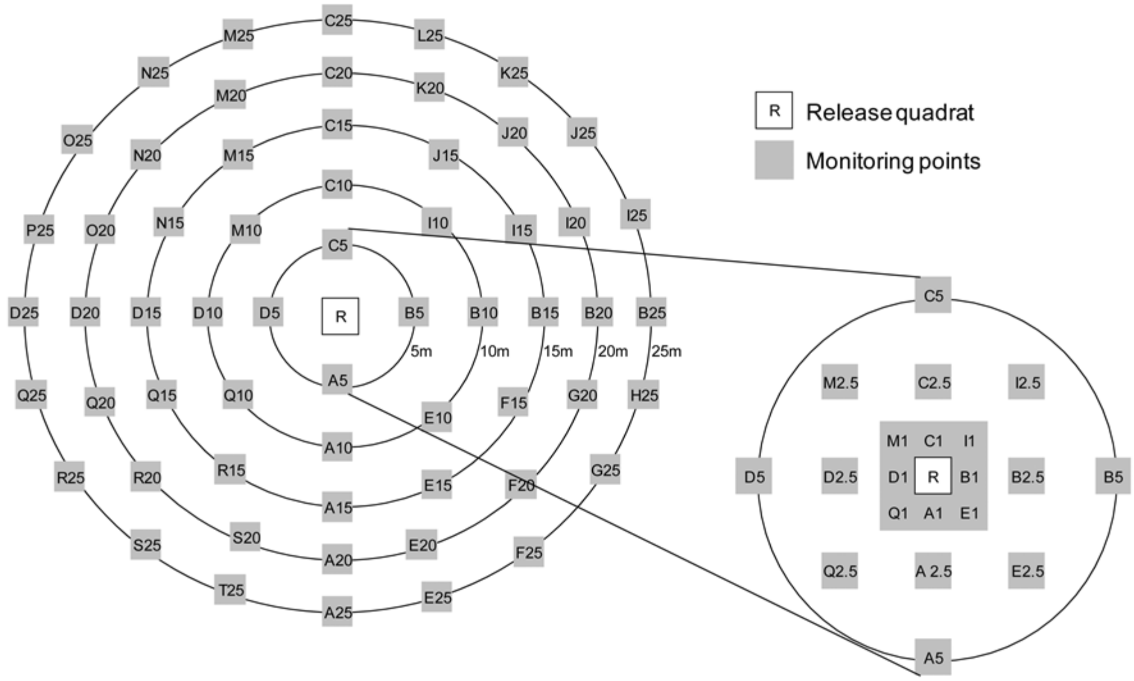

Fig. 1.1. Site design for weevil release arrays. Monitoring points were located on concentric circles 5 m, 10 m, 15 m, 20 m and 25 m from the point of release. The inset illustrates sixteen additional monitoring points located approximately 1 m and 2.5 m from the release.

Four hundred and fifty weevils, the maximum number available, were released at each site on 9 June 2005. Three hundred weevils for each release were obtained from the NJ Department of Agriculture Phillip Alampi Beneficial Insect Rearing Laboratory; the remaining 150 weevils were from the University of Delaware's rearing colony. Both rearing colonies were founded with weevils collected from *P. perfoliata* plants in Hunan Province, China (Hough-Goldstein et al., 2009). Weevils were not sexed prior to release, but samples checked by workers at the Phillip Alampi Beneficial Insect Rearing Laboratory typically had a 1:1 sex ratio (personal communication, Daniel Palmer, NJ Department of Agriculture). Upon release, the majority of weevils crawled onto the mile-a-minute weed and many immediately began to feed; very few were observed flying despite hot, sunny conditions.

A HOBO H8 Pro Series data logger (Onset Computer Corporation, Bourne, Massachusetts) was installed within 5 m of the release at each site. The logger recorded the temperature once per hour, 24 h day $^{-1}$.

Monitoring protocol

During 2005, monitoring began four days post-release and then took place weekly from 16 June through 19 July, and every other week through 1 November. In 2006 through 2010, monitoring was conducted every other week beginning when large numbers of weevils were observed actively feeding in the field sites, and ending with the first sustained frost. Dates were 23 May through 18 October, 2006, 30 May through 10 October, 2007, 17 May through 11 October, 2008, 22 May through 6 October, 2009, and 8 June through 13 October, 2010. Each monitoring point was checked for the following within a 1-m^2 quadrat: percent cover of mile-a-minute weed, number of weevils, and presence or absence of eggs (2005-2007 only). To monitor each point, a 1 x 1 m frame constructed of PVC pipe was placed around the bamboo pole marking the center of the point, oriented in the same direction each time. The frame was cut in half in order to center it around the pole and minimize disturbance to the mile-a-minute plants, because the weevils sometimes drop off the plants when disturbed.

Percent cover was determined by looking down at the 1-m^2 frame, which was marked in 10-cm intervals, and estimating what percentage of the area within the frame was covered by live mile-a-minute weed foliage. The number of mature and immature seed clusters was recorded in the release quadrat and all monitoring points located on the A, B, C, and D transects from 1 m to 25 m (n = 21 maximum points; Fig. 1.1), beginning each year with the onset of seed production. A mature cluster contained at least one blue fruit and an immature cluster contained at least one full-sized but green fruit. In both cases the fruit had to be present in the main cluster, not in an ocrea.

All monitoring points within 5 m of the release point were checked at each site in 2005. Monitoring points on the 10-m ring were then checked for signs of weevil activity, i.e. presence of adult weevils or eggs or nodes damaged by larval feeding. If weevil activity was observed at three or more monitoring points, all points on that ring were monitored and the next ring was checked. This sampling protocol was used throughout 2005. All rings were monitored at each site during 2006-2010. Spring seedling counts were conducted in May of 2006-2010 using a 1 x 0.5 m quadrat frame constructed of PVC, oriented in the same direction each time, in the release quadrat and at all monitoring points located on the A, B, C, and D transects from 1 m to 25 m (n = 21 maximum points; Fig. 1.1).

Generations per year

To determine the number of weevil generations that could develop in the course of a season, wood-framed cages (56 x 56 x 61 cm) were constructed. A fine mesh fabric was stapled to the cage interior and standard plastic window screening was stapled to the exterior. The bottom of each cage was left open and gaps between the edges of the cages and the ground were filled with soil. Two cages were installed at each of the release sites and a large potted mile-a-minute plant was placed in each cage. On 2 June 2005, 20 weevils from the University of Delaware rearing colony were added to each cage. These adults were allowed to feed and oviposit on the plant for 5 d. All adults that could be found were then removed from the cages. The potted plants were watered as needed and were checked for weevil emergence, which took approximately one month from the addition of the original adults. When large numbers of F1 adults were observed in the cages, these weevils were captured, the old plant was removed and a new potted plant was added to the cage. The F1 adults were returned to the cage, oviposited on the plant for one week, and were then removed. The plant was watered and checked for the emergence of F2 adults. This procedure continued through October of 2005.

Dispersal beyond arrays, 2005 and 2006

A limited search for weevil activity on mile-a-minute in areas surrounding the release sites was conducted in mid-October 2005. During late June through July of 2006, the BVA Myrick Conservation Center and the Laurels Preserve were surveyed and an eTrex Vista GPS unit (Garmin Ltd., Olathe, Kansas) was used to create GIS maps of their *P. perfoliata* populations. A worker walked along trails and hedgerows, the main sites of mile-a-minute populations, and examined the plants for weevil activity. Waypoints were recorded at intervals of approximately 5 m and were plotted on aerial orthophotos from the Pennsylvania State University using Arcview 9.1 (Esri, Inc., 2005). Mile-a-minute patches were color coded to indicate if weevil activity was present, and were counted to determine the proportion of patches with weevil activity.

Weather data

Monthly temperature and rainfall data for each year were obtained from the Weather Warehouse, Station West Chester 2Nw, located in West Chester, PA (Weather Source, 2011).

Statistical analysis

To assess the seasonality of egg production, the mean proportion of all monitored quadrats with eggs present from 2005 through 2007 was determined, treating the three release sites as replicates for this variable. The temperature readings from the HOBO data recorders were averaged to obtain mean daily temperatures at each site. Temperatures at the three sites were always very similar, and therefore the mean daily temperatures from mid-August through the end of September were averaged for the three sites in 2005 and 2006 to provide overall temperature data. The 2007 data file from the Laurels became corrupted, so the average temperature for 2007 was based on the two BVA sites.

The maximum distance at which weevil activity was detected during each sample period in 2005 was used to calculate the rate of dispersal for each site. For each sample period, maximum distance from the release point was divided by the number of days since release, to yield an estimate of dispersal per day at each site. A two-way ANOVA followed by Tukey's test was applied to analyze the rate of dispersal by site and sample time (SAS Institute, 2008).

Mile-a-minute percent cover varied greatly at different sites and monitoring points. Therefore weevil populations were expressed as weevils m^{-2} of mile-a-minute cover. This was calculated for each monitoring point on each sample date by dividing the total number of weevils in a given quadrat by the proportion of mile-a-minute cover in that quadrat. Changes in the number of weevils m^{-2} of mile-a-minute weed and percent mile-a-minute cover over time were evaluated in the 21 monitoring points located within 5 m of the release point. This region of the array consisted of a dense patch of mile-a-minute weed in full sun at all three sites. The integral, or area under the curve (AUC), was calculated for each monitoring point, each year, to quantify the cumulative population of multiple generations of weevils and the cumulative amount of mile-a-minute cover over the course of the entire season for each site and year. This technique has been used to compare insect densities among treatments (Parry et al., 2006) and insect populations over time (Hough-Goldstein and McPherson, 1996). Slight adjustments were made to the sample dates in order to analyze the same range of dates each year. Changes in weevil density and percent cover over time were analyzed by applying a one-way ANOVA to the integrals by year at each site using PROC GLM of the SAS system; Tukey's test was used for mean separation (SAS Institute, 2008).

Cumulative seed cluster production during the entire season was also assessed by determining the area under the curve for each site and year, using the release point and all monitoring points on the A, B, C, and D transects at each site over all six years (individual area under the curve calculated for a maximum of 29 points per year). The relationship between cumulative weevil populations and cumulative seed cluster production at each monitoring point was assessed using a regression analysis (PROC REG) of weevils m^{-2} mile-a-minute area under the curve versus seed cluster area under the curve, separately for each site but including all six years, using the monitoring points on the A, B, C, and D transects within the central 5 m radius (maximum of 13 points per year) (SAS Institute, 2008).

To assess changes in seedling production from one year to the next at each site, the mean number of mile-a-minute seedlings 0.5 m^{-2} counted in the spring in the release point and all points along the A, B, C and D transects (Fig. 1.1) were compared using a one-way ANOVA (PROC GLM) followed by Tukey's test (SAS Institute, 2008).

Data were log or square-root transformed as needed to reduce heteroskedasticity of variance residuals. If transformations did not resolve problems with heteroskedasticity, data were analyzed using Wilcoxon ranks. Non-transformed means and standard errors are presented.

RESULTS

Rhinoncomimus latipes egg production and generations per year

Eggs were observed in an average of 65% of monitored quadrats at the three release sites four days after the 9 June release in 2005 (Fig. 1.2). In 2006 and 2007, eggs were found in about 50% of quadrats when monitoring commenced in May, and this proportion stayed relatively high throughout the season. About 60% of quadrats contained eggs in late August, after which egg production ceased (Fig. 1.2). The decline in egg production was highly synchronized from late August to late September all three years (Fig. 1.2). Temperatures remained relatively warm during this period, especially in 2005 (Table 1.1). The first sustained frost occurred in late October in 2005 and 2007, and in mid-October in 2006.

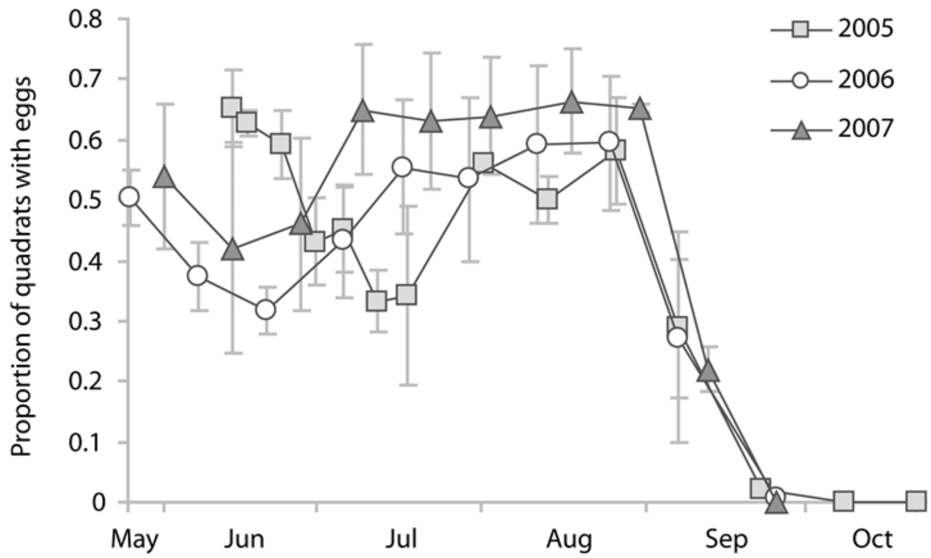

Fig. 1.2. Mean (± SEM) proportion of monitored mile-a-minute quadrats with eggs present at the three release sites from 2005 to 2007.

Table 1.1. Average weekly temperature (ºC) at release sites from mid-August through the end of September, when egg production by *R. latipes* ceased.

	2005	2006	2007
Aug 18-24	20.76	21.02	17.31
Aug 25-31	21.22	21.25	21.28
Sept 1-7	17.96	16.95	18.69
Sept 8-14	17.88	16.57	19.74
Sept 15-21	20.74	16.58	13.13
Sept 22-28	18.01	15.35	18.21

The weevils caged on potted mile-a-minute plants in June 2005 produced a new generation in all six replicate cages (two per site) about one month later. The F2 generation emerged in mid to late August 2005 and the F3 generation was observed in all cages in mid to late September. A few F4 adults were observed in the cages in October, prior to the first hard frost.

Weevil dispersal

Weevils dispersed from the point of release and by the fall of 2005 were detected in monitoring points on the 15 m ring at the BVA CREP site, the 20 m ring at the BVA Wetland, and the 25 m ring at the Laurels. The average weekly rate of dispersal during 2005 differed by both site ($F_{2,20}$ = 10.32, P = 0.008) and time (weeks since release; $F_{10,20}$ = 11.95, P < 0.0001). Average dispersal rates during 2005 ranged from 1.5 to 2.9 m week^{-1}, and dispersal was slower at the BVA Wetland than at the other two sites (Table 1.2a). During the first week, weevils dispersed from the release point at an average rate of 6.0 m wk^{-1}, but by week 16 the overall average dispersal rate from the point of release was only 1.3 m wk^{-1} (Table 1.2b).

Table 1.2. Rate of dispersal from mid-June, one week post release, to early October 2005, a) by site and b) by sample time. Values for each site are means of 11 sample times, and values for each sample time are means of three sites; means followed by the same letter are not significantly different (Tukey's test).

a. Site	Dispersal rate (m day^{-1}) ± SEM	b. Sample Time (week)	Dispersal rate (m day^{-1}) ± SEM
Laurels	0.42±0.09a	1	0.86±0.20a
BVA CREP	0.34±0.06a	2	0.57±0.12ab
BVA Wetland	0.22±0.05b	3	0.39±0.08bc
		4	0.30±0.06bc
		5	0.24±0.05c
		6	0.20±0.04c
		8	0.18±0.05c
		10	0.21±0.08c
		12	0.22±0.04c
		14	0.22±0.02c
		16	0.19±0.01c

In October 2005, weevils were found about 100 m from the BVA CREP release and approximately 200 m from the Laurels release. Fourteen months after the release, in late June and July of 2006, weevil activity was detected 760 m from the Laurels release and nearly 600 m from the release sites at BVA. Dispersing weevils located both large mile-a-minute weed populations and small, isolated patches. At the time, weevil activity was present in 12% (N = 643) of patches at the 771-acre Laurels Preserve and 44% (N = 454) of patches at the 318-acre BVA Myrick Conservation Center.

Changes in weevil populations and mile-a-minute cover over time

The average weevil population density m^{-2} of mile-a-minute weed within 5 m of the release sites decreased at each site after the 9 June 2005 release as weevils dispersed from the release quadrats (Figs. 1.3, 1.4, and 1.5), and increased when the F1 generation began to emerge in the field, approximately one month after the release. The highest density of weevils in 2005 was observed at the Laurels, with a peak of 37.5 weevils m^{-2} of mile-a-minute weed on 4 October (Fig. 1.3). Weevils successfully overwintered at all three release sites.

