AN INTEGRATED APPROACH TO THE RESTORATION OF AREAS INVADED BY MILE-A-MINUTE WEED (*PERSICARIA PERFOLIATA)* USING BIOLOGICAL CONTROL AND HERBACEOUS NATIVE SEEDING

by

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ABSTRACT

Disturbed areas frequently experience invasion by introduced plant species and subsequently a drop in species richness and ecosystem services. Research on the best means of removing these species abounds, but typically does not include techniques to restore the invaded habitat. Without such restoration the introduced species often re-occur, or an alternate introduced invasive plant fills the opened niche. This study investigated an integrated approach to controlling the introduced annual *Persicaria perfoliata* [L.] H. Gross (mile-a-minute weed) using the Asian biocontrol weevil *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) and establishment of native plants from seed. The native seed mix used was comprised of five widely adapted plant species, three grasses and two forbs. A fully factorial design integrated weevils and seeding, using a systemic insecticide as a control. In a field setting, areas of well-established mile-a-minute weed were sown with natives in early spring when the weed germinated. A weevil population had been established at the site one year prior to sowing and was augmented both years of the experiment. By year two there was significantly lower *P. perfoliata* mean biomass and percent cover in plots integrating weevils and native seeds than seed-only or control plots. Further, the integrated plots had about half the mile-a-minute biomass and percent cover of the weevil-only treatment. Greenhouse experiments of the same basic design produced similar results. There was little evidence of native plant establishment during the first year in the field. Year two resulted in significantly higher native plant species richness

in the integrated weevil and native seed-treated plots. These results indicate that removal of introduced invasive plant species should be accompanied by re-vegetation to aid in restoration.

Chapter 1

INTRODUCTION

1.1 *Theoretical Background*

Invasive introduced plants have many deleterious effects in their novel ranges, including altered fire regimes, nutrient cycling, hydrology, energy budgeting, and native species survival (Gooden et al. 2009; Gordon 1998; Mack et al. 2000). Therefore, limiting introduced species via removal or control is beneficial (Morrison 2002; Willis & Birks 2006). If a plant invader is not removed by some means it may even facilitate growth of other non-indigenous affiliated species (Simberloff & Von Holle 1999; but also see Simberloff 2006).

Even when invasive introduced plants are removed, the same or another introduced species will frequently take advantage of the opened niche (Benz et al. 1999; Reeder & Hacker 2004). This dynamic has been especially noted in the biocontrol field (Butler & Wacker 2010; Stephens et al. 2009; Thomas & Reid 2007; McEvoy & Coombs 2000) and has been termed the "invasive species treadmill", in which removal of the problematic species merely leads to alternative invasion as a

result of the habitat disturbance. Because of this, an integrated approach to restoration of areas invaded by introduced plant species has been suggested as important from the standpoints of vegetation (Benz et al. 1999), soil and nutrient cycling (e.g Heneghan et al. 2008) and economics (Lym 2005). In the case of plants, this means replacement of target weeds with beneficial natives is a better solution than removal without follow-up (Hough-Goldstein et al. 2008a; Lonsdale & Farrell 1998; Lym 2005).

Hacker and Dethier (2009) expand on the dynamics of community disturbance through foreign plant invasion by using the alternative stable state theory, a model originally proposed by Lewinton (1969). They suggest that one of three states will occur after introduced species removal, "(1) the invasion state in which the invader dominates the community through positive feedback mechanisms that continue its expansion and dominance (2) a restored state defined as the replacement of the lost species assemblage with the original assemblage, and its function, after the invader is removed, and (3) an alternative state defined as one in which a new species assemblage colonizes (either native or non-native) and persists; it could include reinvasion by the original invading species or other non-indigenous species." Most sites invaded by mile-a-minute weed (*Persicaria perfoliata* [L.] H. Gross) resemble the first state described (Hough-Golstein et al. 2008), and after removal via biocontrol, the third state (reinvasion by itself or other introduced species) (K. Cutting, University of DE, personal observation).

Depending on the ecological legacy of the site, i.e. seed bank, mycorrhizal presence, allelopathic effects, nutrient cycles, etc. (Schaefer 2009), it is rarely possible

to restore to the original plant assemblage (Hilderbrand et al. 2005, Zedler 2000). To attempt the painstaking task of forcing a site to resemble an exact previously documented single state may not be the most practical route in an era when restoration of functional ecosystems is in high demand but funding scarce.

The goal of this study was to assess ways to actively and efficiently restore an area invaded by *P. perfoliata* so it reaches the third state as described by Hacker and Dethier, i.e. that of a new species assemblage of persisting natives that are resilient to reinvasion (the second version of the third state described above). Some of the newly restored plant species may have originally been present prior to the invasion, and may still persist nearby ready to re-colonize via propagule pressure. Restoration designs should consider re-vegetation with locally occurring native species, preferably of regional ecotype (Hufford & Mazer 2003). Our objective is for the new community to provide ecosystem services and function with all the stability of a biodiverse native habitat (Ives & Carpenter 2007; Duffy 2009). If undisturbed, it may remain resistant to another invasion.

