



Establishment and dispersal of the biological control weevil *Rhinoncomimus latipes* on mile-a-minute weed, *Persicaria perfoliata*

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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 US 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 US in 2004 as part of a classical biological control program. The weevil was intensively monitored in three release arrays over 4 years, and 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 1.5–2.9 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. Mile-a-minute cover decreased at the site with the highest weevil density. The production of *P. perfoliata* seed clusters decreased with increasing weevil populations at two sites, and seedling production declined over time at two sites by 75% and 87%. The ability of the weevil to establish, produce multiple generations per season, disperse to new patches, and likelihood of having an impact on plants in the field suggests that *R. latipes* has the potential to be a successful biological control agent.

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1. Introduction

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Freeman and Reveal, 2005), is an invasive annual vine in the US 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 US 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 US in the 1930s at the Gable Nursery in Stewartstown, York County, Pennsylvania, where it emerged with a planting of holly seeds that

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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 6 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 US 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 US (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; Korotyayev, 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 order to track weevil dispersal within 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 4 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.

2. Materials and methods

2.1. 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 4 months of the release, and therefore monitoring was discontinued after 2005.

Mile-a-minute weed has been present at the BVA Wetland release site (39°55'06.66"N, 75°40'41.38"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 (39°54'42.35"N, 75°40'33.30"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 (39°55'48.30"N, 75°47'23.30"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-m² monitoring points located on concentric circles between 1 and 25 m from the release point were established at each release site (Fig. 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). A portion of the 20 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.

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.

2.2. Monitoring protocol

During 2005, monitoring began 4 days post-release and then took place weekly from 16 June through 19 July, and every other week through 1 November. In 2006, 2007, and 2008, 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, and 17 May through 11 October, 2008. Each monitoring point was checked for the following within a 1-m² 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 × 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-m² frame, which was marked in 10-cm intervals, and estimating what

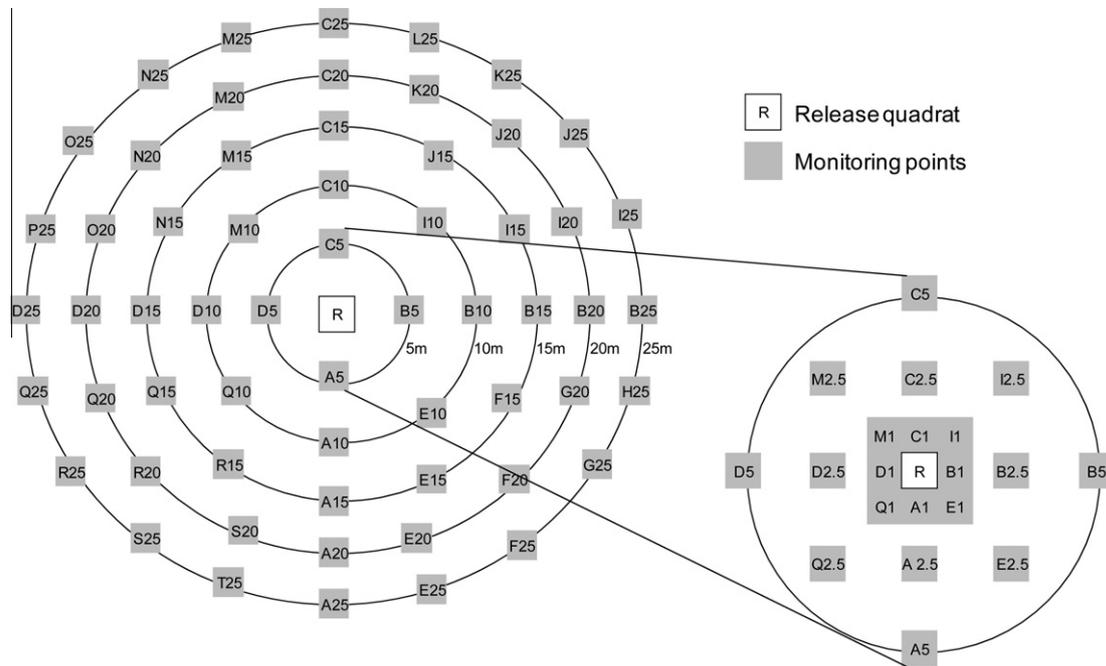


Fig. 1. Site design for weevil release arrays. Monitoring points were located on concentric circles 5, 10, 15, 20 and 25 m from the point of release. The inset illustrates 16 additional monitoring points located approximately 1 and 2.5 m from the release.