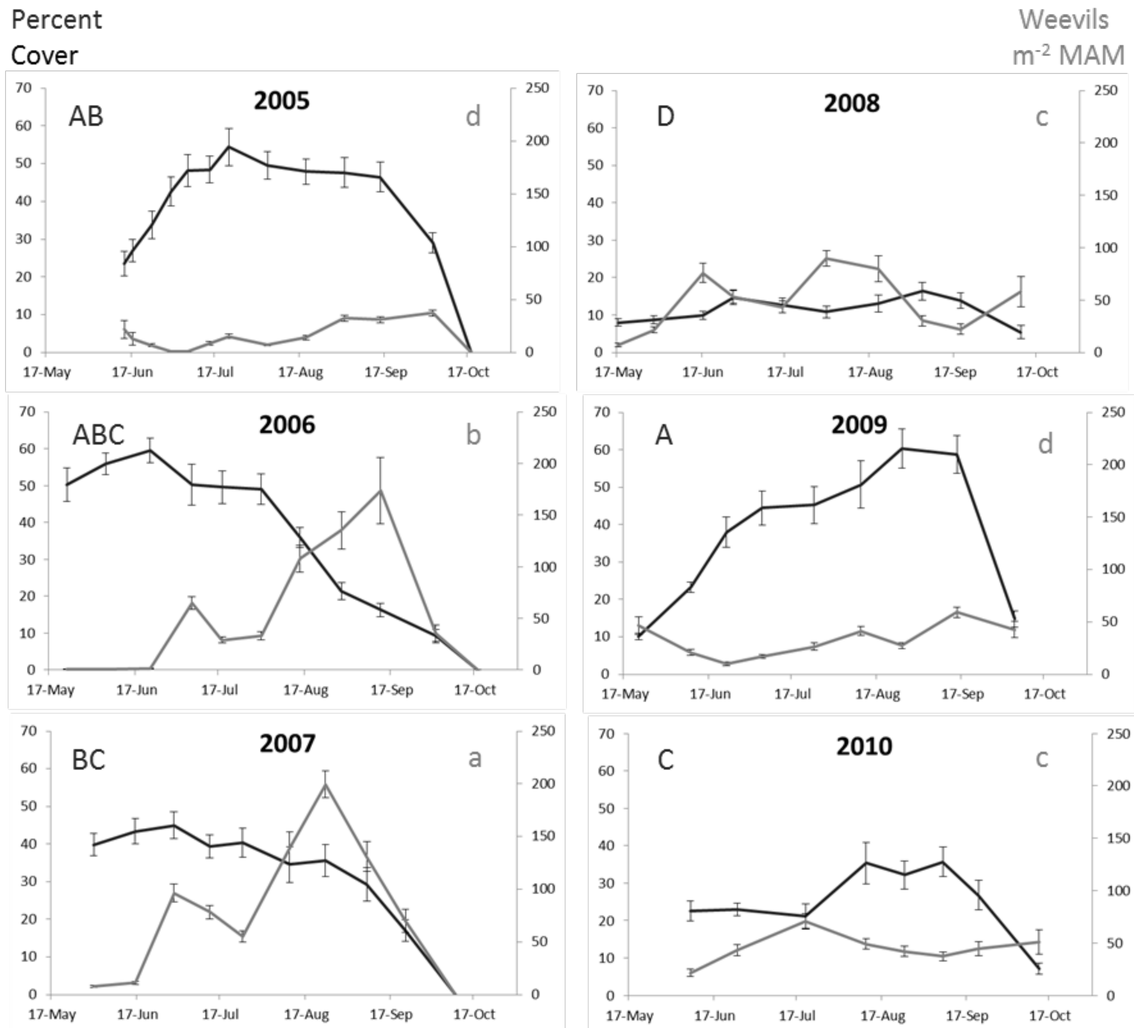

Fig. 1.3. Mean (± SEM) weevils m^{-2} of mile-a-minute weed and percent cover of mile-a-minute within 5 m of the release at the Laurels from 2005 to 2010. Analyses based on the area under the curve; means with the same letter are not significantly different (Tukey's test).

Weevil population density increased at the Laurels from 2005 to 2007 with an average of more than 100 weevils m^{-2} of mile-a-minute for at least one month in 2006 and 2007. The weevil density at the Laurels was low in 2008 and very low in 2009 but increased in 2010 (area under the curve, $F_{5,120}$ = 53.66, P < 0.0001, Fig. 1.3). At the BVA

CREP site, fewer than 30 weevils m^{-2} of mile-a-minute weed were sampled on most dates and years, and there was no significant change in weevil population density from 2005 through 2010 ($F_{5,96} = 0.88$, $P = 0.4990$, Fig. 1.4). The weevil population density increased over time at the BVA Wetland ($F_{5,116} = 14.45$, $P < 0.0001$, Fig. 1.5). An average of fewer than 20 weevils m^{-2} of mile-a-minute were counted on most dates at this site.

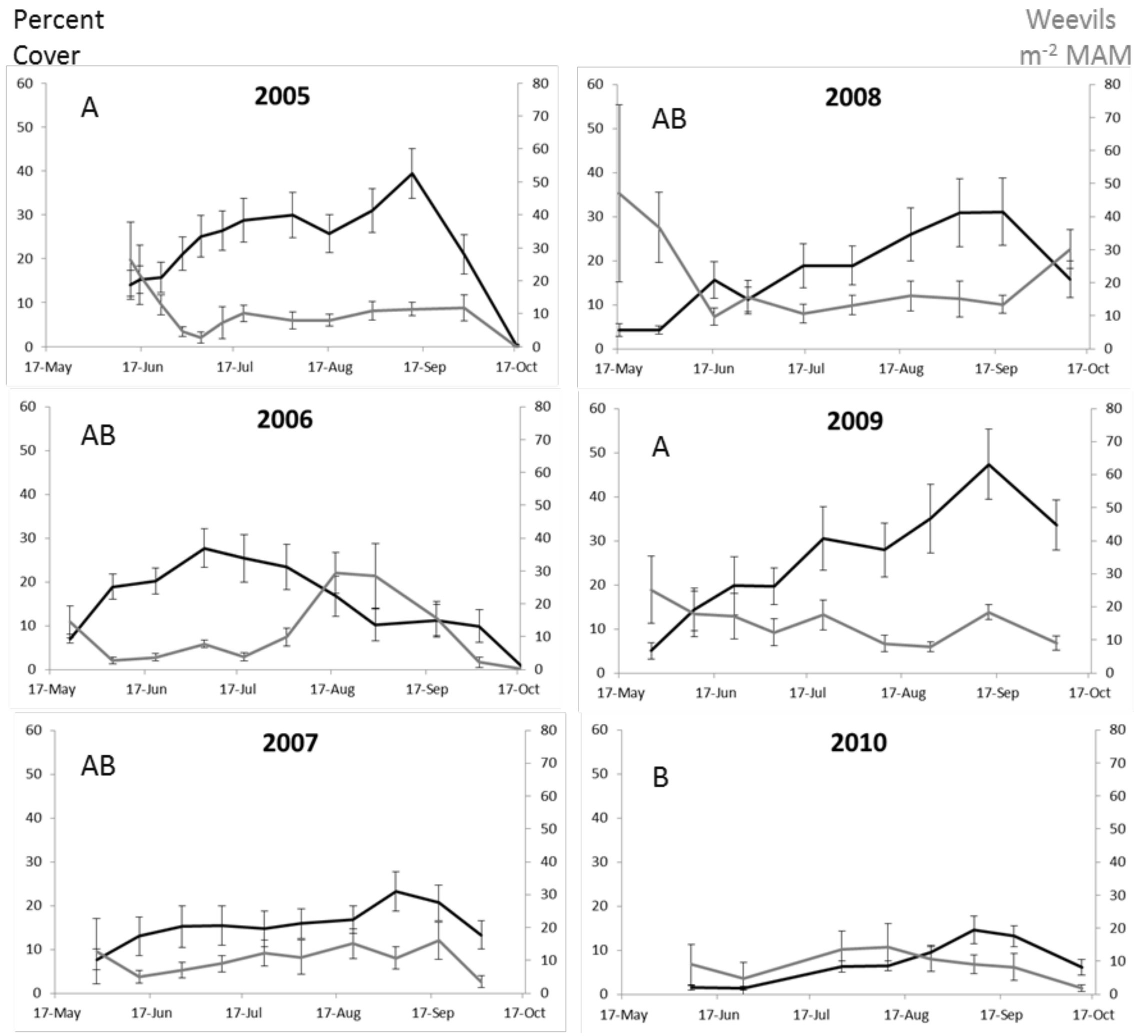

Fig. 1.4. Mean (± SEM) weevils m^{-2} of mile-a-minute weed and percent cover of mile-a-minute within 5 m of the release at the BVA CREP site from 2005 to 2010. Analyses based on the area under the curve; means with the same letter are not significantly different (Tukey's test).

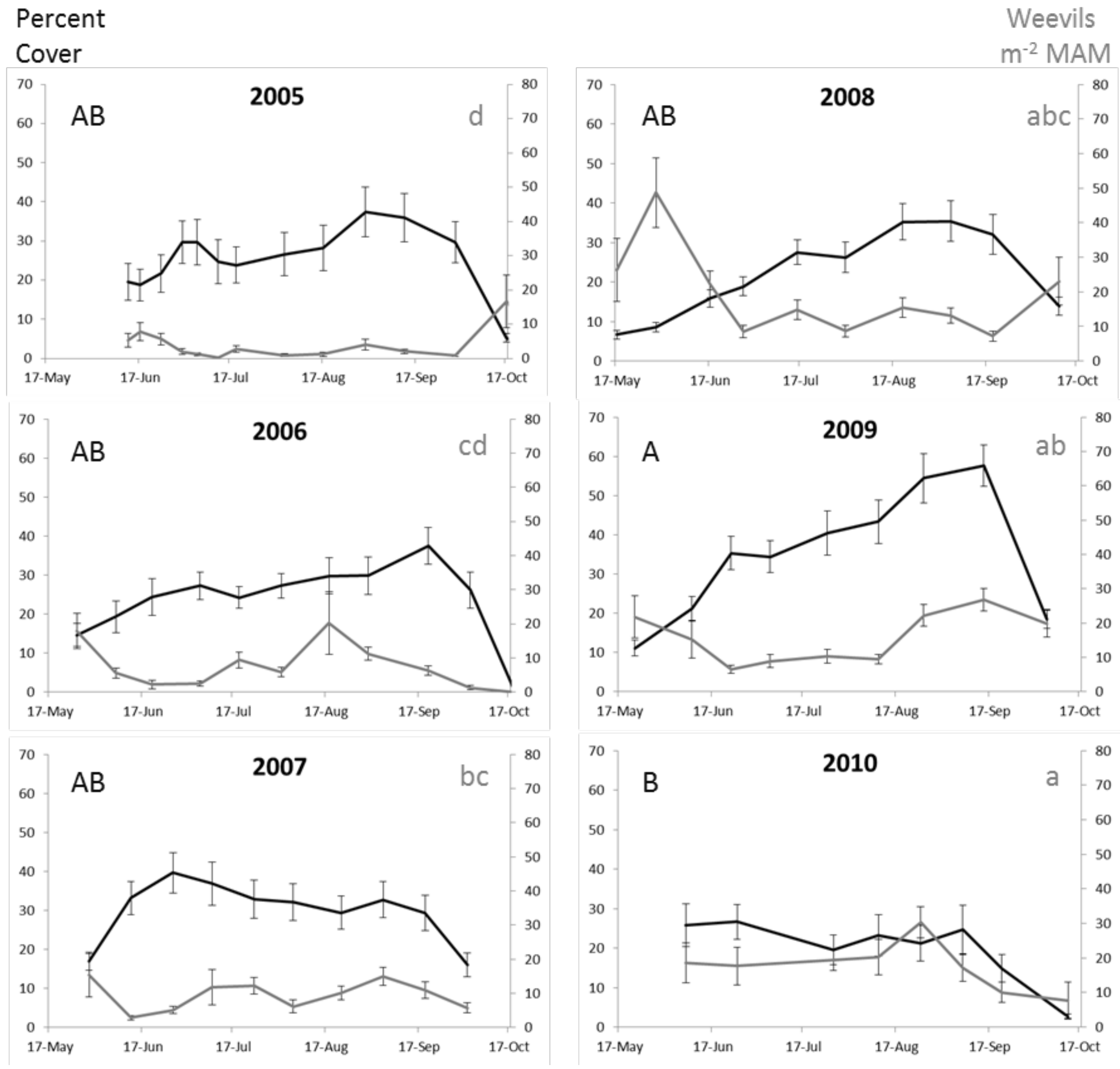

Fig. 1.5. Mean (± SEM) weevils m^{-2} of mile-a-minute weed and percent cover of mile-a-minute within 5 m of the release at the BVA Wetland from 2005 to 2010. Analyses based on the area under the curve; means with the same letter are not significantly different (Tukey's test).

The percent cover of mile-a-minute weed within 5 m of the release site at the Laurels declined from an average of more than 50% in 2005 to less than 20% in 2008. Mile-a-minute cover increased in 2009 but then declined the following year ($F_{5,120}$ = 26.07, P <0.0001, Fig. 1.3). Mile-a-minute cover declined over time at the BVA CREP site ($F_{5,97}$ = 4.30, P = 0.0014, Fig. 1.4); at the BVA Wetland percent cover was lower in 2010 than 2009 ($F_{5,116}$ = 2.88, P = 0.0173, Fig. 1.5). Both of these sites initially had lower percent cover of *P. perfoliata* than the Laurels: mile-a-minute cover averaged 45% at the Laurels, 28% at the BVA Wetland and 26% at the BVA CREP site during the 2005 season prior to plant senescence (Figs. 1.3, 1.4, 1.5). In 2009, the mile-a-minute cover appeared to rebound at all three sites to levels comparable to 2005, but then was once again substantially reduced in 2010 (Figs. 1.3, 1.4, 1.5).

Persicaria perfoliata **seed cluster production and seedling counts**

Seed cluster production declined dramatically between 2005 and 2008 at the Laurels. As with percent cover, the number of seed clusters then increased in 2009 followed by another decline in 2010 (area under the curve, $F_{5,141}$ = 10.93, P < 0.0001, Fig. 1.6). Similar patterns in seed cluster production were observed at the BVA CREP ($F_{5,109}$ = 7.70, P < 0.0001, Fig. 1.7) and BVA Wetland sites ($F_{5,134}$ = 4.02, P = 0.0020, Fig. 1.8).

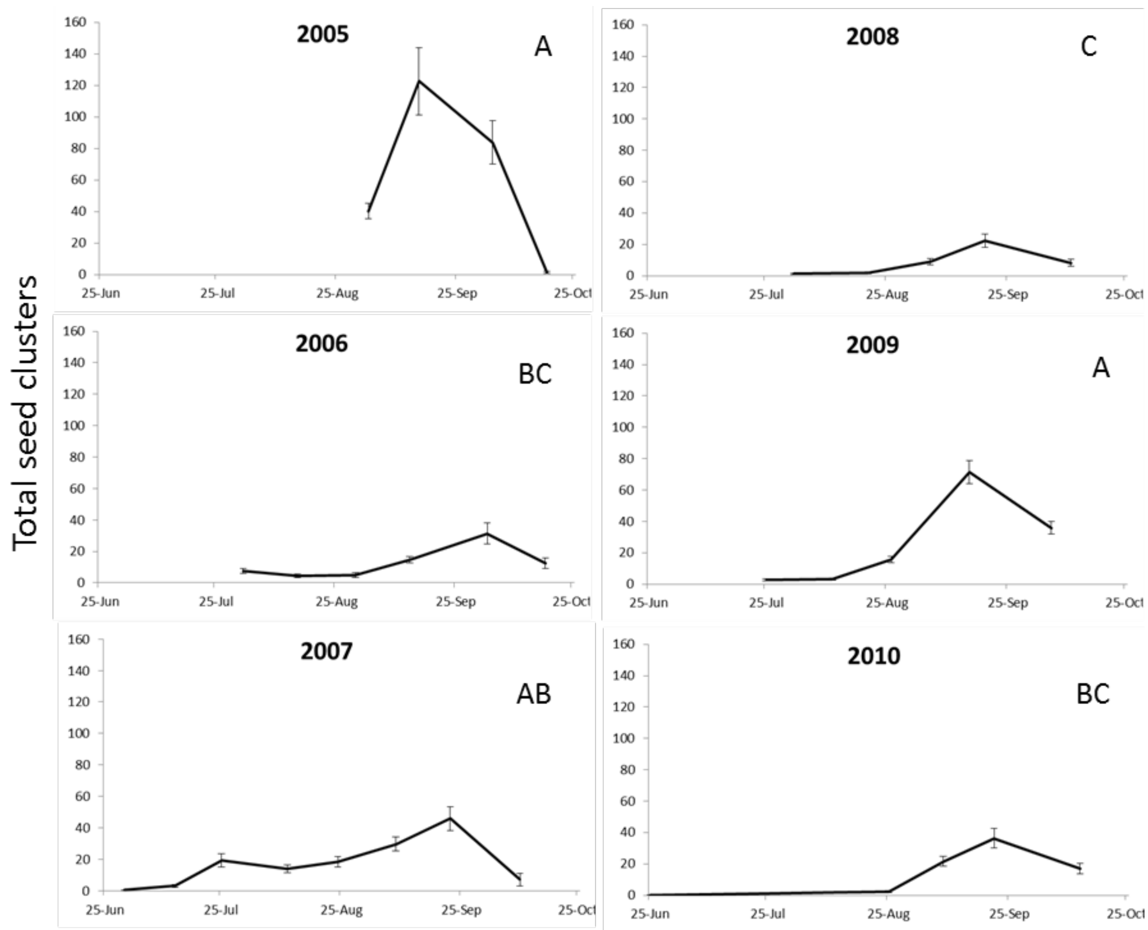

Fig. 1.6. Mean (± SEM) mile-a-minute seed clusters at the Laurels from 2005 to 2010. Analyses based on the area under the curve; means with the same letter are not significantly different (Tukey's test).