1.2 *Study Organisms*

Persicaria perfoliata, or mile-a-minute weed (MAM), is an invasive annual vine accidentally introduced from Asia to York, PA in the 1930s (Moul 1948). Leaves are alternate and triangular, and a diagnostic feature is the presence of saucer-shaped ocreae, or fused stipules that surround the stem at each leaf node (Hough-

Goldstein et al. 2008a). Seeds germinate in early spring, and vines grow rapidly during the summer, with small spines on leaves and stems helping the plant to climb up and over other vegetation. Seed production may occur as early as June, but the majority of the seeds are produced in the fall (Hough-Goldstein et al. 2008a, b).

This plant inhibits commercial and natural forest regeneration, interferes with recreational use of natural areas, reduces wildlife habitat, and may lead to native floral decline (Mountain 1989; McCormick and Hartwig 1995;Wu et al. 2002; Hough-Goldstein et al. 2008a). Because of this, MAM may be considered both a transformer species (Dodson & Fiedler 2006) and ecosystem engineer (Jones et al. 1997).

P. perfoliata proliferation after initial introduction is most likely aided by lack of top-down regulation (i.e. enemy release hypothesis, Williamson 1996) paired with fast growth and nutrient cycling. Its remarkable ability to dominate disturbed plant communities may also be promoted through underground interactions such as allelopathy or mycorrhizal associations. Whatever the means, MAM has spread widely after invasion, characterizing the community dominant species Gordon (1998) describes as one capable of changing ecosystem processes. There is urgency in the need to halt spread of this species (Kumar & Ditommaso 2005).

An approach to limiting the invasion by MAM is development of integrated control strategies based on ecological knowledge of the plant both in its novel and native host ranges (Hyatt & Araki 2006). Therefore we seek to address MAM's pre-disposition to dependence on disturbance (i.e. open niches) and will attempt to reduce plant survival and seed production (Hyatt & Araki 2006).

Hyatt & Araki (2006) suggested control of MAM through labor-intensive mechanical plant removal, but this is somewhat unrealistic given the current state of invasion. McCormick and Hartwig (1995) pose that MAM can and should be controlled with herbicides. However, this method of control is costly and often difficult due to lack of accessibility in some environments (Lym 2005). Herbicides can also cause collateral damage by impacting non-targets (Simmons 2005).

However, without initial control of the target weed by some means, re-vegetation through native seeding may not succeed (Lym 2005). Hence, a vital part of the integrated approach presented here is through establishment of the appropriate biocontrol agent, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) or the mile-a-minute weevil. *R. latipes* feeds only on *P. perfoliata* in both its native and introduced ranges, in both the adult and larval stages (Colpetzer et al. 2004a; Ding et al. 2004; Frye et al. 2010) and was released in the U.S. in 2004.

The small (approx. 2-mm long) adult *R. latipes* weevils emerge in early spring, soon after MAM seedlings appear, and chew characteristic small round holes in the leaves. They lay their eggs on *P. perfoliata* stems, terminals, and leaves, with oviposition beginning about 6 days after adult emergence and continuing at a rate of about 3 eggs per female per day for at least 2 months under laboratory conditions (Colpetzer et al. 2004b). Larvae bore into the stem at nodes soon after hatching, complete their development internally, then exit the stem and drop to the soil for pupation (Hough-Goldstein et al. 2008a). The weevils go through at least three or four overlapping generations during the growing season in the Mid-Atlantic region, with

each generation taking about one month to develop (Lake et al. 2011, in press). Adults stop producing eggs between late August and late September, and adult weevils overwinter in the leaf litter or soil.

1.3 *Plant Competition*

The vacant niche is key in invasions because it represents underutilized resources (Elton 1958; Mack et al. 2000; Tilman 1999). Strongly competing plants quickly fill vacant niches because of the nutrients, water and sunlight they offer. An occupied niche hampers invasion because the resources are taken. This dynamic is exemplified in the invasive species treadmill concept, and underscores the need for intentional replacement of native competitive vegetation if disturbance occurs. Disturbance from a variety of sources initially allows invasion by MAM. After biocontrol weevils are applied to invaded areas, it appears that weevil-induced weakening and mortality of MAM weed is in turn a disturbance that creates an open niche. It is at this point active restoration with native resource competitors will prevent the invasive treadmill from occurring.

Elton (1958) and Tilman (1999) propose that community species richness is inversely proportional to invasibility (diversity-invasibility hypothesis). In greenhouse conditions, Perry & Galatowitsch (2003) showed that a mix of four perennial and annual native cover crops suppressed growth of annual introduced

species. We hypothesized that a mix of hardy annual and perennial native forbs and warm and cool season grasses would facilitate strong resource competition with MAM over time and throughout each growing season. Forbs establish quickly after seeding and create initial competition for light and space. MAM is an early emerger (a trait typical of successful invaders), so the cool season grass provides competition in subsequent springs. Lonsdale and Farrell (1998) showed that perennial grasses competing with an introduced species during germination significantly reduced the number of introduced seedlings germinating, as well as their final biomass. Warm season grasses continue to grow rapidly through the summer, competing for water resources.