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 to 25 m ($n = 21$ maximum points; Fig. 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 2006–2008. Spring seedling counts were conducted in May of 2006–2008 using a 1×0.5 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 to 25 m ($n = 21$ maximum points; Fig. 1).

2.3. Generations per year

To determine the number of weevil generations that could develop in the course of a season, wood-framed cages ($56 \times 56 \times 61$ 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 days. 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 1 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.

2.4. 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.

2.5. 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 quantitate 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 within the central 5-m radius at each site over all 4 years (individual area under the curve calculated for a maximum of 13 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 4 years (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) were compared using a one-way ANOVA (PROC GLM) followed by Tukey's test (SAS Institute, 2008).

Data were log-transformed [$\log(x + 1)$] as needed to reduce heteroskedasticity of variance residuals. Non-transformed means and standard errors are presented.

3. Results

3.1. *R. latipes* egg production and generations per year

Eggs were observed in an average of 65% of monitored quadrats at the three release sites 4 days after the 9 June release in 2005 (Fig. 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. 2). The decline in egg production was highly synchronized from late August to late September all 3 years (Fig. 2). Temperatures remained relatively warm during this period, especially in 2005 (Table 1). The first sustained frost occurred in late October in 2005 and 2007, and in mid-October in 2006.

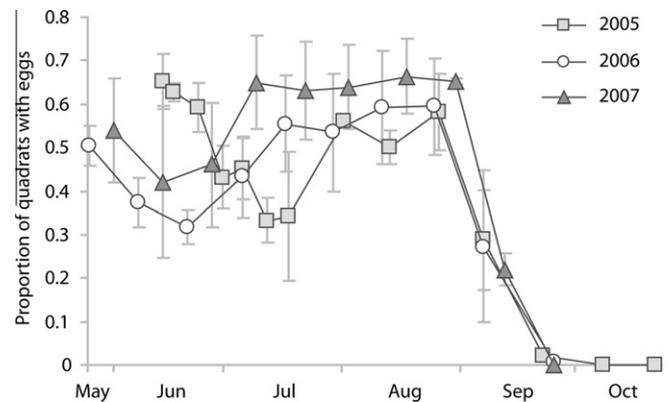


Fig. 2. Mean (\pm SEM) proportion of monitored mile-a-minute quadrats with eggs present at the three release sites from 2005 to 2007.

Table 1

Average weekly temperature ($^{\circ}C$) at release sites from mid-August through the end of September, when egg production by *R. latipes* ceased.

| | 2005 | 2006 | 2007 |
|-----------------|-------|-------|-------|
| August 18–24 | 20.76 | 21.02 | 17.31 |
| August 25–31 | 21.22 | 21.25 | 21.28 |
| September 1–7 | 17.96 | 16.95 | 18.69 |
| September 8–14 | 17.88 | 16.57 | 19.74 |
| September 15–21 | 20.74 | 16.58 | 13.13 |
| September 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 1 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.

3.2. 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 $m wk^{-1}$, and dispersal was slower at the BVA Wetland than at the other two sites (Table 2a). During the first week, weevils dispersed from the release point at

Table 2

Rate of dispersal from mid-June, 1 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 ($m wk^{-1}$) \pm SEM | (b) Sample time (week) | Dispersal rate ($m wk^{-1}$) \pm SEM |
|-------------|--|------------------------|--|
| Laurels | 2.9 \pm 0.6a | 1 | 6.0 \pm 1.4a |
| BVA CREP | 2.4 \pm 0.4a | 2 | 4.0 \pm 0.8ab |
| BVA Wetland | 1.5 \pm 0.3b | 3 | 2.7 \pm 0.6bc |
| | | 4 | 2.1 \pm 0.4bc |
| | | 5 | 1.7 \pm 0.3c |
| | | 6 | 1.4 \pm 0.3c |
| | | 8 | 1.2 \pm 0.4c |
| | | 10 | 1.5 \pm 0.6c |
| | | 12 | 1.5 \pm 0.3c |
| | | 14 | 1.6 \pm 0.1c |
| | | 16 | 1.3 \pm 0.1c |

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 2b).