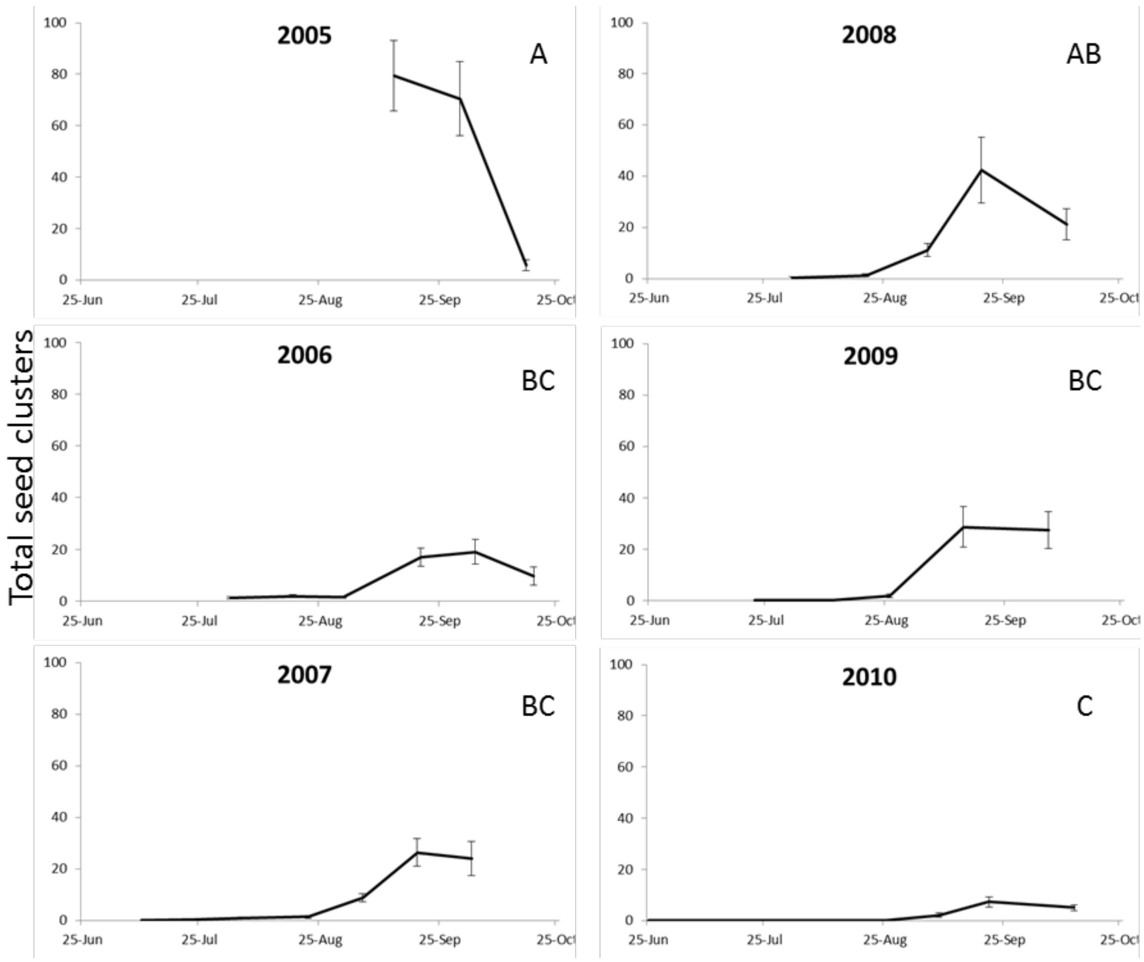

Fig. 1.7. Mean (± SEM) mile-a-minute seed clusters at the BVA CREP site from 2005 to 2010. Analyses based on the area under the curve; means with the same letter are not significantly different (Tukey's test).

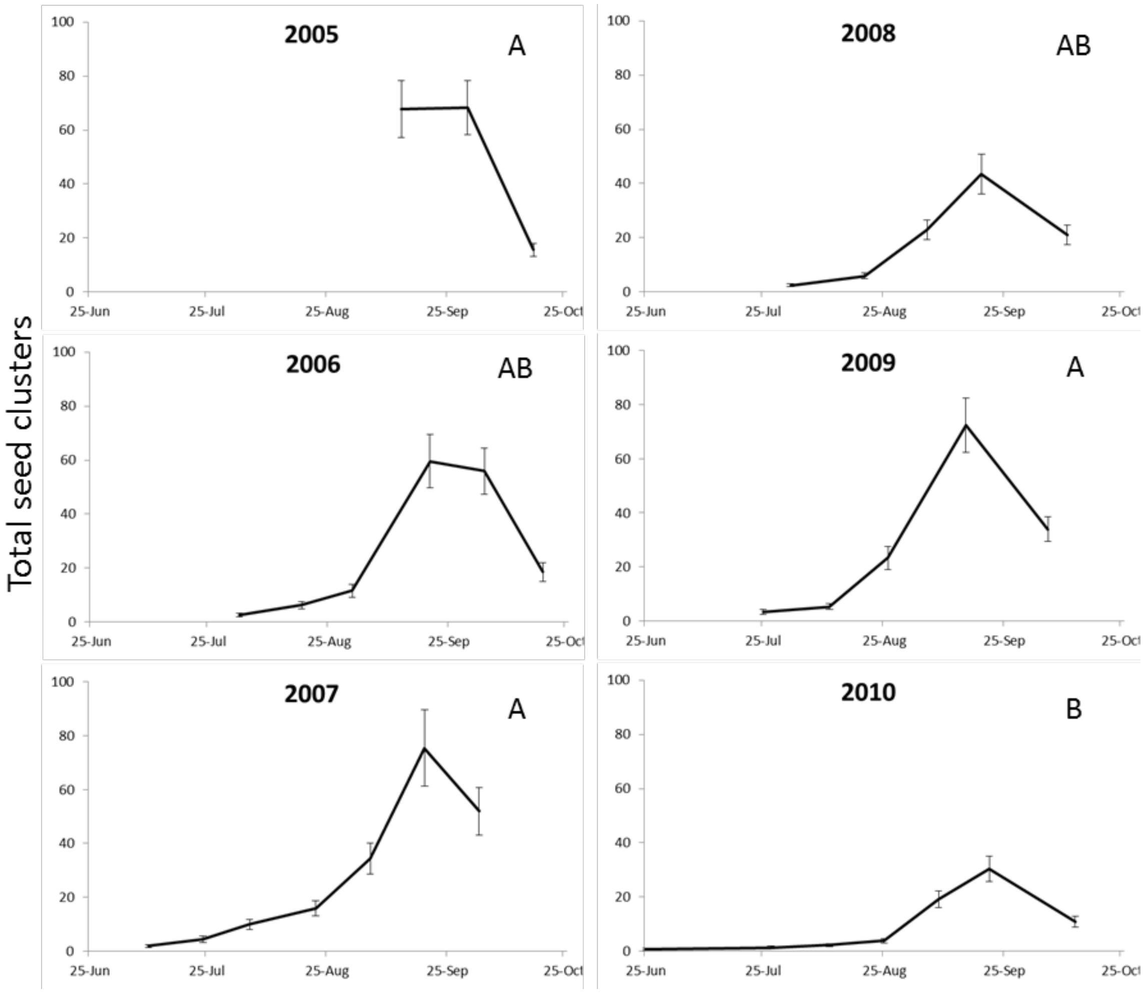

Fig. 1.8. Mean (± SEM) mile-a-minute seed clusters at the BVA Wetland from 2005 to 2010. Analyses based on the area under the curve; means with the same letter are not significantly different (Tukey's test).

Seed cluster production declined with increasing weevil population at the Laurels ($P < 0.0001$, $R^2 = 0.2319$, Fig. 1.9A) and BVA Wetland ($P = 0.0042$, $R^2 = 0.1082$, Fig. 1.9C) but this relationship was not significant at the BVA CREP site ($P = 0.9590$, $R^2 = 0.0$, Fig. 1.9B).

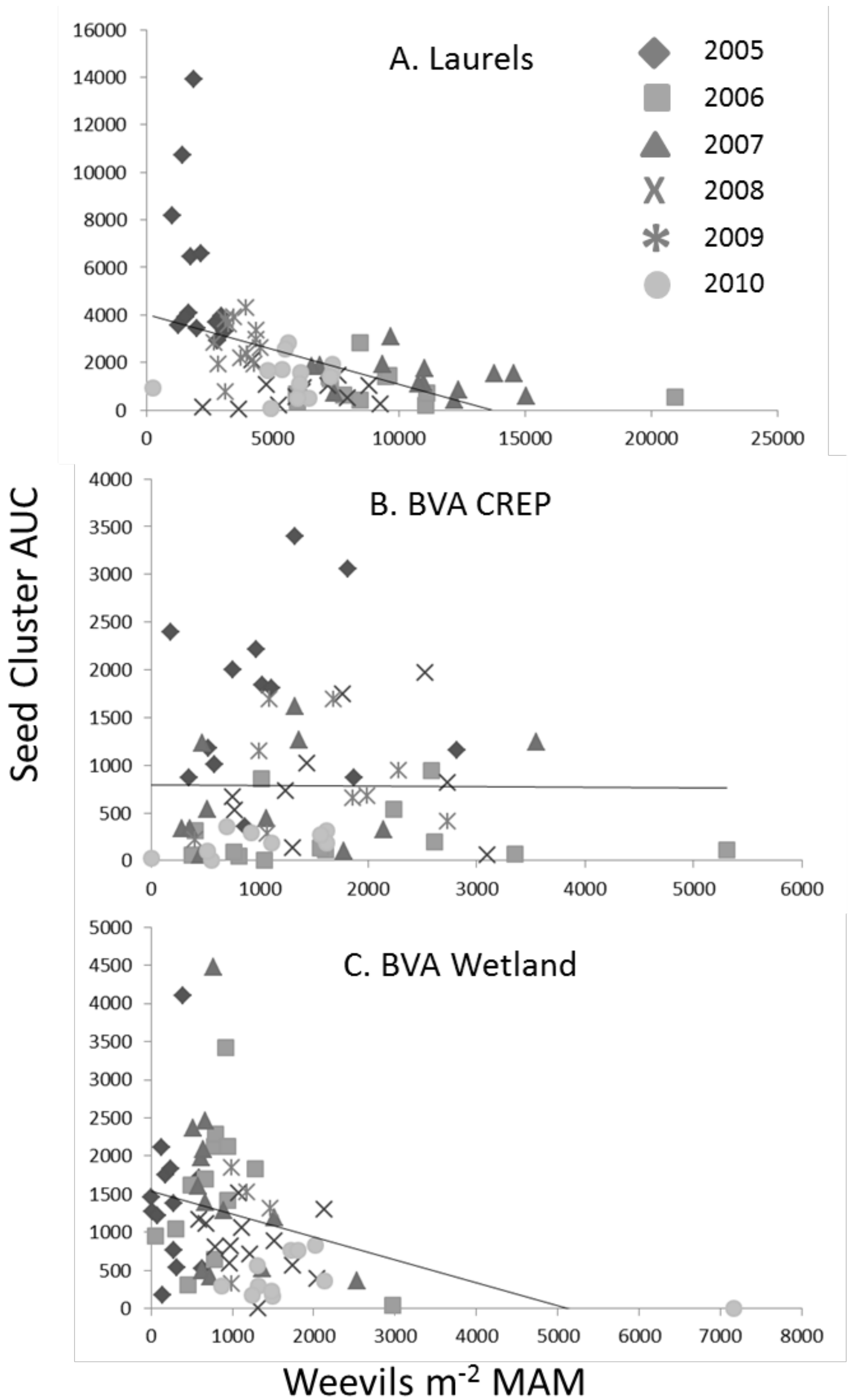

Fig. 1.9. Comparison of weevils m^{-2} mile-a-minute weed area under the curve and seed cluster area under the curve for the release site and points on the A, B, C, and D transects within 5 m of the release from 2005 to 2010.

The number of mile-a-minute weed seedlings 0.5 m^{-2} counted each spring decreased at the Laurels by 87%, from an average of approximately 100 seedlings in 2006 and 2007 to fewer than 15 in 2008. The number of seedlings then increased in 2009 and 2010 ($F_{4,120} = 17.11$, $P < 0.0001$, Fig. 1.10A). At the BVA CREP site the average number of seedlings declined by more than 90% from around 18 in 2006 to approximately 1.5 seedlings 0.5 m^{-2} in 2010 ($F_{4,107} = 9.06$, $P < 0.0001$, Fig. 1.10B). The BVA Wetland site showed a pattern similar to that of the Laurels, with a decline in spring seedling counts through 2008 but an increase in 2010 following the rebound in percent cover and seed cluster production observed in 2009 ($F_{4,118} = 4.39$, $P = 0.0024$, Fig. 1.10C).

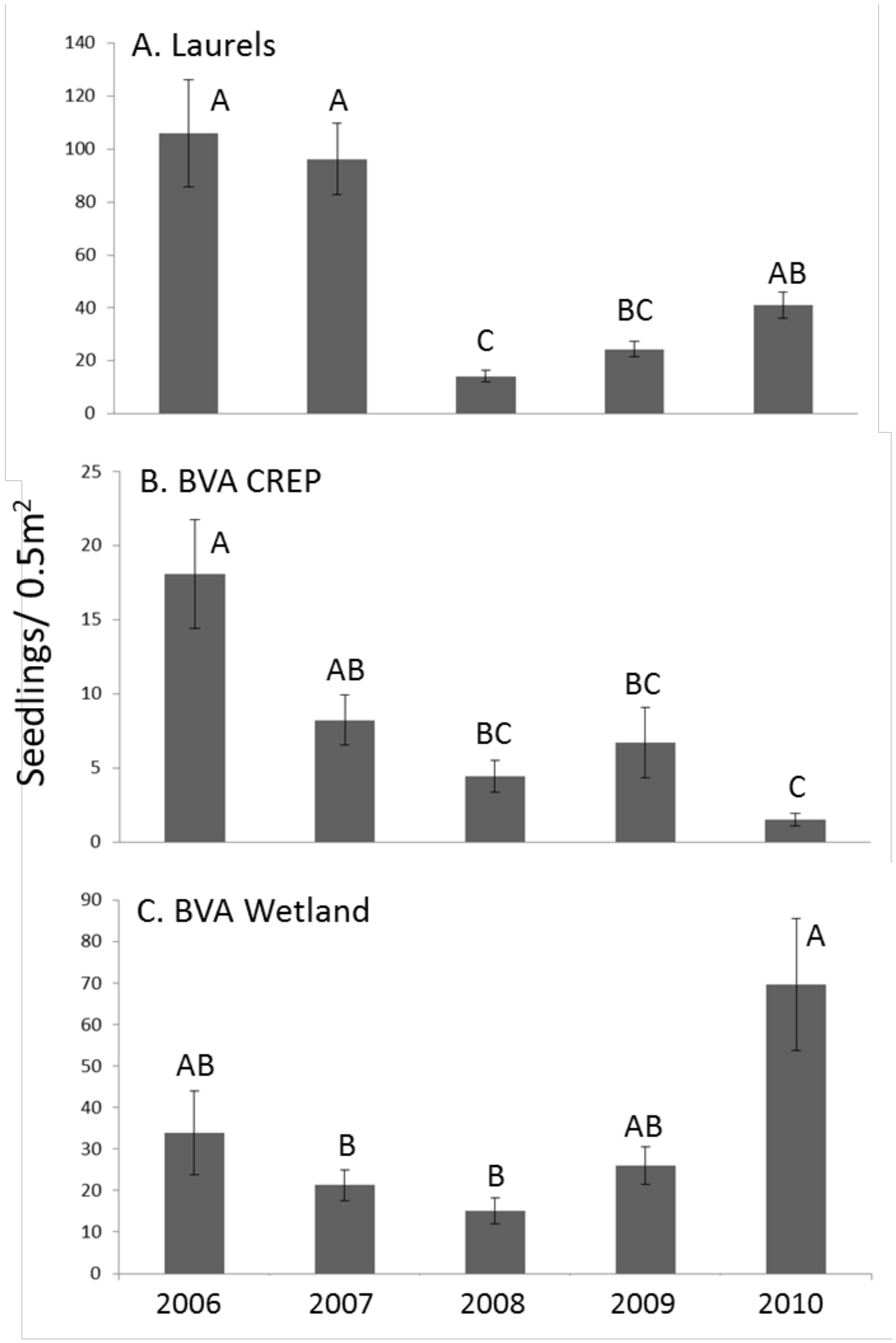

Fig. 1.10. Mean (± SEM) number of mile-a-minute seedlings counted in May from 2006 to 2010. Note the difference in scale at the three sites.

Weather data

The highest annual total rainfall occurred in 2009 (Table 1.3). In most months in 2009, the mean monthly temperature was on the lower end of the range for all years, particularly during June and July (Table 1.4).