Plants proposed for re-vegetation are more than a placeholder or resource competitor. Plant nativity has important ramifications for wildlife species and diversity due to the nature of its chemical composition and expression. Native plants have been shown to support a significantly greater abundance and species richness of native Lepidoptera due to a shared evolutionary history (Burghardt et al. 2010). It has been suggested that the same is true of other arthropods (Tallamy 2004). Native insects, therefore, may be the primary means by which energy is transferred up through the trophic levels, supporting an array of invertebrates and vertebrates (Burghardt et al. 2009). The ecosystem services provided by the natural world, largely comprised of native plants, have been estimated at a value of $33 trillion per annum (Costanza et al.1997). Plants in their native context have evolved interacting with a broad community, becoming regulated through top-down control and interspecific

competition. They therefore should not exhibit domineering qualities when in their native range.

Interspecific competition is caused by plant neighbors and their impacts both above and below the soil surface. Murphy & Dudley (2007) have shown that soybean plants respond to above and below ground competitive cues independently. This suggests that both light competition and root neighbors may be important to trigger competitive phenotypic responses in plants (Callaway 2002). A significant difference in root growth occurs in root neighbors when compared with plants grown separately but with equal resource access (Gersani et al. 2001). This non-resource based interaction can trigger heavy use of resources allotted to stem elongation and root mass growth (Murphy & Dudley 2007). These events could increase a plant's immediate competitive strength, but at the cost of negatively influencing processes such as leaf and seed production (Murphy & Dudley 2007; Gersani et al. 2001). It is expected that such a competitive reaction would be heightened in the case of MAM's weak root system (Mountain 1989) in the presence of native grasses' strong sod formation (Benz et al. 1999; Burton et al. 2006). Compromised reproduction has severe implications in an annual plant such as MAM where seed production is key to population persistence and propagation over time (Mountain 1989).

Various studies have explored the compromising effects of herbivory on plants' abilities to compete with surrounding vegetation, whether intraspecific (Center et al. 2005) or interspecific (Bacher & Schwab 2000). Bacher & Schwab found that augmentative biocontrol of *Cirsium arvense* (L.) Scop. (Canada thistle) combined with

herbaceous seeded plant competition reduced problematic thistle populations in Switzerland by 50%. The perennial plant, Canada thistle, uses substantial root masses to store nutrients for survival. If the combined pressure of herbivory and seeded plant competition caused this level of mortality in plants with such robust roots, it is reasonable to believe that *P. perfoliata*, with its relatively weak root systems, may struggle more so under similar treatment.

This additive effect of herbivory and competition is due to the fact that a plant's tolerance to herbivory is most likely linked to resource availability (Hawkes & Sullivan 2001; Sun et al. 2010). It has already been demonstrated that plant competition in concert with *R. latipes* feeding causes weakening of *P. perfoliata* (Hough-Goldstein et al. 2008b). We hypothesize that resource competition via surrounding native plants may render *P. perfoliata* vulnerable to extensive *R. latipes* damage. This could initiate a positive feedback cycle, as native plants respond to continual release from MAM via weevil feeding. Ultimately, we hypothesize that this integration of weevil feeding and native seeding will transform an introduced species monoculture to a restored, biodiverse habitat that provides vital wildlife and ecosystem services.

Chapter 2

MATERIALS AND METHODS

2.1 *Field Experiment*

A field site with a mile-a-minute weed (MAM) infestation of ~118 m^2 was selected in 2008. It was present on Longwood Gardens (LWG) land, a public garden located in the piedmont in Kennett Square, Lancaster County, PA (39°52'48.44"N 75°40'07.12"W).

The LWG site was a long, narrow patch of MAM situated between a fence line and a steep bank adjacent to Route 926. The patch was approximately 2.5 meters wide and 59 meters long. Initially the predominant vegetation within the patch besides MAM was *Dennstaedtia punctilobula* (Michx.) T. Moore (eastern hayscented fern), various *Rubus* spp., *Microstegium vimineum* (Trin.) A. Camus (Japanese stiltgrass), *Lonicera japonica* Thunb. (Japanese honeysuckle), and *Phytolacca americana* L. (American pokeweed). An aerial photograph shows the patch within the surrounding landscape (Fig. 1).

Fig. 1 An aerial photograph of the Longwood Gardens field site.

2.1.1 *Field Experiment Site Preparation*

In spring 2009 the patch of MAM was divided into twenty 4 m^2 plots. Four treatments were replicated five times in a complete randomized block design, with PVC pipe used to mark corners of plots. *P. perfoliata* was the most abundant plant growing in every plot. A one-meter wide buffer between plots was maintained throughout the experiment by applying glyphosate (Roundup®, Monsanto Co., St. Louis, MO) at the beginning of the growing season and hand weeding throughout. Any *Rubus* spp. present in the experimental plots was spot treated with glyphosate in March 2009. MAM vines were restricted from growing into neighboring plots by weekly redirection back into their plot of origin. A systemic insecticide was used to exclude *R. latipes* from some plots. MAM weevils and a native seed mix were applied

in a fully factorial design. The four combinations (treatments) and their corresponding abbreviations were: weevils/no seed mix (Wnoseed), no weevils (treated with dinotefuron)/no seed mix (Dnoseed), weevils/seed mix (Wseed), and no weevils (treated with dinotefuron)/seed mix (Dseed).