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.

3.3. 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 (Fig. 3), and increased when the F1 generation began to emerge in the field, approximately 1 month after the release. The highest density of weevils in 2005 was observed at the Laurels, with a peak of $37.5 \text{ weevils m}^{-2}$ of mile-a-minute weed on 4 October (Fig. 3). Weevils successfully overwintered at all three release sites.

Weevil population density increased at the Laurels from 2005 to 2007 with an average of more than $100 \text{ weevils m}^{-2}$ of mile-a-minute for at least 1 month in 2006 and 2007. Weevil density was lower at the Laurels in 2008, though still significantly higher overall than in 2005 (area under the curve, $F_{3,80} = 56.63$, $P < 0.0001$, Fig. 3). At the BVA CREP site, fewer than $30 \text{ weevils m}^{-2}$ 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 2008 ($F_{3,70} = 0.94$, $P = 0.4282$, Fig. 3). The weevil population density increased over time at the BVA Wetland ($F_{3,80} = 14.91$, $P < 0.0001$, Fig. 3). An average of fewer than $20 \text{ weevils m}^{-2}$ of mile-a-minute were counted on most dates at this site.

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 ($F_{3,80} = 34.36$, $P < 0.0001$, Fig. 3). There were no changes over time in the percent cover of mile-a-minute weed at the BVA CREP ($F_{3,71} = 1.90$, $P = 0.1372$, Fig. 3) or BVA Wetland sites ($F_{3,80} = 0.30$, $P = 0.8274$, Fig. 3). 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 (Fig. 3).

3.4. *P. perfoliata* seed cluster production and seedling counts

Seed cluster production declined with increasing weevil population at the Laurels ($P < 0.0001$, $R^2 = 0.2814$, Fig. 4) and BVA Wetland ($P = 0.0374$, $R^2 = 0.0837$, Fig. 4) but this relationship was not significant at the BVA CREP site ($P = 0.5794$, $R^2 = 0.0070$, Fig. 4).

The number of mile-a-minute weed seedlings 0.5 m^{-2} counted in May each year decreased at the Laurels by 87%, from an average of approximately 100 seedlings in 2006 and 2007 to fewer than 15 in 2008 ($F_{2,73} = 26.47$, $P < 0.0001$, Fig. 5). At the BVA CREP site the average number of seedlings declined by 75%, from around 18 to approximately 4.5 seedlings per 0.5 m^2 ($F_{2,72} = 7.61$, $P = 0.0010$, Fig. 5). The decline in seedling counts observed at the BVA Wetland site was not significant ($F_{2,74} = 2.16$, $P = 0.1221$, Fig. 5).

4. 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). *R. latipes* overwinters in the adult stage in leaf litter and/or the top few centimeters of topsoil (personal communication, Fu Weidong, Institute of Environment and Sustainable Agricultural