Table 1.3. Total monthly precipitation (inches) for West Chester, PA during the mile-a-minute growing season.

	April	May	June	July	August	Season Total
2005	5.15	1.81	2.35	5.69	2.13	17.13
2006	3.85	2.02	11.33	3.49	2.63	23.32
2007	8.24	3.17	3.65	4.19	4.61	23.86
2008	2.77	4.53	2.37	4.42	2.44	16.53
2009	4.16	6.15	4.68	2.70	9.28	26.97
2010	2.80	3.67	1.89	8.30	3.06	19.72

Table 1.4. Mean monthly temperature (°F) for West Chester, PA during the mile-a-minute growing season.

	April	May	June	July	August
2005	52.2	56.7	73.1	77.5	76.9
2006	52.4	60	68.7	76.4	74.5
2007	48.1	61.8	71.1	73.1	73.5
2008	52.9	56.9	72.6	75.1	70.1
2009	51.6	61.1	68.4	71.6	75.0
2010	54.3	63.3	74.1	76.9	73.8

DISCUSSION

The three releases of *R. latipes* conducted in this experiment resulted in weevil establishment at all three sites. Multiple demographic and environmental conditions as well as Allee effects interact to determine whether individual populations of biological control agents will establish following release (Hopper and Roush, 1993; Grevstad, 1999a,b). Failure to successfully overwinter has been a problem with some biological control agents including the stem-boring weevil *Mecinus janthinus* Germar, on Dalmatian toadflax, *Linaria dalmatica* (L.) Mill (De Clerck-Floate and Miller, 2002). *Rhinoncomimus latipes* overwinters in the adult stage in leaf litter and/or the top few centimeters of topsoil (personal communication, Fu Weidong, Institute of Environment & Sustainable Agricultural Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China). It overwinters throughout China, including Heilongjiang province in the northeast, where low winter temperatures can range from minus 30-40 ºC (Ding et al., 2004). *Rhinoncomimus latipes* established in 96.9% of monitored releases sites in the mid-Atlantic U.S. (Hough-Goldstein et al., 2009).

Field populations of *R. latipes* consist of multiple overlapping generations, which increases the likelihood of establishment and the potential for rapid population growth. Three to four generations of weevils developed in field cages in 2005, and this experiment may have underestimated the potential number of generations since it started later in the spring than weevils could be active. Also, light and therefore temperature conditions varied among the cages, leading to long periods of time between observation of the first weevil to emerge and removal of all adult weevils. Based on the limitations of this experiment, three to four generations per season is a conservative estimate of potential weevil population growth.

In this study, weevils began to oviposit soon after emergence from overwintering in the early spring, and the proportion of quadrats with eggs remained high for most of the summer. The highly synchronous decline in oviposition in late summer suggests that the fall reproductive diapause is cued more by changing day length and possibly declining food quality than temperature. Based on sunrise and sunset data obtained for West Chester, Pennsylvania, day length declined from about 13 hours in late August to approximately 12 hours in late September-early October, when fewer than 2% of quadrats contained eggs (United States Naval Observatory, 2010). A similar relationship between reproductive diapause and day length has been found in other insects, including the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (de Kort, 1990) and the tamarisk leaf beetle, *Diorhabda carinulata* (Desbrochers) (Coleoptera: Chrysomelidae) (Dalin et al., 2010). Declining food quality also appears to play a role in inducing diapause in the Colorado potato beetle (Voss et al., 1988).

Price et al. (2003) hypothesized that decreased egg production from the F1 to F3 generations in quarantine under constant light conditions was due to declining quality of *P. perfoliata* plant material in September and October. Female weevils preferentially feed on developing mile-a-minute capitula, presumably using protein from the pollen they consume for ovogenesis (Colpetzer et al., 2004b). Pollen resources decline later in the season as the majority of capitula consist of ripe or ripening seeds rather than flowers (personal observation). As the mile-a-minute plants mature and seed ripens, the plant stems get woodier near the terminals (personal observation). Colpetzer et al. (2004b) observed dead larvae while rearing the weevil and attributed their deaths to an inability to bore into the semi-woody portions of mile-a-minute stems. Larvae that hatch from eggs produced late in the season may have difficulty finding a non-woody stem to enter and have little time to complete development prior to the first frost.

In this experiment, weevil dispersal within the arrays consisted of a gradual radiation from the release quadrat to the outer rings, at a rate of 1.5 to 2.9 m wk^{-1}. The highest dispersal rate was measured during the first two weeks following release, as the initial 450 weevils defoliated the central mile-a-minute plants and moved to nearby plants. These estimated rates of dispersal are conservative due to the sampling methodology, and weevil activity was found beyond the 25-m radius of the monitored arrays by four months post-release. The type and structure of unsuitable habitat can strongly influence dispersal rates and may account for both the observed differences among the arrays and the presence of longer distance dispersers in the broader landscape (Jonsen et al., 2001 and references therein).

Hough-Goldstein et al. (2009) estimated *R. latipes* dispersal to be 4.3 km/year between one and three years following release. Initial rates of spread can be low compared to later calculations for the same organism. For example, the weevil *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae), a control agent for *Melaleuca quinquenervia* (Cav.) Blake, dispersed at a rate of 2.8 km yr^{-1} two years post-release (Pratt et al., 2003).

Twelve years post-release, dispersal was estimated at 13.8 km yr^{-1} (Balentine et al., 2009). The combination of increasing competition for limited resources and the coalescence of founding populations can lead to a drastic rise in the dispersal rate over time (Balentine et al., 2009).

The ability of a biocontrol agent to disperse through a complex and diverse landscape that includes patches of unsuitable habitat can influence the agent's metapopulation dynamics, the design of release strategies, and ultimately the success of the biological control program (Jonsen et al., 2001). Dispersing *R. latipes* navigated obstacles in the landscape including streams, tree lines, and hay fields. Weevils were able to locate both large mile-a-minute infestations and isolated patches, which suggests that the weevil is capable of finding small mile-a-minute populations before they have the opportunity to expand. Declining food resources can trigger *R. latipes* dispersal, and females are more likely to disperse long distances from deteriorating host patches than males (Paras, 2009). Both factors may facilitate colonization of additional mile-a-minute populations.

In this study, weevils were found 760 m from the point of release within 14 months, so weevils could have moved between the two BVA sites after the first season. This is reinforced by the presence of weevil activity at 44% of sites monitored outside the arrays during the second year at the BVA property. Nevertheless, the two BVA sites were independent during the first season, when dispersal was assessed, and continued to provide two different estimates of weevil population density and mile-a-minute cover change over time.

Six years post-release, mile-a-minute weed cover was significantly reduced at the Laurels and BVA CREP site and the number of mile-a-minute seed clusters declined at all three sites. There appeared to be a rebound in mile-a-minute percent cover at all three sites in 2009, followed by an apparent resumption of mile-a-minute suppression in 2010. A cool and wet year in 2009 provided good growing conditions for mile-a-minute and probably also contributed to a smaller weevil population, possibly reducing the total number of weevil generations produced that year. The rebound in mile-a-minute cover in 2009 was reflected in higher numbers of seed clusters that year and increased spring seedling numbers in 2010 at the Laurels. At all three sites, mile-a-minute cover was reduced in 2010, when weather conditions appeared to be more favorable for weevil population growth and less favorable for mile-a-minute compared to 2009.

We cannot say definitively that the overall reduction in mile-a-minute cover and seed production were the result of weevils, because control plots were colonized by dispersing weevils very early in the experiment. The lack of control plots for comparison with release plots due to dispersing biological control agents is a common problem in weed biocontrol research (Lesica and Hanna, 2009; Morin et al., 2009 and references therein). However, a significant correlation was observed between weevil populations and seed cluster production over time at the Laurels and at the BVA Wetland sites, lending support to the hypothesis that changes in mile-a-minute populations were due to weevils. Weevils have been shown in other studies to reduce mile-a-minute growth and reproduction in field cages (Hough-Goldstein et al., 2008b), at monitored release sites compared with control sites (Hough-Goldstein et al., 2009), and in a common garden experiment in China (Guo et al., 2011).

Based on the current findings, the ability of the weevil to establish, produce multiple generations per season, disperse to new patches, and probably have an impact on plants in the field suggests that *R. latipes* has the potential to be a successful biological control agent for mile-a-minute weed. A missing component in this study is the response of the remaining plant community to the reduction in mile-a-minute weed populations. Observations suggest that the combination of competition from the plant community and weevil herbivory led to the decline in mile-a-minute weed at the BVA CREP site, which now consists of a diverse plant community. In contrast, the plant community at the BVA Wetland and Laurels release sites appeared to be less diverse at the start of the experiment, and exotic plants such as *Microstegium vimineum* (Trin.) A. Camus, Japanese stiltgrass, and *Rosa multiflora* Thunb., multiflora rose, increased in abundance as mile-a-minute declined. Therefore the restoration of mile-a-minute sites using a combination of control techniques, including revegetation, is the subject of ongoing research.

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Chapter 2

INTEGRATING BIOLOGICAL CONTROL, HERBICIDE APPLICATION AND NATIVE PLANTINGS TO RESTORE SITES INVADED BY MILE-A-MINUTE WEED, *PERSICARIA PERFOLIATA*, IN THE MID-ATLANTIC U.S.

INTRODUCTION

Classical biological control is often discussed as a tool for the protection and management of natural ecosystems (Headrick and Goeden, 2001; Carson et al., 2008; Van Driesche et al., 2010). The desired outcome of weed control in these areas is a decrease in the target weed plus indirect effects such as an increase in species diversity, the abundance of native plant species and ecosystem services (Denslow and D'Antonio, 2005; Hulme, 2006; Van Driesche et al., 2008). Despite this goal of ecosystem restoration, the field of weed biocontrol has been criticized for failing to conduct sufficient post-release monitoring to evaluate the impact of biological control on the plant community and other ecosystem characteristics (Denslow and D'Antonio, 2005; Thomas and Reid, 2007; Carson et al., 2008; Morin et al., 2009).

It is sometimes assumed that using biocontrol or another management strategy to reduce the abundance of an invasive weed will result in the return of the desired native plant community (Zavaleta et al., 2001; Lesica and Hanna, 2004). In many cases, however; the target weed may be reduced only to be replaced by another undesirable plant (McEvoy and Coombs, 2000; Lesica and Hanna, 2004; Reid et al., 2009; Stephens et al., 2009), the invasive treadmill effect (Thomas and Reid, 2007). One way to combat this invasive species treadmill and restore native plant communities is through integrated weed management, including restoration planting (Denslow and D'Antonio, 2005; Müller-Schärer and Schaffner, 2008; Reid et al., 2009).

In a review of biological control programs that succeeded in reducing the abundance or distribution of invasive weeds, Denslow and D'Antonio (2005) found that successful programs often used integrated weed management strategies. Planting native competitors as part of an integrated strategy may help to increase species diversity (Lesica and Hanna, 2004), reduce the abundance of an invasive weed (Price and Weltzin, 2003), and prevent invasion by exotics and facilitate colonization by natives (Bakker and Wilson, 2004). Native plantings, when combined with biological control, can enhance the effectiveness of the biological control agent by increasing plant competition (Hulme, 2006) against a weed that has already been stressed by herbivory (Van Driesche et al., 2008). Integrating biological control with herbicides, mechanical control or grazing, can be challenging since application must be timed to impact the plant without having deleterious effects on the biocontrol agent (Collier et al., 2007). Biological control has been successfully combined with herbicide application to control *Lythrum salicaria* L., purple loosestrife, (Henne et al., 2005) and with herbicides plus other management strategies to control *Mimosa pigra* L. (Paynter and Flanagan, 2004) and *Euphorbia esula* L., leafy spurge (Lym, 2005).

This study incorporated the management of an invasive species into a broader goal to restore native plant communities (Zavaleta et al., 2001; Reid et al., 2009). The objectives of this experiment were to evaluate the effect of biological control alone and in combination with pre-emergent herbicide and/or native plant competition on mile-a-minute weed and the surrounding plant community.

MATERIALS AND METHODS

Study species

Persicaria perfoliata (Polygonaceae), mile-a-minute weed, is an annual vine of Asian origin (Wu et al., 2002 and references therein) that established in York County, PA in the 1930s (Moul, 1948) and has since invaded the eastern U.S. (Hough-Goldstein et al., 2008a; EDDMapS, 2011). Backward projecting thorns on mile-a-minute's leaves and stems facilitate the vine's climbing habit (Moul, 1948). *Persicaria perfoliata* germinates earlier in the spring than many native plants and can grow to a length of 6 m (Mountain, 1989). A single mile-a-minute plant can produce more than 2200 seeds (achenes) (Hough-Goldstein et al., 2008b) and its seeds can persist for at least 6 years in the seedbank (Hough-Goldstein et al., 2008a). Mile-a-minute weed can form dense monocultures and outcompete native plants in a variety of habitats and thus poses a threat to natural ecosystems (Mountain, 1989; Oliver, 1996; Hough-Goldstein et al., 2008a).

Rhinoncomimus latipes Korotyaev (Coleoptera: Curculionidae) is a host-specific Asian weevil that was introduced to the U.S. in 2004 by the U.S. Forest Service as part of a classical biological control program against mile-a-minute weed (Wu et al., 2002; Ding et al., 2004). The host-specificity of the weevil was demonstrated in multiple studies (Price et al., 2003; Colpetzer et al., 2004a), including a field study in the introduced range with closely related members of the Polygonaceae (Frye et al., 2010). Adult weevils feed on the leaves, ocrea, and capitula of mile-a-minute and oviposit on the capitula, leaves and stems. *Rhinoncomimus latipes* larvae bore into and feed within mile-a-minute stems, then exit the stem and then drop to the soil to pupate (Colpetzer et al., 2004b). Under laboratory conditions, weevil development from egg to adult takes about 26 days (Colpetzer et al., 2004b). The weevil can complete at least 3-4 generations per season in the field in the mid-Atlantic U.S. and reach densities of 200 weevils m^{-2} mile-a-minute weed (Lake et al., 2011). Weevil feeding damage reduces mile-a-minute weed growth and reproduction and decreases its competitive ability (Hough-Goldstein et al., 2008b; Hough-Goldstein et al., 2009; Guo et al., 2011; Lake et al., 2011; Hough-Goldstein and LaCoss, unpublished data).

Euthamia graminifolia (L.) Nutt. (Asteraceae), formerly *Solidago graminifolia* (L.) Salisb. (USDA ARS, 2011), flat-top goldentop, is a forb native to much of the U.S. and Canada (USDA NRCS, 2011). It was selected for use as native plant competition for this experiment because it is a vigorous perennial. *Euthamia graminifolia* is deer resistant, tolerates poor soils, is somewhat drought tolerant, attains a height of 0.6 – 0.9 m and spreads 0.3 - 0.6 m (North Creek Nurseries, Inc., 2011). Plugs were purchased in September 2008 from a local nursery (North Creek Nurseries, Inc., Landenberg, PA). *Euthamia graminifolia* was also naturally present at all study sites prior to this study.

Ulmus americana L., the American elm, was widely distributed throughout the U.S. (USDA NRCS, 2011) in both forests and urban settings prior to the introduction of the fungal pathogens *Ophiostoma ulmi* and *O. nova-ulmi*, Dutch Elm Disease, which arrived on diseased logs from Europe in the 1930s (Schlarbaum et al., 1997). The American elm was selected for this experiment because of its fast growth (eFloras, 2011) and the opportunity to use seeds from trees tolerant of Dutch elm disease (V. D'Amico, personal communication). The elm trees planted in this experiment were started from seed in May 2008 and were approximately 0.6 m tall at the time of planting in October 2008.

Site history, preparation and planting

Three sites in southeastern Pennsylvania were used for this experiment. The Laurels site was located at the Brandywine Conservancy's Laurels Preserve in a meadow along Buck Run (39°55'40.58"N, 75°46'51.97"W). Mile-a-minute established in this meadow before 2003 and was found in patches throughout the site. The second site was located at another Brandywine Conservancy property, Waterloo Mills Preserve (40°01'10.29"N, 75°24'57.41"W). The study site was bush-hogged and sprayed with Roundup in 2002 to control woody invasives, and mile-a-minute established following this disturbance. Multiple and sometimes dense patches of mile-a-minute were present at the start of the study. The third site was located at the Kendal-Crosslands Communities (39°53'02.81"N, 75°39'01.51"W), in a meadow along a small unnamed tributary to the

Brandywine Creek. Mile-a-minute established in this site before 2001 and was present in high densities throughout much of the meadow.

Four 6.1 x 6.1 m plots were flagged in mile-a-minute patches at each site in September 2008. The post-emergent herbicides glyphosate and Garlon 3A (Dow AgroSciences LLC, Indianapolis, IN) were applied between 18 and 23 September 2008, at rates of 9.5 liters $ha^{-1}$and 4.75 liters ha^{-1}, respectively. A methylated seed oil (MSO) surfactant was also applied at a rate of 2.375 liters ha^{-1} along with Bullseye® at rate of 0.75 liters ha^{-1} (Milliken Chemical, Division of Milliken & Company, M-206, Spartanburg, SC). Bullseye® is a blue liquid colorant that facilitates uniform application of the herbicide. Surfactants facilitate spread of the herbicide spray over the leaf surface, increase adherence to the leaf and aid in penetration of the leaf's waxy outer cuticle, all of which increase uptake of the herbicide and thus improve its effectiveness (Hough-Goldstein et al., 2008a). The combination of glyphosate and Garlon 3A killed all vegetation in the study plots. All herbicides used in this experiment were applied by Weeds, Inc. (Aston, PA). Dead plant material was raked from the plots prior to planting.