With the diversity-invasibility hypothesis in mind, we chose to re-vegetate with a variety of native plants. Our five species (Table 1) represent five genera from two different plant families, varying in life histories, resource needs, rooting behaviors and statures (Burton et al. 2006). This mix includes widely adapted, hardy annual and perennial forbs, as well as warm and cool season perennial grasses. Only plant species native to the U.S. mid-Atlantic region were used in this study.

The mix was purchased from Ernst Conservation Seeds (Meadville, PA) who recommended a seeding rate of 25 lbs/acre, or ~2.81 g/m^2. This custom mix of five species was of local ecotype and was seeded at a rate of ~ 11.3 g/plot (4 m^2) in April 2009.

Table 1. Plant species and ratios used in the native seed mix.

Scientific Name **(Common Name)**	**Classification**	**% Seed Mix**
Elymus canadensis (Canada Wild Rye)	Cool Season Grass	25%
Andropogon gerardii (Big Bluestem)	Warm Season Grass	20%
Panicum virgatum (Switchgrass)	Warm Season Grass	15%
Heliopsis helianthoides (Ox Eye Sunflower)	Broadleaf Forb	20%
Rudbeckia hirta (Black Eyed Susan)	Broadleaf Forb	20%

Pine wood flakes were purchased by the bale (Southern States, Newark, DE) and used as a broadcast medium for the native seed mix. The shavings were mixed with the seeds and distributed to a depth of ~1 cm. A thatch of dead MAM vines from previous years' growth (~3-5 cm thick) was removed to apply the seed/shavings mixture and then replaced.

Because the growth of natives seeded in 2009 appeared to be minimal in early 2010, plugs of the same species (Table 1) were planted in the native seed-treated plots in spring 2010. The plugs were ordered from North Creek Nurseries (Landenberg, PA) and planted in the same ratios (Table 1) as the seeds in the earlier mix. The plugs were planted in a random pattern that was the same in all natives-treated plots. Plugs were marked with plastic in-ground labels to allow for ease of monitoring. Each week the plugs were located via the markers and assessed as dead or alive. As with the MAM plants, percent cover of the native plugs was also estimated each week.

In order to create 'no weevil' treatments a systemic insecticide was used. Dinotefuron is a neonicotinoid compound (Safari® 20 SG, Valent U.S.A. Corp., Walnut Creek, CA) that is highly water-soluble and fast acting. According to a pilot study (see Appendix A) it eliminated weevils more quickly than imidacloprid and did not cause a significant difference in plant biomass compared to untreated plants without weevils. It was applied as a soil drench at a rate of 51 g per 28.4 liters water (1.8 oz per 7.5 gal of water) three times in the 2009 season (15 May, 16 July, and 14 August) and two times in the 2010 season (28 April and 8 July). When dinotefuron was applied to plots to eliminate weevils during the 2010 season, the same amount of

water (~7.5 gal), without the chemical, was applied to the plots where weevils were present.

Weevils were initially released at the site in July 2008 and weevil feeding damage was assessed as high (extensive damage on most leaves) in October 2008, six months before the experiment began. Although clearly established by spring 2009, the *R. latipes* population at the field site appeared to be sparse so additional weevils were introduced upon emergence of MAM seedlings in May 2009 at the rate of 100 weevils/plot, totaling 1,000 weevils. The following spring in May 2010, weevils were again added to the plots, but releases were staggered (two releases, two weeks apart) and weevils were distributed among plots according to the percent cover of MAM weed. A total of 1,100 weevils were released in May 2010. In both 2009 and 2010 the weevils were reared and shipped overnight from the NJ Department of Agriculture Philip Alampi Beneficial Insects Laboratory (Trenton, NJ).

Additional summer releases of weevils were made. In August 2009, 340 weevils were collected from a separate location and added to the field study population at the rate of ~0.5 weevils/1% cover of MAM. In June 2010 a total of 1,100 weevils were distributed among the 'weevil treated' plots according to the percent cover of MAM present.

In 2010 supplemental watering occurred on two occasions (6 July and 9 September). Each plot received 38 L of water, except those that were about to be re-dosed with dinotefuron. Dinotefuron was applied as a drench of 38 L of water and dissolved insecticide.

Popillia japonica Newman (Japanese beetles) were present at the site and can sometimes feed heavily on MAM (Hough-Goldstein 2008a). In order to prevent

their feeding effects from confounding those of the weevils, Japanese beetle traps (Spectrum Brands, Alpharetta, GA) were hung ~92 m away on two sides from the experiment during July and August each year.

2.1.2 *Field Experiment Data Collection*

MAM seedlings in each plot were counted shortly after germination each spring (15 -21 May 2009 and 10 -17 April 2010) as soon as clear identification was possible.

Data for all plots were collected within a single day, at weekly intervals, and included: number of adult *R. latipes*, weevil foliar feeding damage, percent cover MAM, number of immature and mature seed clusters, and native plug percent cover and mortality. Native seeded plant cover was not measured. During 2010 monthly plant species richness surveys were conducted and at the conclusion of the 2010 season a final destructive harvest occurred.