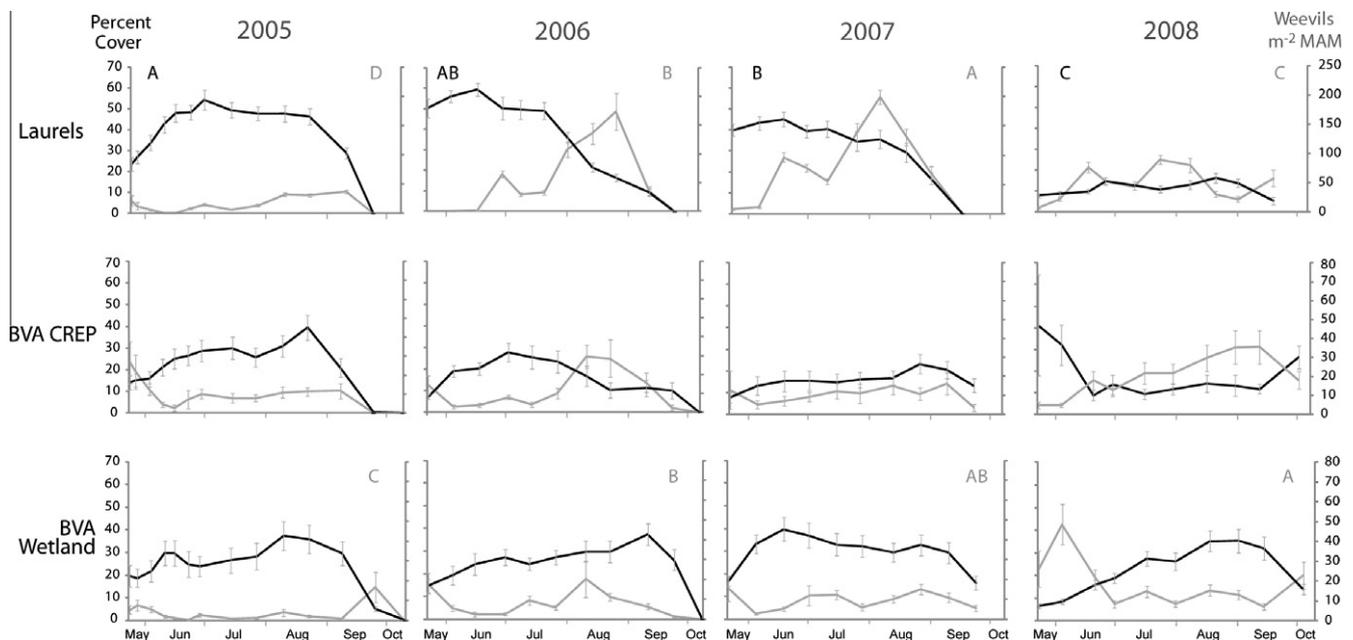


Fig. 3. Mean (\pm SEM) weevils m^{-2} of mile-a-minute weed and percent cover of mile-a-minute within 5 m of the release from 2005 to 2008. Analyses based on the area under the curve; means with the same letter within a site are not significantly different (Tukey's test).

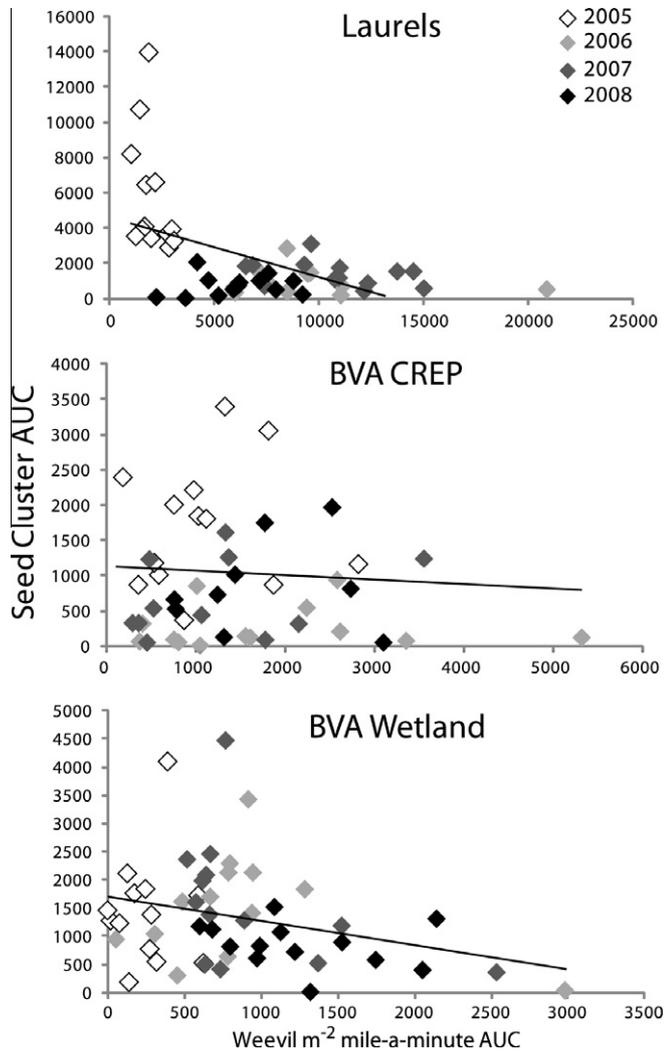


Fig. 4. Comparison of weevils m^{-2} mile-a-minute weed area under the curve and seed cluster area under the curve for the release site and points on the A, B, C and D transects within 5 m of the release from 2005 to 2008.