Treatments were randomly assigned to plots using PROC PLAN (SAS Institute, 2008). A deer fence (Benner's Gardens, Phoenixville, PA) was installed around the elm plot at each site the week of 29 September 2008. A metal pole was secured at the corners of the plots and heavy duty polypropylene mesh netting 6.1 x 2.3 m was attached to the poles with zip ties. The finished fence was approximately 2.1 m tall with about 0.15 m of additional of fencing staked to the ground around the outer perimeter of the plot.

Orange marking chalk (Rust-oleum® Industrial Brands, Vernon Hills, IL) was used to mark the spot where each *E. graminifolia* plug was to be planted. In the low and elm plots, plugs were spaced 0.6 m apart for a total of 100 plugs per plot (Fig. 2.1). In the high plots, *E. graminifolia* plugs were spaced 0.3 m apart, for a total of 400 plugs per plot. Twenty-five elm trees were planted in each elm plot and were spaced approximately 1.1 m apart. The elm trees and plugs were planted between 1 and 13 October 2008. All plant material was watered the day it was planted and as needed thereafter.

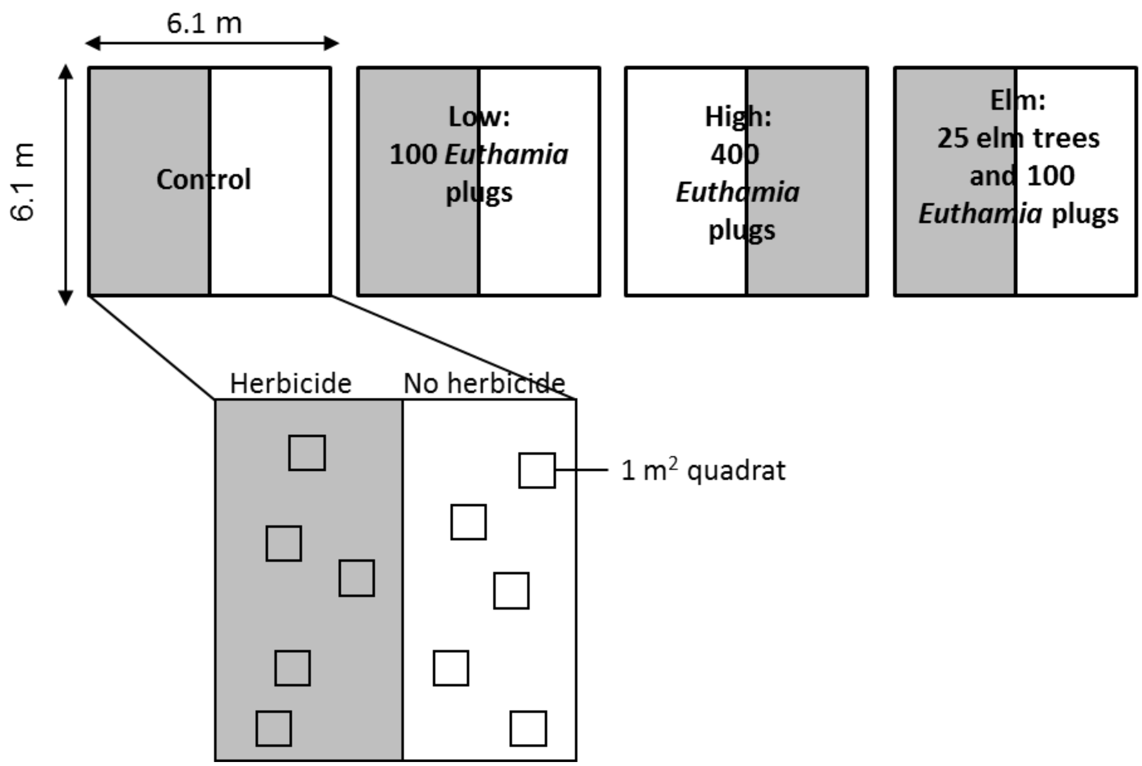

Fig. 2.1. Restoration experiment site design. Each site consisted of four planting treatment plots. The inset illustrates the herbicide and no herbicide treatment areas and the five monitoring quadrats that were established within each herbicide and planting treatment combination.

The majority of *E. graminifolia* plugs heaved over the winter and were firmed back into the soil when the ground thawed in late March 2009. At this time, each plot was divided in half according to the dominant landscape feature, i.e. parallel, not perpendicular to slope. One side of the plot was randomly assigned to receive pre-emergent herbicide and the perimeter of the area to be sprayed was marked with flags. On 1 April 2009, the pre-emergent herbicide Barricade® 65WG (Syngenta Crop Protection, LLC, Greensboro, NC) was applied at a rate of 1.125 kg ha^{-1}. The herbicide was applied with water and Bullseye®. The active ingredient in Barricade® is prodiamine. It provides pre-emergent control of susceptible grasses and broadleaf weeds by preventing growth and development of newly germinated seeds.

Weevil population and mile-a-minute cover

Monitoring quadrats were marked at the center by a bamboo pole labeled with an alphanumeric code indicating the herbicide and planting treatment and the quadrat number. Five quadrats were randomly positioned within the herbicide and no herbicide areas of the planting plots. The monitoring plots did not overlap and were not within 0.25 m of the edge of the planting treatment plot or within 0.5 m of the line demarking the division between the herbicide and no herbicide side of the plots (Fig. 2.1).

Mile-a-minute seedlings were counted each year (2009, 2010, and 2011) between early-May and mid-June, depending on seasonal weather conditions and plant phenology. Seedlings were counted in the monitoring quadrats using a 0.5 x 1 m frame constructed of PVC pipe oriented in the same direction each time.

Weevils were obtained from the NJ Department of Agriculture Phillip Alampi Beneficial Insect Rearing Laboratory. Their rearing colony was founded with weevils collected from *P. perfoliata* plants in Hunan Province, China (Hough-Goldstein et al., 2009). Weevils were not sexed prior to release, but samples checked by workers at the Phillip Alampi Beneficial Insect Rearing Laboratory typically had a 1:1 sex ratio (personal communication, Daniel Palmer, NJ Department of Agriculture). During June of 2009, 125 weevils were released weekly in each planting treatment plot along the herbicide application line, for a total of 500 weevils per plot, 2000 weevils per site. Weevils were present at low densities at all sites prior to the start of this experiment.

The sites were monitored monthly from 15 July through 2 October, 2009, and 1 June through 12 October, 2010. Each 1-m^2 quadrat was checked for: number of weevils, percent cover of mile-a-minute and other plants, and presence or absence of weevil eggs and larval feeding damage. A 1 x 1 m frame constructed of PVC pipe was centered around the bamboo pole in the same orientation each time the plot was monitored. The weevils sometimes drop from the plants when they are disturbed, so the frame was cut in half to minimize disturbance to the plant as the frame was centered around the pole. The percent cover of live foliage in each quadrat was estimated by looking down at the frame, which was marked in 10-cm intervals. Using this bird's-eye view, plant cover was not permitted to exceed 100%.

Plant community assessment

The sites were surveyed with a botanist between 7 and 16 September, 2010. Each plant within the monitoring quadrats was identified to species and the cover was estimated. The scientific and common names, plant family, duration, growth habit and native status of each species are according to the PLANTS Database (USDA NRCS, 2011; Table 2.1). The Polygonaceae family is under revision and updated scientific names from the Germplasm Resources Information Network are presented for mile-a-minute weed and other Polygonaceae identified in the study plots (USDA ARS, 2011).

Estimates of plant cover were made by one individual in order to minimize observer error; 1%, 5%, and 10% cover templates were used to help calibrate cover estimates (Wilson, 2007). Absolute cover was recorded for each species. In some quadrats the total of all plant cover was less than 100% due to bare ground, and in others total cover exceeded 100% due to layering of plants. Relative cover was calculated by dividing the absolute cover by the total plant cover in each quadrat to determine the percent cover of natives, *P. perfoliata*, *E. graminifolia* and *Microstegium vimineum* (Trin.) A. Camus, Japanese stiltgrass. Plants classified as both native and introduced in the PLANTS Database usually represented a very low percentage of plant cover and neither these plants nor elm trees were included in any measures of plant cover.

Elm tree survival and growth

Elm tree survival was determined in October 2010 by counting the number of live elms on the herbicide and no herbicide side of the plots, maximum of 10 trees in each treatment. Elm height was measured to the nearest 5 cm with lengths of PVC pipe marked in 10 cm increments. Some elms were attaining the characteristic vase-shape (eFloras, 2011) and height was measured at the highest point in the crown without manipulating the branches.

Statistical analysis

The mean of the five subsample quadrats was calculated for each combination of herbicide and planting treatment at each site (Fig. 2.1). PROC MIXED was used to analyze this split plot experiment, with herbicide and planting treatments considered fixed effects and sites considered random (Littell et al., 2002; SAS Institute, 2008). Mile-a-minute percent cover varied greatly at different sites and quadrats, and weevils were found only on mile-a-minute weed. Therefore weevil populations were expressed as weevils m^{-2} of mile-a-minute cover. This was calculated for each quadrat on each sample date by dividing the total number of weevils in a given quadrat by the proportion of mile-a-minute cover in that quadrat. Differences in weevil density and percent cover of mile-a-minute were analyzed using the REPEATED statement (SAS Institute, 2008; SAS noted that the estimated G matrix was not positive definitive in all PROC MIXED analyses).

The annual seedling counts, species richness, and the cover of native species, *Euthamia graminifolia*, and *Microstegium vimineum* were also analyzed by PROC MIXED, using the mean of the five subsample quadrats for each plot. The CONTRAST statement was used to compare the control to all the planting treatments as a group within the herbicide and no herbicide plots. The LSMEANS statement with the Tukey-Kramer adjustment was used to compare the individual planting treatments to the control and each other within the herbicide and no herbicide plots (Littell et al., 2002; SAS Institute, 2008). Seedling count and species richness data were square-root transformed and percent cover data were arcsine-square-root transformed prior to analysis. Non-transformed means and standard errors are presented in all figures.

At the Laurels, naturally occurring *Euthamia graminifolia* was abundant, and *E.graminifolia* cover was sometimes higher in the controls than in the treatments where *E. graminifolia* had been planted. The cover of Japanese stiltgrass was also very low at the Laurels compared to the other sites. Therefore, the plant cover data is presented treating all three sites as replicates and also with only Crosslands and Waterloo Mills as replicates. The proportion of total, native and introduced species at all three sites was analyzed using a chi-square test of goodness of fit (assuming equal proportions) (PROC FREQ, SAS Institute, 2008).

Elm tree survival was assessed using a chi-square test of independence (PROC FREQ) and tree height in 2010 was analyzed using a two-way ANOVA by site and herbicide treatment (PROC GLM) (SAS Institute, 2008).

RESULTS

Weevil population and mile-a-minute cover

The pre-emergent herbicide application in 2009 was not 100% effective since some mile-a-minute seedlings had started to germinate prior to application. In 2009, mile-a-minute seedling counts were higher in the no herbicide than herbicide plots ($F_{1,8}$ = 48.72, P = 0.0001, Fig. 2.2A). There were no differences by planting treatment ($F_{3,6}$ = 1.77, P = 0.2521) and there was no significant interaction. In 2010, mile-a-minute seedling counts were much lower overall, but again were higher in the no herbicide plots than the herbicide plots ($F_{1,8}$ = 14.12, P = 0.0056, Fig. 2.2B), with no difference by planting treatment ($F_{3,6}$ = 0.69, P = 0.5921) and there was no significant interaction. This same pattern was observed in 2011, with more mile-a-minute seedlings in the no herbicide than herbicide plots ($F_{1,8}$ = 15.90, P = 0.0040, Fig. 2.2C), no difference by planting treatment ($F_{3,6}$ = 0.71, P = 0.5784) and no significant interaction.

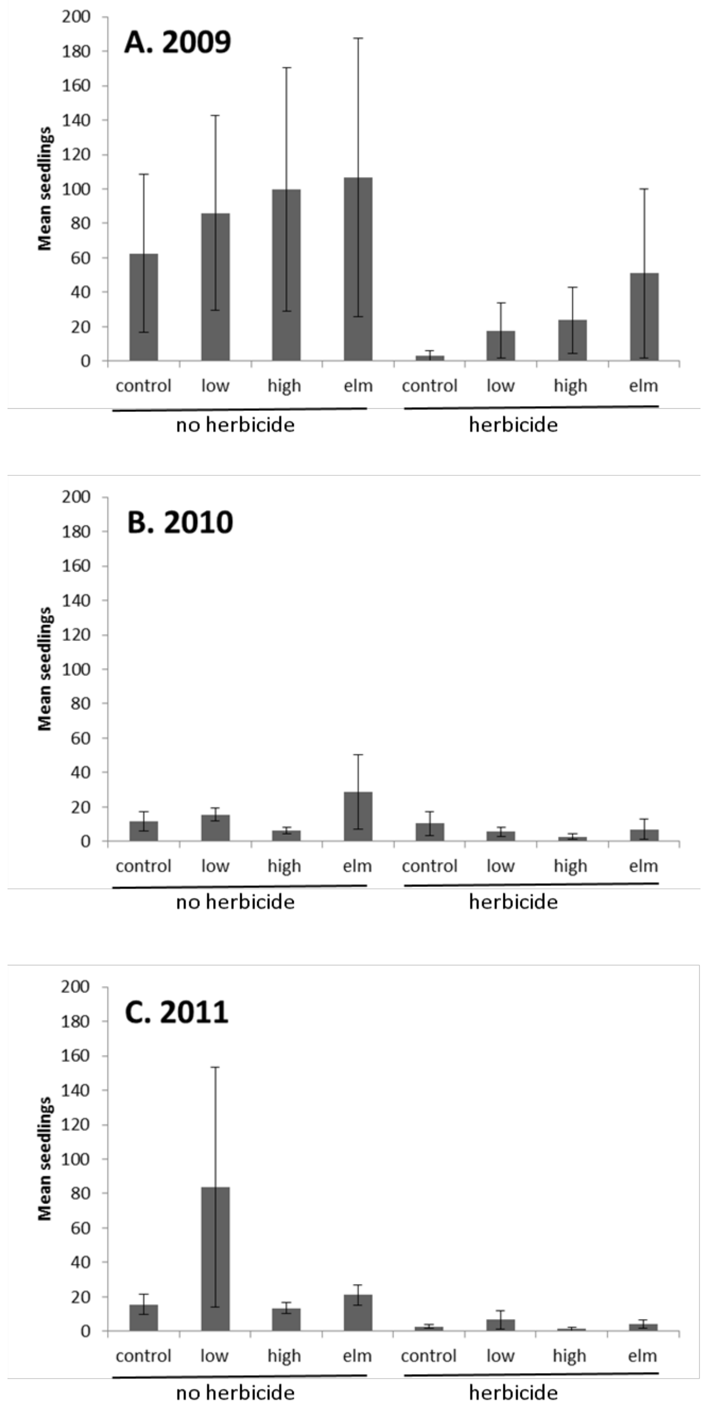

Fig. 2.2. Spring seedling counts in (A) 2009, (B) 2010 and (C) 2011. Mean ± SEM seedlings 0.5 m^{-2} of the 5 monitoring quadrats in each herbicide and planting treatment combination. There were significantly more seedlings in the no herbicide than herbicide plots in all years but there were never any significant differences by planting treatment.

Mile-a-minute weed cover was also higher in the no herbicide than herbicide plots in 2009 (repeated measures, $F_{1,8.91} = 71.43$, $P < 0.0001$, Fig. 2.3) with no difference by planting treatment ($F_{3,6.09} = 0.69$, $P = 0.5906$) or month ($F_{2,22.2} = 2.18$, $P = 0.1371$), but with a significant interaction between herbicide and month ($F_{2,22.2} = 14.13$, $P = 0.0001$). In 2010, mean mile-a-minute cover was less than 18% in all plots, but again differed by herbicide (repeated measures, $F_{1,8.51} = 8.69$, $P = 0.0173$, Fig. 2.4) and month ($F_{4,29.7} = 3.26$, $P = 0.0249$) but not by planting treatment ($F_{3,8.08} = 0.86$, $P = 0.5000$). The interaction of herbicide and month was marginally significant ($F_{4,29.7} = 2.58$, $P = 0.0576$).