Adult weevils were counted via a thorough visual search of all *P. perfoliata* within a plot, using a tally clicker. The same investigator measured this variable both years because search skill and technique differs between observers.

Weevil feeding damage was rated on a scale of 1-4 in 2009: (1) = no damage, (2) = holes in a few scattered leaves, (3) = holes in many leaves, (4) = extensive damage on most leaves. In 2010 the scale was expanded to 1-6 to allow for more descriptive and statistical power, as follows: (1) = no damage, (2) = holes in a

few scattered leaves, (3) = holes in half the leaves, (4) = holes in many leaves, (5) = holes in most leaves, (6) = extensive damage on most leaves.

MAM seed clusters were counted during the entire season using a tally clicker, with a minimum of three seeds defining a cluster, and immature and mature seed clusters tallied separately. An immature cluster was defined as one with at least one fully formed immature seed present, and mature clusters as those with at least one blue seed present.

The percent cover of MAM was estimated visually for each plot, and always by the same observer. The remaining plot area consisted of various plants, bare ground, or detritus.

In 2010 monthly plant surveys were conducted to determine species biodiversity in each plot. The United States Department of Agriculture (http://plants.usda.gov/java/) plant database was used for species common names and assignment of nativity. Between 11and 18 Oct 2010 all MAM was harvested by cutting the vines at soil level. This vegetation was placed in large brown paper bags in a drying oven at 95 - 100°C for 11 days, and then weighed.

2.1.3 *Field Experiment Statistical Analyses*

For all experiments, data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance residuals using Levene's test. Where necessary, data were either log or square root transformed to improve homogeneity or normality.

The arcsine-square root transformation was applied to percentages where at least some were more than 70% or less than 30% (Snedecor and Cochran, 1980). If transformed data failed to meet assumptions of normality, then data were ranked and statistical tests were applied to the ranks. Non-transformed data are shown in figures and tables. The 2009 and 2010 *P. perfoliata* seedlings counted at germination were compared using a 2-way analysis of variance (ANOVA) followed by a Tukey's test for mean separation.

Data collected weekly were compared by repeated measures ANOVA, using the REPEATED statement in PROC GLM of the SAS system (Littell et al. 2002). This analysis was applied to data collected during the peak of the season for each variable, when treatment effects were most likely to be observed (e.g. data collected for MAM cover was tested over August and September dates, when cover was at its highest before plant senescence). Data for different variables could not be tested over identical time frames due to differences in phenology (i.e. time of peak seed production and peak percent cover are different). Data for the same variable were tested over roughly the same time frame each year.

For immature seed cluster data the dates tested were late in the season, 28 August - 29 September (2009) and 17 August - 23 September (2010), during the peak of seed production. Percent cover of MAM was compared for the time periods of 3 August - 10 September (2009) and 4 August - 9 September (2010), before characteristic plant senescence.

Plant species richness and destructively harvested MAM biomass were analyzed using two-way ANOVAs followed by Tukey's test for mean separation, to compare the four treatments independently. They were also analyzed with an ANOVA for a two-way factorial experiment where factors (or main effects) were weevil vs. no-weevil and seed vs. no-seed (Littell et al. 2002). For some tests, effects of each factor were determined using the SLICE option in the LSMEANS statement of SAS to obtain *F*-tests for simple effects (Littell et al. 2002). Either the main effects (differences within weevil treatment or within seed treatment), or the simple effects (e.g. differences of weevil treatment within seed treatment) are reported for each test, but never both (Littell et al. 2002).

2.2 *Greenhouse Experiments*

For the greenhouse experiments, plants were grown in heavy-duty flats (Kardon Corp., now owned by Buckhorn Inc., http://www.buckhorninc.com/contact/), 35.6 cm wide x 50.8 cm long x 15.3 cm deep (0.18 m^2 surface area). Pro-Mix (Premier Horticultural Inc., Quakertown PA) was used as the growth medium.

2.2.1 *Greenhouse Main Experiment Preparation*

The MAM seed used in the greenhouse experiment was collected in fall 2008 and again in fall 2009 in sufficient quantities to replicate seed density found in

the field. The seed was collected from multiple sites, mixed together, dried for 6 weeks, and refrigerated for 4 weeks before distribution into flats. MAM seed was distributed into the flats at a rate of 300 seeds/m^2, since 300-500 stems/m^2 have been found in severe MAM infestations (Hough-Goldstein et al. 2008a; Hyatt & Araki 2006). In 2008, 1,080 seeds were used (0.18 m^2 /flat x 20 flats = 3.6 m^2), or 54 MAM seeds/flat. Seeds were dropped onto the soil surface of each flat and then sprinkled with additional pro-mix. Flats were placed outdoors to overwinter next to the UD greenhouse in December of 2008 and 2009.

While the same procedure was applied in year two, several alterations were made due to poor MAM germination in year one. The seeding rate was increased to 75/flat, totaling 1,500 seeds. Also the flats were completely encased in a fine mesh screen to prevent contamination by debris including unwanted seeds during the overwintering process. The mesh also prevented seed predation by rodents. While the flats were outside they were watered to prevent seed desiccation and rotated monthly to avoid bench effects. Immediately after germination the flats were brought into the greenhouse, which occurred in early May in 2009 and early April in 2010.