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 °C (Ding et al., 2004). *R. latipes* established in 96.9% of monitored releases sites in the mid-Atlantic US (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,

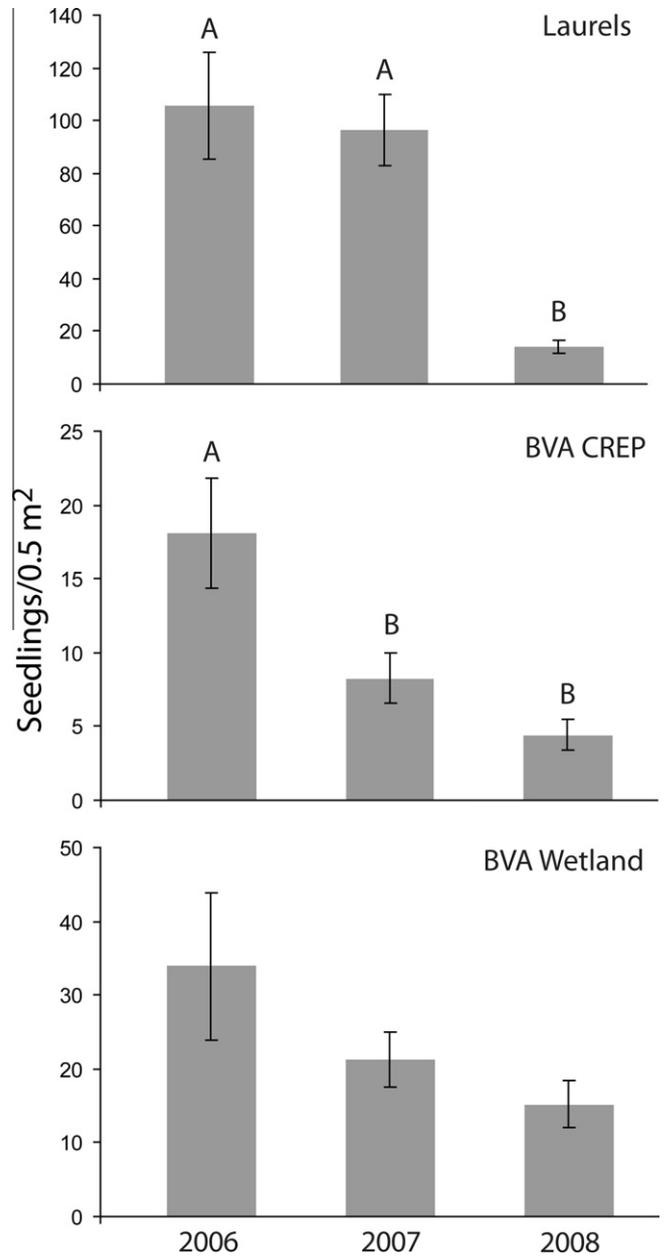


Fig. 5. Mean (\pm SEM) number of mile-a-minute seedlings counted in May from 2006 to 2008.

day length declined from about 13 h in late August to approximately 12 h 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 three 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 4 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 yr⁻¹ between one and 3 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 maximum rate of 2.8 km yr⁻¹ 2 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.

Four years post-release, mile-a-minute weed cover was significantly reduced at the Laurels, and spring seedling counts declined at the Laurels and BVA CREP sites. We cannot say definitively that these reductions were the result of weevils, because control plots were colonized by dispersing weevils and therefore not available for comparison with release plots. 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. Long-term monitoring of weevil release sites and the restoration of mile-a-minute sites are the subjects of ongoing research.

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