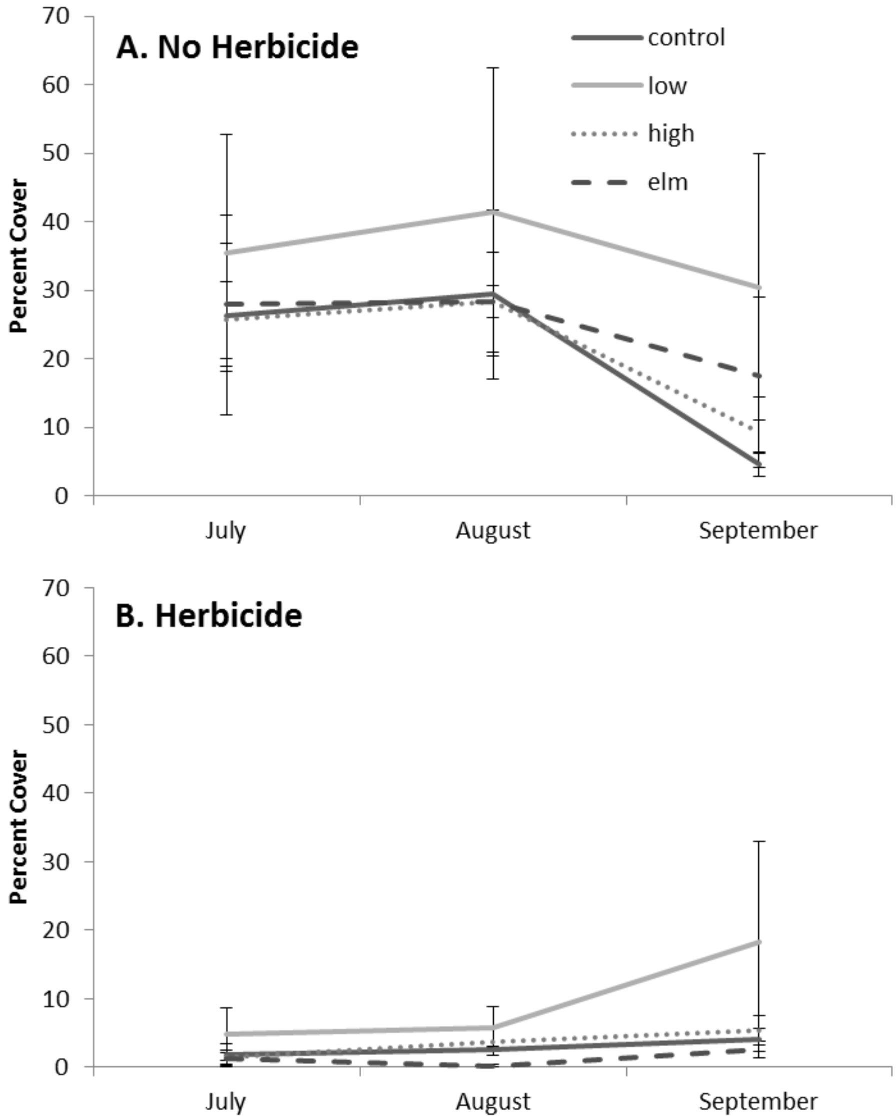

Fig. 2.3. Mean ± SEM mile-a-minute weed percent cover in 2009 in (A) no herbicide and (B) herbicide plots. Mile-a-minute cover was higher in the no herbicide than herbicide plots (repeated measures, $P < 0.0001$) but there was no difference by planting treatment ($P = 0.5906$) or month ($P = 0.1371$).

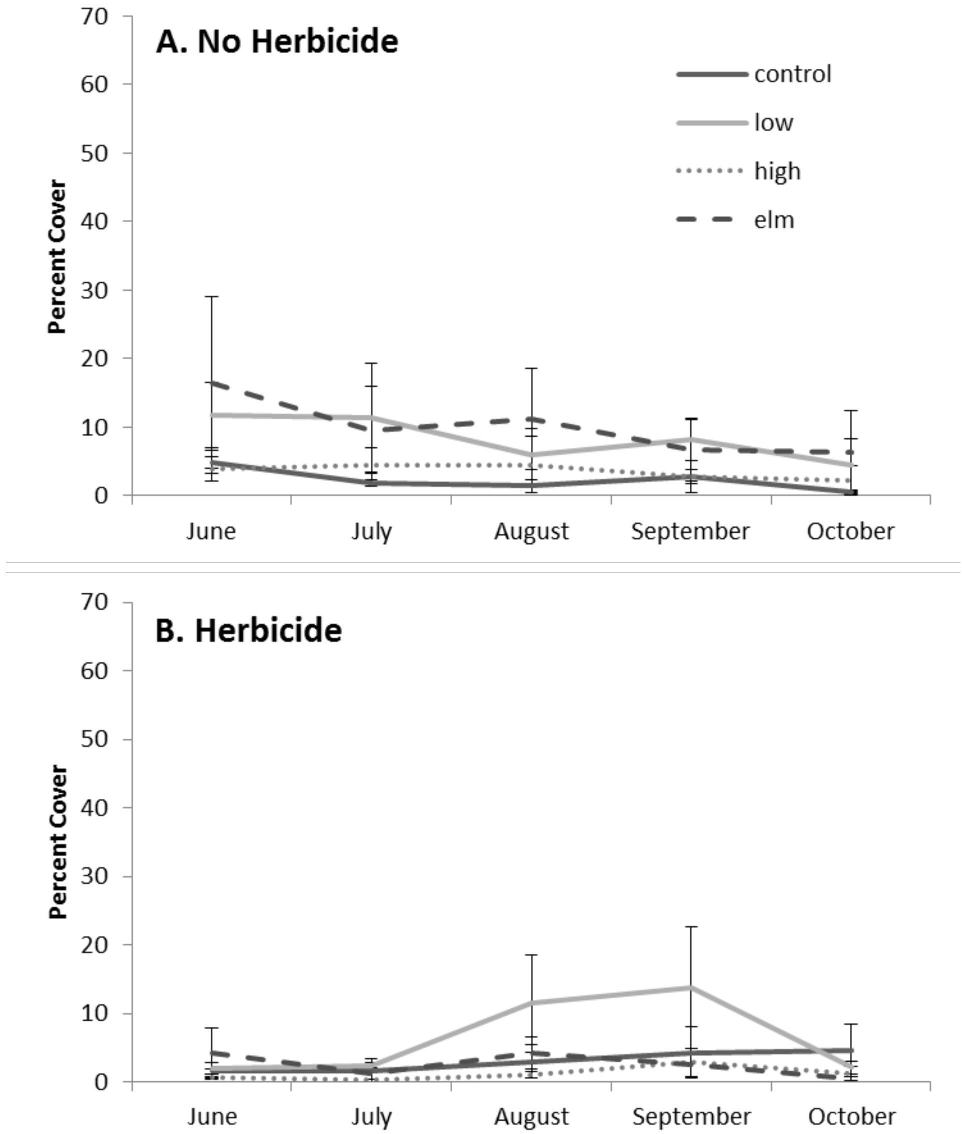

Fig. 2.4. Mean ± SEM mile-a-minute weed percent cover in 2010 in (A) no herbicide and (B) herbicide plots. Mile-a-minute cover was higher in the no herbicide than herbicide plots (repeated measures, P = 0.0173) and there was an effect by month (0.0249) but not planting treatment (P = 0.5000).

In 2009, the establishment year for the weevil population, weevil density was higher in the plots not treated with herbicide (repeated measures, $F_{1,7.83} = 17.28$, P = 0.0033, Fig. 2.5). The mean weevil density was below 20 weevils m^{-2} mile-a-minute in the herbicide plots over the course of the season while mean weevil density exceeded this level in most of the no herbicide plots (Fig. 2.5). Weevil density differed by month ($F_{2,23.3}$ = 4.68, P = 0.0196) but not by planting treatment ($F_{3,5.89}$ = 0.38, P = 0.7690) and there were no significant interactions. In 2010, weevil density was substantially higher overall, and again was higher in the no herbicide than herbicide plots (repeated measures, $F_{1,14.9}$ = 4.66, P = 0.0476, Fig. 2.6). Weevil density in 2010 differed by planting treatment ($F_{3,14.9}$ = 6.23, P = 0.0059) and month ($F_{4,32.6}$ = 11.95, P < 0.0001). There were significant interactions between planting treatment and month ($F_{12,39.5}$ = 2.33, P = 0.0226) and herbicide and month ($F_{4,32.6}$ = 2.71, P = 0.0468).

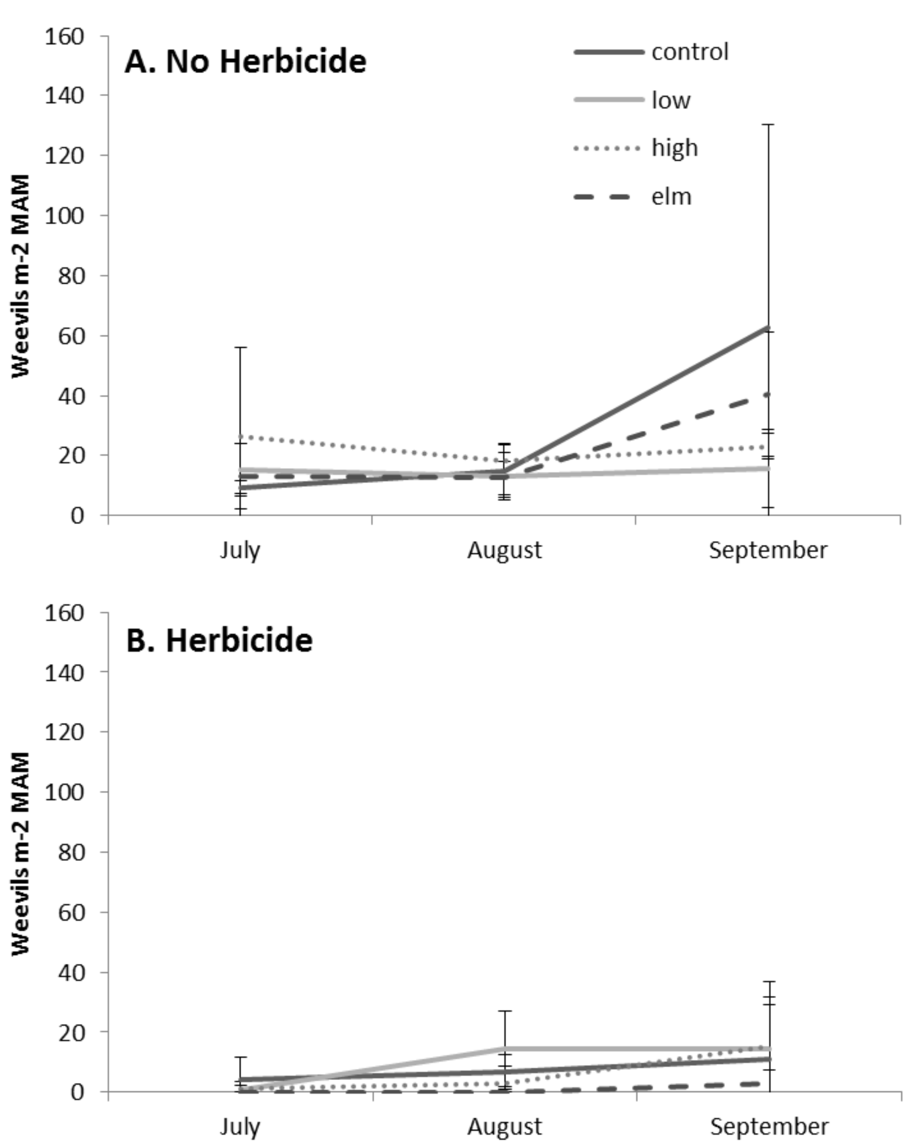

Fig. 2.5. Mean ± SEM weevils m^{-2} mile-a-minute weed in 2009 in (A) no herbicide and (B) herbicide plots. Weevil density was higher in the no herbicide than herbicide plots (repeated measures, $P = 0.0033$) and differed by month ($P = 0.0196$) but not planting treatment ($P = 0.7690$).

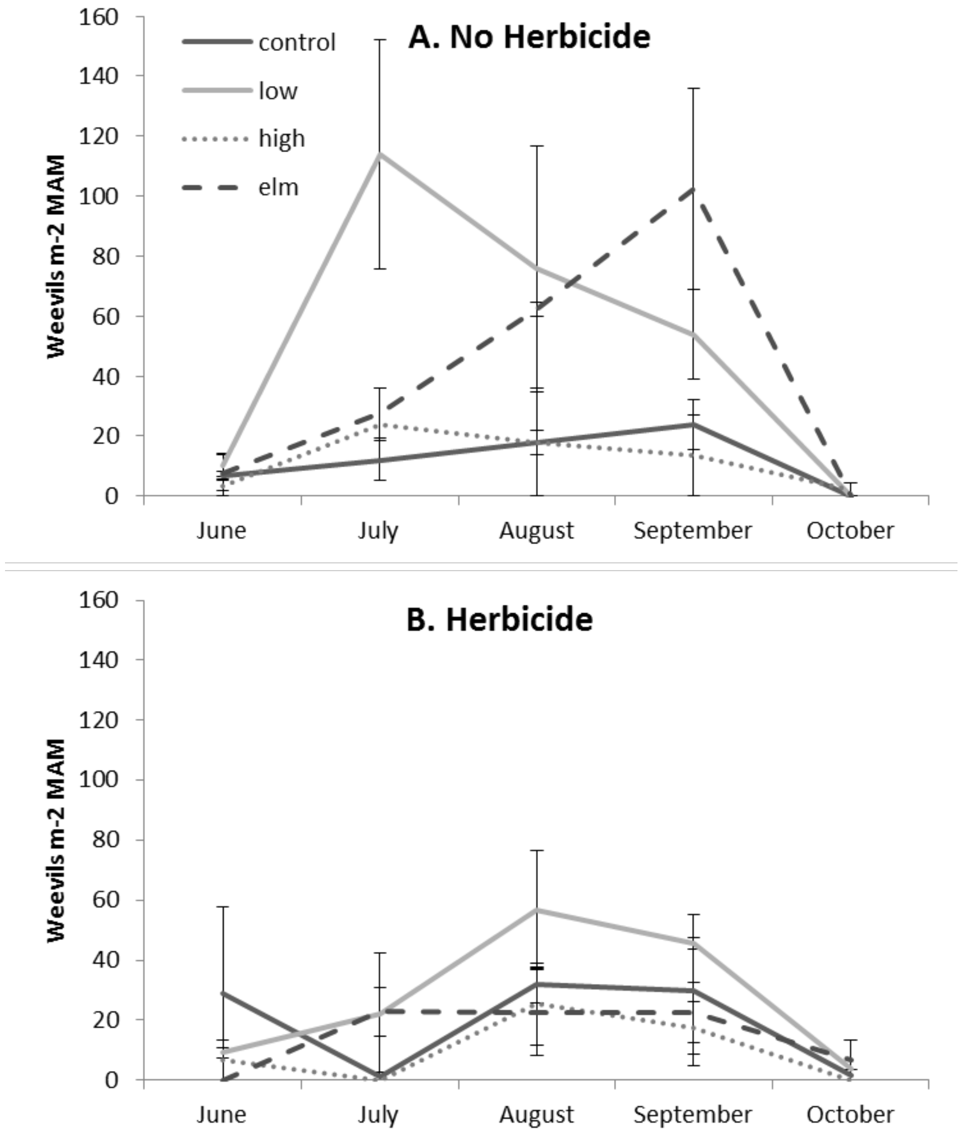

Fig. 2.6. Mean weevils m^{-2} ± SEM mile-a-minute weed in 2010 in (A) no herbicide and (B) herbicide plots. Weevil density was higher in the no herbicide than herbicide plots (repeated measures, $P = 0.0476$) and differed by planting treatment ($P = 0.0059$) and month ($P < 0.0001$).

Plant community assessment

A total of 127 plant species from 48 families were identified in the study plots (Table 2.1; 4 unknowns were not included). There was no difference in the proportion of total, native, or introduced species among the study sites; more than 60% of plant species at each site were native (Table 2.2). Species richness did not differ by herbicide ($F_{1,16}$ = 1.53, P = 0.2341) or planting treatment ($F_{3,16}$ = 0.19, P = 0.9025) and there was no significant interaction (Fig. 2.7).

Table 2.1. List of species identified during plant surveys. All information on plant family, scientific and common names, duration, growth habit and native status is from the PLANTS Database (USDA NRCS, 2011). Native status describes the status of the plant in the lower 48 U.S. states, N = native, I = introduced. The Polygonaceae family is under revision and updated scientific names from the Germplasm Resources Information Network are provided for members of this family (USDA ARS, 2011).