Where necessary, MAM seedlings were culled (in 2009 some were transplanted) to adjust to seven seedlings per flat. Flats were arranged in blocks so that one of the four treatments was in a randomly selected position within each block. Treatments were replicated five times.

The same native seed mix composition (Table 1) as used in the field experiments was planted into the native-seed treated flats as soon as possible after MAM germinated. The total amount of native seed mix needed for the 10 flats was ~5.06 g [(10 flats x 0.18 m^2 = 1.8 m^2) x 2.81 g/m^2 ~ 5.06 g]. This is ~0.5 g/flat, when

seeded at the same rate as in the field. Flats were watered and lightly fertilized (Peters 21-5-20 fertilizer at a rate of 200 ppm nitrogen) weekly.

As soon as possible after native seeding, weevils were introduced to weevil-treated flats while dinotefuron was applied to no-weevil flats. Dinotefuron was reapplied in July, 2009. In 2009 five weevils were released on each flat within 2 days of native seeding. This density (a rate of ~25 weevils/m^2 of MAM weed) has been found capable of suppressing MAM (Hough-Goldstein 2008b). The rate of 5 weevils per weevil-treated flat resulted in a total of 50 weevils initially released in the greenhouse. Large thermoregulation fans present in the greenhouse walls apparently caused weevils to be sucked out periodically during the 2009 season. Due to this unplanned weevil removal, supplemental weevils were added weekly to ensure that at least 5 weevils were present on the each weevil-treated flat throughout the experiment.

Despite this attempt to compensate for the fans, weevils had uncharacteristically low numbers, feeding damage, and overall impact in 2009. Because of this, the weevil number was increased to 8/flat in 2010 and weevils were restricted to flats via use of white mesh cages measuring 61x61x61 cm (bug dorms, BioQuip, Rancho Dominguez, CA) instead of using dinotefuron. To equalize cage effects, every flat was covered with an individual cage to exclude or include weevils, depending on the treatment requirements. In 2010, dinotefuron was not needed to eliminate weevils due to use of cages so treatment nomenclature was changed from a "D" designating no-weevils to "No Weevil" for that year and experiment alone.

Greenhouse pests infested the experimental flats on several occasions. Early in 2009 aphids became problematic on flats not treated with dinotefuron. Ladybugs collected from the field were released on these flats and brought the aphids

under control. At the end of both the 2009 and 2010 seasons, mealy bugs infested senescing plant material and so the destructive harvest occurred immediately. In spring 2010, slugs infested the *R. hirta* plants so beer in dishes was used to control their population. Later in 2010 white flies were controlled by use of yellow sticky cards.

2.2.2 *Greenhouse Allelopathy Experiment*

In year two (2010) an additional treatment was added to the greenhouse experiment to investigate possible allelopathic effects of MAM weed. When native seeds were planted into flats with germinating MAM in early spring 2010, each block also included a flat growing just the native seed mix. This was to allow a comparison of native plant biomass when grown with and without MAM. The flats with MAM were overwintered to cold-stratify the seeds. The flats that had only native seeds did not overwinter because those seeds did not require stratification to germinate. The natives-only treatment was rotated with the other four treatments in each block and then harvested at the end of the season, dried, and weighed for comparison.

2.2.3 *Greenhouse Disturbance Experiment*

One further experiment was conducted in the greenhouse to determine how disturbance might influence the establishment of MAM weed. There were two

treatments in this experiment, and the same native seed mix and application rate was used as was in the previous experiments. In 2009 the seed mix was distributed into ten flats of pro-mix and was watered and fertilized weekly, allowing the plants to establish throughout the season without additional manipulation. In late fall 2009, 75 MAM seeds were dropped into each one of these established-natives flats. Seventy-five MAM seeds were also placed into each of ten additional flats of plain pro-mix (the same flats used for the main greenhouse experiment). All twenty flats were then placed outside in fall 2009 to overwinter (see section 2.2.1). When the MAM began to germinate in early spring 2010 the twenty flats were brought inside. Of these twenty flats, ten were exposed to weevils (five from each treatment). Weekly supplements of weevils kept the populations of weevil-treated flats to a minimum of eight. Cages were kept on each flat to control weevil movement.

2.2.4 *Greenhouse Experiments Data Collection*

As in the field experiments, data variables for all plots were collected within a single day, at weekly intervals. Variables were the same as in the field except that no native plugs were planted in the greenhouse and MAM seedling survival was monitored in the disturbance experiment only. Variables were measured using the same protocol as described in section 2.1.2. The 2010 expanded feeding damage rating scale was used in year two of the greenhouse experiment. A destructive harvest of all species grown in the greenhouse experiment occurred 14 September 2009 and 24 September 2010.

For the disturbance experiment, the number of MAM seedlings that germinated in each flat was counted and seedlings were not moved or manipulated as was done in the other treatments. In the disturbance experiment MAM seedlings were a dependent (i.e. response) variable of interest, whereas in the rest of the treatments they were an independent variable.

At the end of each growing season, greenhouse flats were destructively harvested, air dried, and weighed for biomass, with MAM, native grasses, and native forbs weighed separately. Harvests occurred on 14 September, 2009 and 24 September, 2010.

The allelopathy experiment only ran one year and hence was harvested once (2010), and the disturbance experiment did not need to be harvested because all MAM died before the end of the season. When the flats were harvested native biomass was divided into grasses and forbs to compare which grew better.

2.2.5 *Greenhouse Experiments Statistical Analyses*

For all greenhouse experiments, data were analyzed as described in 2.1.3. For weekly data tested using repeated measures in SAS, immature seed clusters were compared for 11 August - 14 September (2009) and 16 August - 21 September (2010). The percent cover of MAM dates tested were 22 June - 28 Jul (2009) and 21 June – 27 July (2010).

Destructively harvested MAM and native biomass were also analyzed using the methods in 2.1.3. Native biomass was analyzed using a two-way ANOVA to

compare total grasses and total forbs for both 2009 and 2010. Total native biomass was also analyzed by treatment in 2009 to determine if the dinotefuron used that year had a stimulatory effect on native plant growth.

A two-way ANOVA was used to compare seedling germination numbers between the two treatments in the Disturbance Experiment.

Chapter 3

RESULTS

3.1 *Field Experiment*

The number of MAM seedlings in spring did not significantly differ among treatments when tested for either 2009 ($F_{3,12} = 3.24$, $P = 0.0603$) or 2010 ($F_{3,12} = 2.33$, $P = 0.1265$; Table 2). *P. perfoliata* seedling numbers were more than ten-fold greater in 2010 than 2009.

Table 2. Number of MAM seedlings per 2m by 2m plot (mean ±SEM), field experiment.

	Mean ± SEM	
Treatment	**2009**	**2010**
Wseed	107.4 ± 60.7	1693.0 ± 802.5
Dseed	272.4 ± 128.2	3041.6 ± 1027.1
Dnoseed	278.0 ±172.5	3375.8 ± 1241.5
Wnoseed	439.4 ± 109.5	4016.6 ± 928.2

The number of adult weevils did not increase greatly throughout 2009. In fact, the average number of weevils (combined Wseed and Wnoseed treatments) per plot averaged across the season was ~52, with minimal population growth over time.

In 2010 numbers grew steadily, and mean number of weevils for a given plot over the entire season was ~80. There was a steep increase in population at the end of the season. Weevils were rarely present in the dinotefuron-treated plots. On a few occasions individuals were found on these plots, usually displaying signs of insecticide exposure. However, if they were found and continued behaving normally after a 30 min observation period, dinotefuron was re-applied soon after.

Foliar feeding damage followed a similar trend over 2009 and 2010. In 2009 there was little difference in damage levels between beginning and end. Mean rated damage throughout the season was a 2.83 (on a 1-4 scale), while the following year it was 3.65 (on a 1-6 scale). Mirroring weevil abundance, in 2010 there was a marked increase in feeding damage as the season progressed, and the *P. perfoliata* plants displayed clear signs of stress.

3.1.1 *Field Experiment MAM Weed Response*

The difference in percentage cover of *P. perfoliata* was marginally significant in a repeated measures ANOVA for 3 August – 10 September, 2009 ($F_{3,16}$ = 2.47, P = 0.0991; Fig. 2A), and was significant over 4 August – 9 September, 2010 ($F_{3,16}$ = 6.73, P = 0.0038; Fig. 2B). For both years the Wseed treatment had the lowest mean value for percent cover.