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Aceraceae	*Acer negundo* L.	boxelder	Perennial	Tree	N		√	
	Acer rubrum L.	red maple	Perennial	Tree	N	√	√	
	Acer L. spp.	maple	Perennial	Tree			√	
Anacardiaceae	*Toxicodendron radicans* (L.) Kuntze	poison ivy	Perennial	Shrub/Forb/ Herb/ Subshrub/ Vine	N			√
Apiaceae	*Conium maculatum* L.	poison hemlock	Biennial	Forb/Herb	I	√		
Apocynaceae	*Apocynum cannabinum* L.	Indianhemp	Perennial	Forb/Herb	N			√
Asteraceae	*Achillea millefolium* L.	common yarrow	Perennial	Forb/Herb	N/I		√	√
	Ageratina altissima (L.) King & H. Rob.	white snakeroot	Perennial	Forb/Herb	N		√	√
	Cirsium arvense (L.) Scop.	Canada thistle	Annual	Forb/Herb	I			√
	Cirsium discolor (Muhl. ex Willd.) Spreng.	field thistle	Biennial/ Perennial	Forb/Herb	N	√		√
	Cirsium vulgare (Savi) Ten.	bull thistle	Biennial	Forb/Herb	I	√		√
	Conyza canadensis (L.) Cronquist	Canadian horseweed	Annual/ Biennial	Forb/Herb	N	√	√	√
	Erechtites hieraciifolia (L.) Raf. ex DC.	American burnweed	Annual	Forb/Herb	N	√	√	√
	Erigeron L. spp.	fleabane			N	√		
	Eupatoriadelphus fistulosus (Barratt) King & H. Rob.	trumpetweed	Perennial	Forb/Herb	N		√	

Table 2.1. continued

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Asteraceae	*Eupatorium serotinum* Michx.	lateflowering thoroughwort	Perennial	Forb/Herb	N			√
	Eupatorium L. spp.	thoroughwort					√	
	Euthamia graminifolia (L.) Nutt.	flat-top goldentop	Perennial	Forb/Herb	N	√	√	√
	Lactuca biennis (Moench) Fernald	tall blue lettuce	Annual/ Biennial	Forb/Herb	N		√	
	Solidago canadensis L.	Canada goldenrod	Perennial	Forb/Herb	N	√	√	
	Solidago gigantea Aiton	giant goldenrod	Perennial	Forb/Herb	N	√	√	
	Solidago rugosa Mill.	wrinkleleaf goldenrod	Perennial	Forb/Herb	N	√	√	√
	Solidago L. spp.	goldenrod			N	√	√	
	Symphyotrichum lateriflorum (L.) A. Löve & D. Löve	calico aster	Perennial	Forb/Herb	N			√
	Symphyotrichum pilosum (Willd.) G.L. Nesom	hairy white oldfield aster	Perennial	Forb/Herb	N			√
	Symphyotrichum Nees spp.	aster			N	√		
Balsaminaceae	*Impatiens capensis* Meerb.	jewelweed	Annual	Forb/Herb	N	√		
Boraginaceae	*Hackelia virginiana* (L.) I.M. Johnst.	beggarslice	Biennial/ Perennial	Forb/Herb	N	√		√
Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	garlic mustard	Annual/ Biennial	Forb/Herb	I	√	√	√
	Barbarea vulgaris W.T. Aiton	garden yellowrocket	Biennial	Forb/Herb	I	√		
	Cardamine impatiens L.	narrowleaf bittercress	Annual/ Biennial	Forb/Herb	I			√
Campanulaceae	*Lobelia inflata* L.	Indian-tobacco	Annual	Forb/Herb	N		√	
	Lobelia siphilitica L.	great blue lobelia	Perennial	Forb/Herb	N	√	√	
	Lobelia L. spp.	lobelia			N		√	

Table 2.1. continued

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Cannabaceae	*Humulus japonicus* Siebold & Zucc.	Japanese hop	Annual/ Perennial	Vine/Forb/ Herb	I	√		
Caprifoliaceae	*Lonicera japonica* Thunb.	Japanese honeysuckle	Perennial	Vine	I	√	√	√
Caryophyllaceae	*Silene latifolia* Poir.	bladder campion	Biennial/ Perennial	Forb/Herb	I			√
Celastraceae	*Celastrus orbiculatus* Thunb.	Oriental bittersweet	Perennial	Vine	I	√	√	√
Chenopodiaceae	*Chenopodium album* L.	lambsquarters	Annual	Forb/Herb	N/I			√
Clusiaceae	*Hypericum mutilum* L.	dwarf St. Johnswort	Annual/ Perennial	Forb/Herb	N	√	√	√
	Hypericum punctatum Lam.	spotted St. Johnswort	Perennial	Forb/Herb	N	√	√	
	Hypericum L. spp.	St. Johnswort			N	√		
Convolvulaceae	*Calystegia sepium* (L.) R. Br.	hedge false bindweed	Perennial	Vine	N/I		√	√
Cucurbitaceae	*Sicyos angulatus* L.	oneseed bur cucumber	Annual	Vine/Forb/ Herb	N		√	
Cyperaceae	*Carex hirtifolia* Mack.	pubescent sedge	Perennial	Graminoid	N		√	
	Carex lurida Wahlenb.	shallow sedge	Perennial	Graminoid	N	√		
	Carex L. spp. 1	sedge			N		√	√
	Carex L. spp. 2	sedge			N	√	√	√
	Carex L. spp. 3	sedge			N			√
	Carex L. spp. 4	sedge			N		√	
Dennstaedtiaceae	*Dennstaedtia punctilobula* (Michx.) T. Moore	eastern hayscented fern	Perennial	Forb/Herb	N		√	
Dryopteridaceae	*Onoclea sensibilis* L.	sensitive fern	Perennial	Forb/Herb	N		√	
Euphorbiaceae	*Acalypha rhomboidea* Raf.	common threeseed mercury	Annual	Forb/Herb	N	√	√	√

Table 2.1. continued

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Fabaceae	*Amphicarpaea bracteata* (L.) Fernald	American hogpeanut	Annual/ Perennial	Vine/Forb/ Herb	N			√
	Desmodium perplexum B.G. Schub.	perplexed ticktrefoil	Perennial	Forb/Herb	N			√
	Gleditsia triacanthos L.	honeylocust	Perennial	Tree/Shrub	N	√		
	Securigera varia (L.) Lassen	crownvetch	Perennial	Forb/Herb/ Vine	I	√		√
Fagaceae	*Fagus grandifolia* Ehrh.	American beech	Perennial	Tree	N		√	
Hamamelidaceae	*Liquidambar styraciflua* L.	sweetgum	Perennial	Tree	N	√		
Iridaceae	*Sisyrinchium angustifolium* Mill.	narrowleaf blue-eyed grass	Perennial	Forb/Herb	N		√	
Juglandaceae	*Juglans nigra* L.	black walnut	Perennial	Tree	N			√
Juncaceae	*Juncus effusus* L.	common rush	Perennial	Graminoid	N	√	√	
	Juncus L. spp.	rush			N	√		
	Juncus tenuis Willd.	poverty rush	Perennial	Graminoid	N	√	√	
Lamiaceae	*Clinopodium vulgare* L.	wild basil	Perennial	Forb/Herb	N			√
	Glechoma hederacea L.	ground ivy	Perennial	Forb/Herb	I		√	
	Lycopus virginicus L.	Virginia water horehound	Perennial	Forb/Herb	N		√	
	Mentha pulegium L.	pennyroyal	Perennial	Forb/Herb	I			√
	Perilla frutescens (L.) Britton	beefsteakplant	Annual	Forb/Herb	I		√	
Lauraceae	*Lindera benzoin* (L.) Blume	northern spicebush	Perennial	Tree/Shrub	N	√	√	
Liliaceae	*Maianthemum racemosum* (L.) Link	feathery false lily of the valley	Perennial	Forb/Herb	N	√		
Magnoliaceae	*Liriodendron tulipifera* L.	tuliptree	Perennial	Tree	N	√	√	√
Moraceae	*Morus alba* L.	white mulberry	Perennial	Tree/Shrub	I	√		

Table 2.1. continued

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Oleaceae	*Fraxinus americana* L.	white ash	Perennial	Tree	N	√		
	Fraxinus L. spp.	ash			N		√	
Onagraceae	*Circaea lutetiana* L.	broadleaf enchanter's nightshade	Perennial	Forb/Herb	N	√		
	Epilobium coloratum Biehler	purpleleaf willowherb	Perennial	Forb/Herb	N	√		
	Oenothera biennis L.	common evening primrose	Biennial	Forb/Herb	N	√		
Oxalidaceae	*Oxalis dillenii* Jacq.	slender yellow woodsorrel	Perennial	Forb/Herb	N	√	√	√
Papaveraceae	*Sanguinaria canadensis* L.	bloodroot	Perennial	Forb/Herb	N	√		
Phytolaccaceae	*Phytolacca americana* L.	American pokeweed	Perennial	Forb/Herb	N		√	
Platanaceae	*Platanus occidentalis* L.	American sycamore	Perennial	Tree	N		√	
Poaceae	*Arthraxon hispidus* (Thunb.) Makino	small carpgrass	Annual	Graminoid	I			√
	Dichanthelium clandestinum (L.) Gould	deertongue	Perennial	Graminoid	N		√	
	Microstegium vimineum (Trin.) A. Camus	Japanese stiltgrass	Annual	Graminoid	I	√	√	√
	Muhlenbergia schreberi J.F. Gmel.	nimblewill	Perennial	Graminoid	N		√	√
	Phalaris arundinacea L.	reed canarygrass	Perennial	Graminoid	N		√	
	Poa L. spp.	bluegrass					√	
	Setaria faberi Herrm.	Japanese bristlegrass	Annual	Graminoid	I	√		√
	Setaria pumila (Poir.) Roem. & Schult.	yellow foxtail	Annual	Graminoid	I			√

Table 2.1. continued

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Poaceae	*Tridens flavus (L.)* Hitchc.	purpletop tridens	Perennial	Graminoid	N			√
Polygonaceae	*Fallopia scandens* (L.) Holub	climbing false buckwheat	Perennial	Vine/Forb/ Herb	N			√
	Persicaria longiseta (Bruijn) Kitag.	Oriental lady's thumb	Annual	Forb/Herb	N/I	√	√	√
	Persicaria maculosa Gray	spotted ladysthumb	Annual/ Perennial	Forb/Herb	I	√		
	Persicaria perfoliata (L.) H. Gross	mile-a-minute weed	Annual	Forb/Herb	I	√	√	√
	Persicaria punctata (Elliot) Small	dotted smartweed	Annual/ Perennial	Forb/Herb	N	√		√
	Persicaria sagittata (L.) H. Gross	arrowleaf tearthumb	Annual/ Perennial	Vine/Forb/ Herb	N	√	√	
	Polygonum aviculare L.	prostrate knotweed	Annual/ Perennial	Forb/Herb	I			√
Rosaceae	*Duchesnea indica* (Andrews) Focke	Indian strawberry	Perennial	Forb/Herb	I		√	√
	Geum canadense Jacq.	white avens	Perennial	Forb/Herb	N			√
	Malus Mill. Spp.	apple			I	√		√
	Prunus serotina Ehrh.	black cherry	Perennial	Tree/Shrub	N	√		
	Rosa multiflora Thunb.	multiflora rose	Perennial	Vine/ Subshrub	I	√		√
	Rubus allegheniensis Porter	Allegheny blackberry	Perennial	Subshrub	N	√	√	
	Rubus flagellaris Willd.	northern dewberry	Perennial	Subshrub	N		√	√
	Rubus occidentalis L.	black raspberry	Perennial	Subshrub	N	√	√	√
	Rubus phoenicolasius Maxim.	wine raspberry	Perennial	Subshrub	I	√	√	√
	Rubus L. spp.	blackberry			N	√		

Table 2.1. continued

Family	Scientific name	Common name	Duration	Growth habit	Native Status	Cross-lands	Laurels	Waterloo Mills
Scrophulariaceae	*Mimulus ringens* L.	Allegheny monkeyflower	Perennial	Forb/Herb	N	√		
	Paulownia tomentosa (Thunb.) Siebold & Zucc. ex Steud.	princesstree	Perennial	Tree	I		√	
	Scrophularia marilandica L.	carpenter's square	Perennial	Subshrub/ Forb/ Herb	N	√		
	Verbascum thapsus L.	common mullein	Biennial	Forb/Herb	I			√
	Veronica serpyllifolia L.	thymeleaf speedwell	Perennial	Forb/Herb	N/I	√		
	Veronica L. spp.	speedwell			I	√		
Solanaceae	*Physalis longifolia* Nutt.	longleaf groundcherry	Perennial	Forb/Herb	N		√	
	Solanum carolinense L.	Carolina horsenettle	Perennial	Subshrub/ Forb/ Herb	N	√	√	√
	Solanum nigrum L.	black nightshade	Annual/ Perennial	Subshrub/ Forb/ Herb	I			√
Staphyleaceae	*Staphylea trifolia* L.	American bladdernut	Perennial	Tree/Shrub	N			√
Urticaceae	*Pilea pumila (L.)* A. Gray	Canadian clearweed	Annual	Forb/Herb	N	√		√
Verbenaceae	*Verbena urticifolia* L.	white vervain	Perennial	Forb/Herb	N	√		√
Violaceae	*Viola* L. spp.	violet			N		√	
Vitaceae	*Parthenocissus quinquefolia* (L.) Planch.	Virginia creeper	Perennial	Vine	N	√		
	Vitis vulpina L.	frost grape	Perennial	Vine	N	√		√

Table 2.2. The total number, and number (and percent) of native and introduced species at the three sites. Data were analyzed using a chi-square test of goodness of fit, assuming equal proportions at each site. Plant status according to the PLANTS Database (USDA NRCS, 2011).

		Number (%)	
Site	Total[a]	Native	Introduced
Crosslands	66	47 (73.4%)	17 (26.6%)
Laurels	63	46 (82.1%)	10 (17.9%)
Waterloo Mills	63	35 (62.5%)	21 (37.5%)
X^2	0.0938	2.0781	3.875
P	0.9542	0.3538	0.1441

[a] The total includes unknowns and plants categorized as native and introduced.

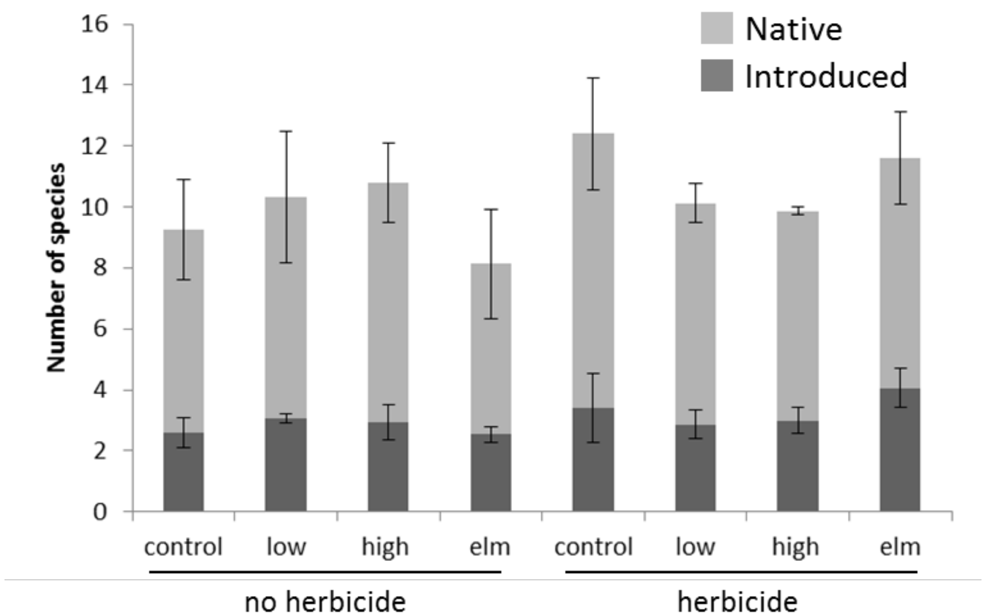

Fig. 2.7. Species richness at the three study sites. Mean ± SEM number of per m^{-2} in each herbicide and planting treatment combination. Only species classified as native or introduced are illustrated in the figure.

When all three sites were used as replicates, the cover of native plants was higher in the herbicide than no herbicide plots ($F_{1,8}$ = 29.64, P = 0.0006), but there were no differences by planting treatment ($F_{3,6}$ = 0.42, P = 0.7466) and no significant interaction. *Euthamia graminifolia* cover was higher in the herbicide than the no herbicide plots ($F_{1,8}$ = 22.95, P = 0.0014), but with no effect of planting treatment ($F_{3,6}$ = 2.63, P = 0.1443) and no interaction. Stiltgrass cover was higher in the no herbicide plots ($F_{1,8}$ = 44.48, P = 0.0002), again with no difference by planting treatment ($F_{3,6}$ = 0.54, P = 0.6714) and no significant interaction effect.

When the Laurels site, which had higher naturally occurring *E. graminifolia* cover and less stiltgrass than Crosslands and Waterloo Mills, is eliminated as a replicate, a planting treatment effect emerges. Native plant cover was much higher in the herbicide than no herbicide plots ($F_{1,8}$ = 271.51, P < 0.0001, Fig. 2.8). There was also a planting treatment effect ($F_{3,8}$ = 11.92, P = 0.0025) and no significant interaction. The control plots had less native cover than the planting treatments within the herbicide plots ($F_{1,8}$ = 27.29, P = 0.0008) and the no herbicide plots ($F_{1,8}$ = 6.48, P = 0.0344). Within the herbicide plots, the plots with *E. graminifolia* planted at high density had more native cover than the control (P = 0.0079) and the elm and low-density *E. graminifolia* plots had marginally higher native cover than the control (P = 0.0727 and P = 0.0752, respectively).

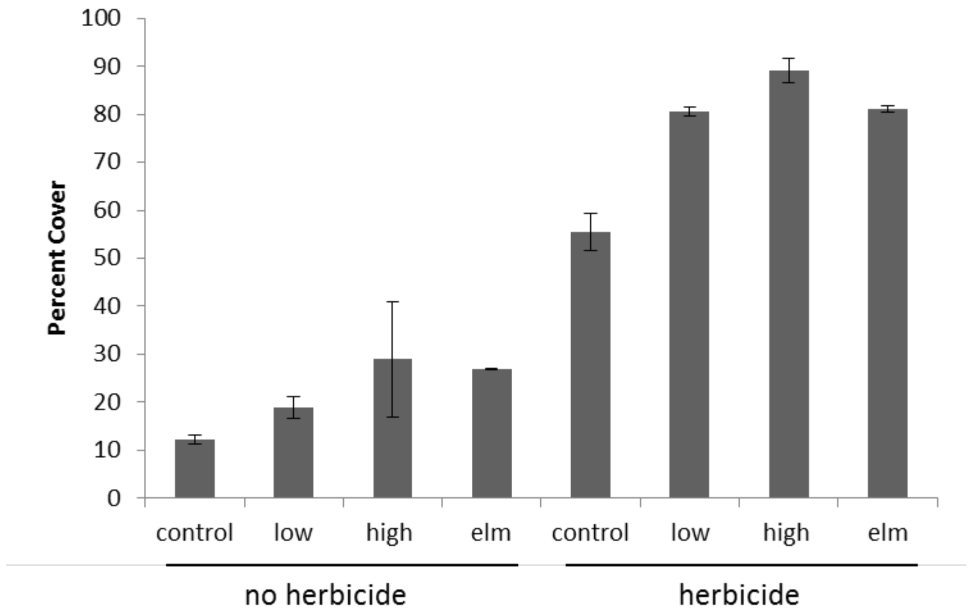

Fig. 2.8. Percent native cover, including *Euthamia graminifolia*, at Crosslands and Waterloo Mills. The control plots had less native cover than the planting treatments in the herbicide (P = 0.0008) and no herbicide plots (P = 0.0344). Within the herbicide plots, the high treatment had more native cover than the control (P = 0.0079).

The cover of *E. graminifolia* at Crosslands and Waterloo Mills was higher overall in the herbicide than no herbicide plots ($F_{1,4}$ = 54.20, P = 0.0018, Fig. 2.9), and was higher in the planting treatments than in the control within the herbicide plots ($F_{1,6.2}$ = 15.33, P = 0.0073) but not in the no herbicide plots ($F_{1,6.2}$ = 1.38, P = 0.2825). Within the herbicide plots, *E. graminifolia* cover was higher in the high-density than in the control plots (P = 0.0410).

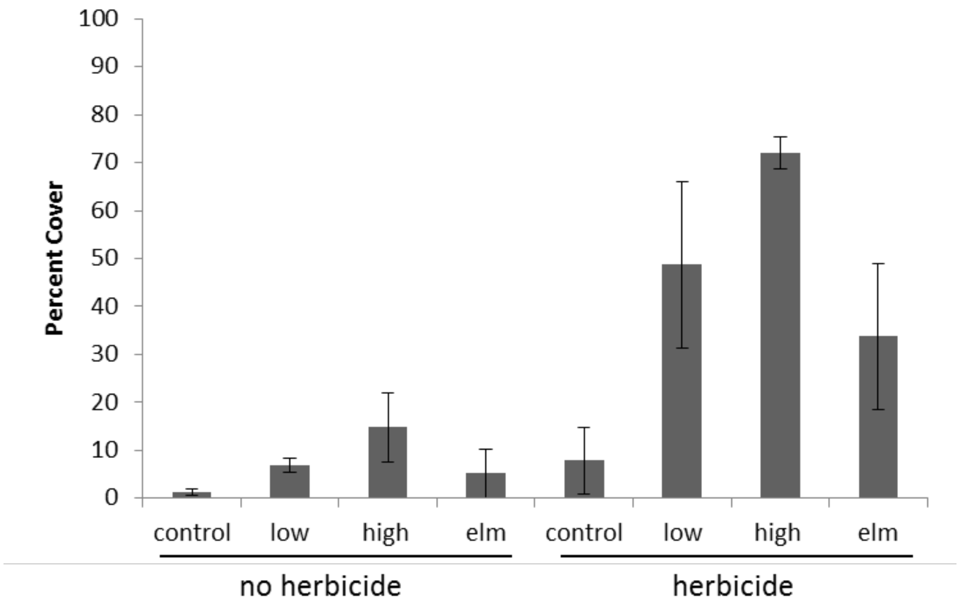

Fig. 2.9. Percent *Euthamia graminifolia* cover at Crosslands and Waterloo Mills. *Euthamia graminifolia* cover was higher in the planting treatments considered together than the control within the herbicide plots (P = 0.0073) but not in the no herbicide plots (P = 0.2825). Within the herbicide plots, *E. graminifolia* cover was higher in the high plots than the control plots (P = 0.0410).

Japanese stiltgrass cover was higher in the no herbicide than herbicide plots at Crosslands and Waterloo Mills ($F_{1,7}$ = 264.31, P < 0.0001, Fig. 2.10). Stiltgrass cover also differed by planting treatment ($F_{3,7}$ = 17.50, P = 0.0012) and there was no significant interaction. Stiltgrass cover was higher in the control than in the planting treatments in the herbicide plots ($F_{1,7}$ = 35.77, P = 0.0006) and no herbicide plots ($F_{1,7}$ = 11.36, P = 0.0119). In the herbicide plots, stiltgrass cover was higher in the control than in the elm (P = 0.0119), high (P = 0.0485) and low (P = 0.0174) planting treatments. In the no herbicide plots, stiltgrass cover was higher in the control than in the elm treatment (P=0.0297).

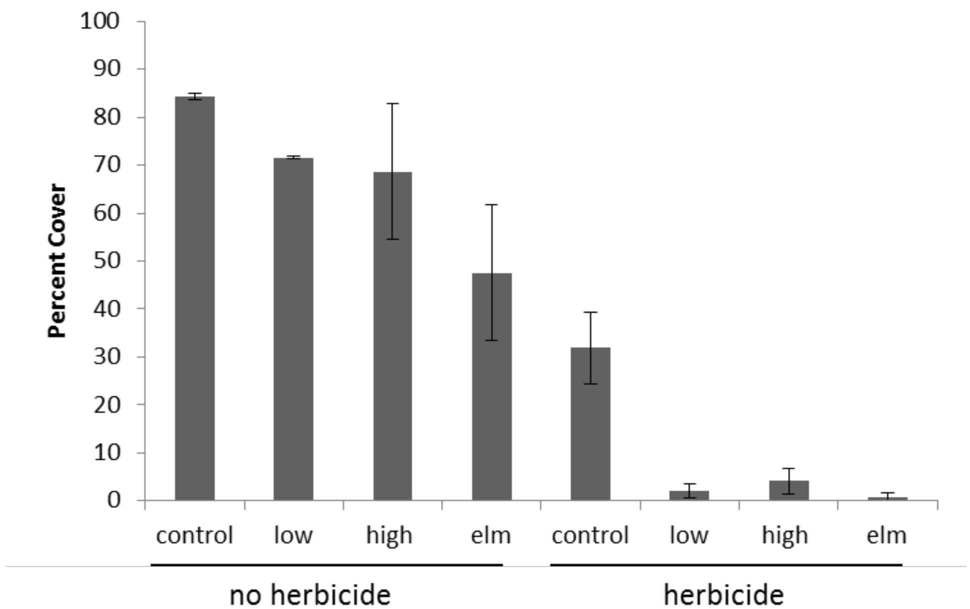

Fig. 2.10. Percent Japanese stiltgrass cover at Crosslands and Waterloo Mills. Stiltgrass cover was higher in the control than in the planting treatments in the herbicide plots (P = 0.0006) and no herbicide plots (P = 0.0119). In the herbicide plots, stiltgrass cover was higher in the control than in the elm (P = 0.0119), high (P = 0.0485) and low (P = 0.0174) treatments. In the no herbicide plots, stiltgrass cover was higher in the control than in the elm treatment (P = 0.0297).

Elm tree survival and growth

Elm tree survival did not differ by herbicide treatment or site (X^2 = 0.8857, P = 0.6422), with an overall survival rate of 88%. Elm height differed by herbicide treatment (two-way ANOVA, $F_{1,46}$ = 9.50, P = 0.0035, Fig. 2.11) but not site ($F_{2,46}$ = 1.30, P = 0.2833) and there was a significant interaction ($F_{2,46}$ = 3.41, P = 0.0418). Overall elms planted in the herbicide plots were more than 0.4 m taller than those planted in the no herbicide plots by October 2010, at which point the elms were 2.5 years old.

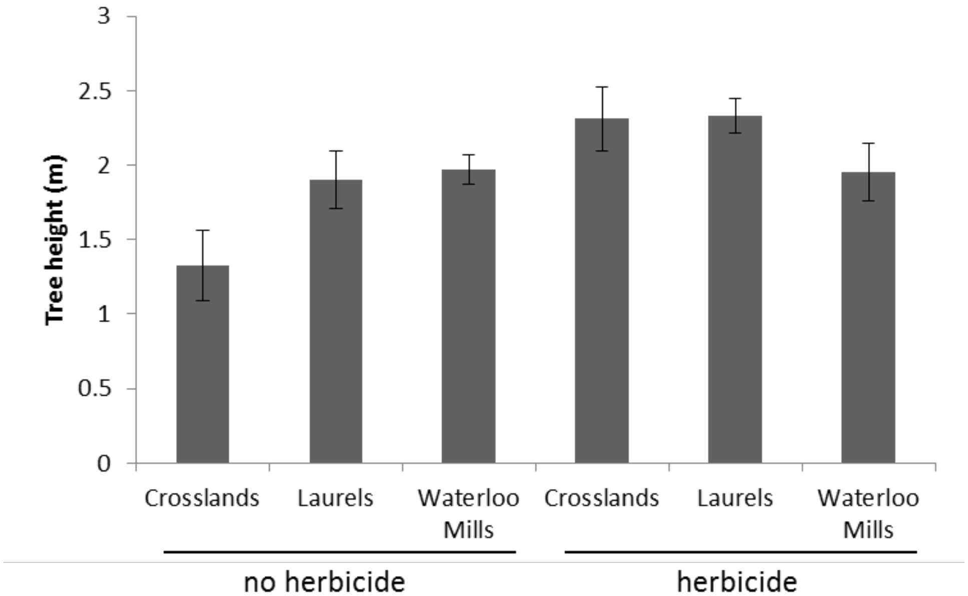

Fig. 2.11. Mean ± SEM height of elm trees at the three study sites. Elms planted in the herbicide plots were taller than those planted in the no herbicide plots (two-way ANOVA, $P = 0.0035$).

DISCUSSION

While pre-emergent herbicide in the presence of the biocontrol weevil *R. latipes* reduced the abundance of mile-a-minute weed compared to the no-herbicide treatments, there were no differences in reduction depending on planting treatments. However, the resulting plant community differed greatly by treatment. The selection of management strategies determined the extent of recovery of the native plant community and whether or not the invasive treadmill effect occurred in this experiment.

Feeding damage by *R. latipes* decreases the competitive ability of mile-a-minute by stressing the plant (Van Driesche et al., 2008) and altering plant architecture (Hough-

Goldstein et al., 2008b; personal observation). Stem-boring by the larvae of *R. latipes* decreases mile-a-minute internode distance, while larval damage coupled with the feeding of adult weevils in the terminal tips may disrupt apical dominance (Hough-Goldstein et al., 2008b). Plants may respond to the loss of apical dominance via the production of secondary and tertiary terminals (Benner, 1988; Irwin and Aarssen, 1996a,b; Lortie and Aarssen, 2000). This branching increases access to light and provides a means for regrowth after the apical meristem is damaged or lost (Fay and Throop, 2005). Apical dominance may be critical for plants competing for light since it promotes vertical growth (Irwin and Aarssen, 1996a).

Heavy feeding damage in the spring negates the competitive advantage that mile-a-minute gains by germinating earlier than many other plants (personal observation), and mile-a-minute plants damaged by *R. latipes* produce large numbers of side terminals (Hough-Goldstein et al., 2008b; personal observation). The combination of weevil feeding plus competition delayed the onset of seed production and killed more than 60% of *P. perfoliata* plants in field cages (Hough-Goldstein et al., 2008b). Thus, if changes to mile-a-minute's architecture limits its vertical growth and prevents it from reaching the plant canopy and necessary light resources, seed production is reduced or eliminated.

Reducing mile-a-minute seed production is critical to the management of this annual weed because the seedbank is crucial for the persistence of mile-a-minute populations. The existence of a seedbank can enable one successful colonization event to result in long-term persistence of a population despite short-term environmental stochasticity (Turnbull et al., 2000). More mile-a-minute seedlings germinated from the no herbicide than the herbicide plots in all years of this study, with a mean of fewer than 11 seedlings in 2010 and 2011 in plots that integrated biological control with herbicide. Low and stable seedling numbers in most plots suggest that herbivory and competition are currently preventing mile-a-minute from producing enough seed for the population to increase in any of the herbicide plots 2 years after a single pre-emergent treatment. The herbicide-control plots also had low numbers of mile-a-minute seedlings, but in this case plant competition was due largely to introduced species such as Japanese stiltgrass.

The reduction of the dominance of mile-a-minute weed had very different consequences for the plant community depending on whether or not other management strategies were used along with biological control. Although more than 60% of species present in the plots were native, the relative cover of native species was less than 30% in plots not treated with herbicide, while in the planted treatments following pre-emergent herbicide application native plant cover was greater than 80% after only 2.5 years.

In two of the three sites, as mile-a-minute cover declined in the no-herbicide plots it was largely replaced by Japanese stiltgrass, an invasive annual grass that was abundant at those sites. Japanese stiltgrass alters the composition of plant communities by inhibiting the establishment and growth of native plants (Flory and Clay, 2010a) and tree regeneration (Flory and Clay, 2010b). In addition to direct competition from live stiltgrass, senesced stiltgrass forms a dense mat, which may have limited native plant germination and growth in the no herbicide plots (Flory and Clay, 2009; Flory and Clay, 2010b).

The post-emergent herbicide applied in September 2008 to prepare the plots for planting decreased a portion of the contribution of mile-a-minute and stiltgrass seed to the seedbank that year. The combination of pre- and post-emergent herbicide applications essentially negated two years of the mile-a-minute and stiltgrass seedbanks. Although *P. perfoliata* seeds can be viable for up to six years in the seedbank, approximately 60% of seeds germinate in the first two years (Hough-Goldstein et al., 2008a). Thus the herbicide applications may have had a large impact on the mile-a-minute seedbank. Japanese stiltgrass has a seedbank that persists for at least 3 years (Barden, 1987) and this species was therefore able to re-invade the herbicide control plots at Crosslands and Waterloo Mills during 2010.

Flory (2010) repeatedly applied a pre-emergent herbicide in combination with a post-emergent herbicide for control of Japanese stiltgrass and found that although the stiltgrass was effectively controlled, the recovery of the native plant community was inhibited by the pre-emergent herbicide. In the current study, the one-time pre-emergent application largely prevented recruitment of both native and non-native seeds from the seedbank in 2009; however, in the planting treatments the herbicide enabled the perennial *Euthamia graminifolia* plugs and the elm tree seedlings to establish with reduced competition. These plantings helped to stabilize the disturbance created during the installation of the experimental plots, and appear to have subsequently facilitated recruitment of other, mostly native plants. In contrast, by fall 2010, stiltgrass cover in no-herbicide plots ranged from 48-84%. As a result, the cover of *Euthamia graminifolia*, specifically selected as a robust competitor, was greatly reduced in the no-herbicide plots, and the elms were much shorter than in the herbicide plots.

This study demonstrates that the selection of the strategy used to manage an invasive weed may determine the fate of the native plant community. In other studies, the resulting plant community also differed depending on the management strategy used to control invasive plants, including Japanese stiltgrass (Flory and Clay, 2009; Flory, 2010), *Hedera helix* L., English ivy (Biggerstaff and Beck, 2007), and *Chrysanthemoides monilifera* spp. *rotundata*, bitou bush (Mason and French, 2007). The act of managing an invasive plant often causes a disturbance (Mason and French, 2007), whether through collateral damage in the case of chemical or mechanical controls (Bush et al., 2007), by enabling the invasive treadmill effect (Reid et al., 2009) or by other means. These disturbances may lead to changes in resource availability, which may increase the invisibility of a community (Davis et al., 2000). Integrated weed management, particularly management that includes revegetation, offers a way to mitigate the impact of disturbance on a community and decrease its invasibility by reducing resource availability (Davis et al., 2000) and niche opportunities (Shea and Chesson, 2002).

Integrating management techniques may provide more effective control of the invasive weed than any technique in isolation (Paynter and Flanagan, 2004; Henne et al., 2005; Lym, 2005) and may increase recovery of the native plant community, particularly if revegetation with natives is part of the management plan (Biggerstaff and Beck, 2007). A restored native plant community may be better able to resist re-invasion by exotic weeds and facilitate colonization by native plants (Bakker and Wilson, 2004). This diverse plant community also has broader ecosystem-wide benefits, as it is able to support greater arthropod abundance and diversity, which then supports higher trophic levels (Haddad, et al., 2009; Tallamy et al., 2010). In this study, the integration of biological control, pre-emergent herbicide and revegetation with competitive natives resulted in suppression of mile-a-minute weed, prevention of the invasive treadmill effect with Japanese stiltgrass, and increased native plant abundance.

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