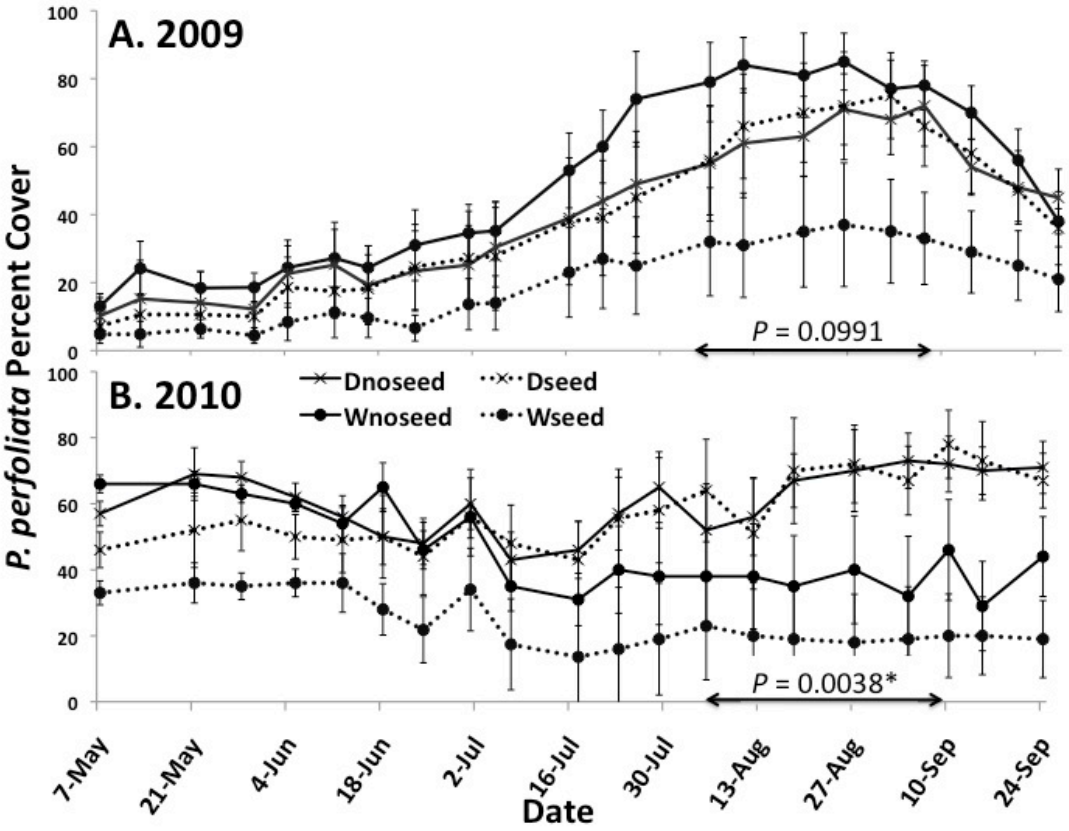

Fig. 2 Percent cover of *P. perfoliata* in (A) 2009 and (B) 2010, field experiment.

The number of immature *P. perfoliata* seed clusters was not significantly different for 28 August – 29 September, 2009 ($F_{3,16} = 2.26$, $P = 0.1204$; Fig. 3A), but was different by treatment for 17 August – 23 September, 2010 ($F_{3,16} = 12.45$, $P = 0.0002$; Fig. 3B). Both years the Wseed treatment produced the fewest seed clusters.

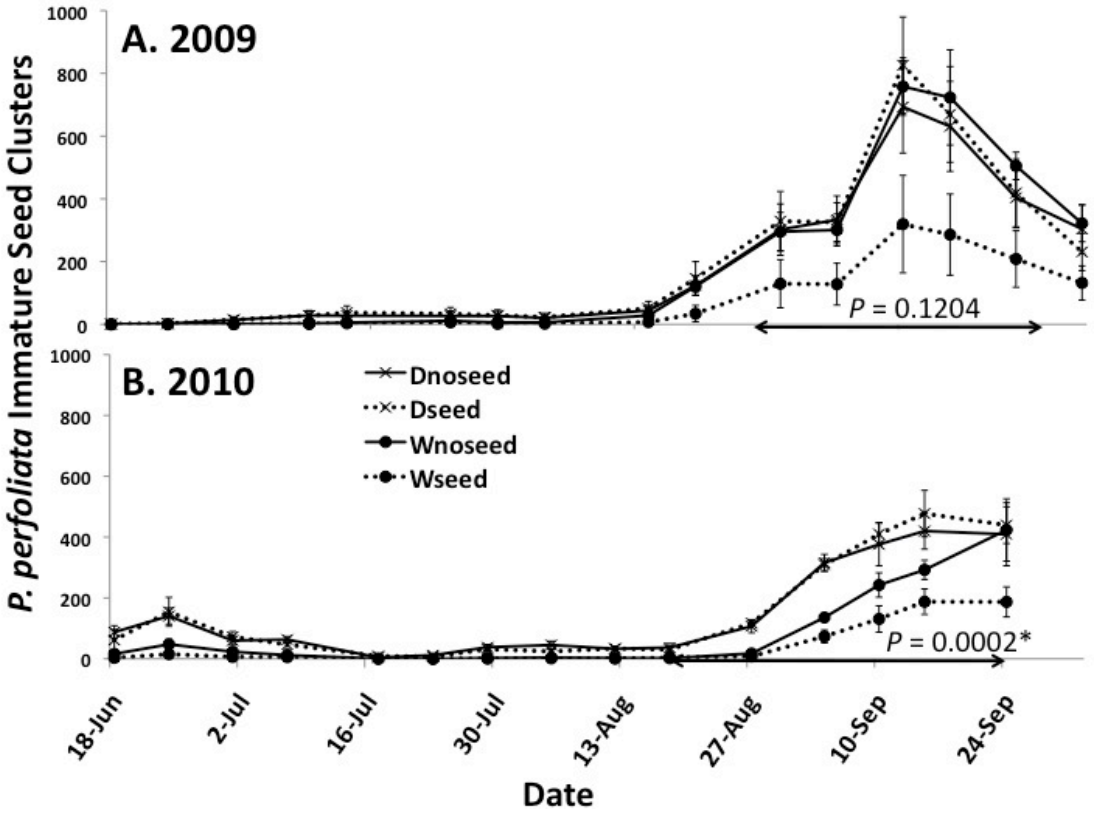

Fig. 3 Number of immature seed clusters in (A) 2009 and (B) 2010, field experiment.

MAM biomass for the Wseed treatment was significantly lower than either dinotefuron treatment ($F_{3, 12} = 7.74$, $P = 0.0039$; Fig. 4). The dinotefuron-treated plots had four to five times as much MAM biomass as the Wseed plots (Fig. 4).

The factorial ANOVA followed by the slice procedure indicated significant effects of the seed mix treatment in plots with weevils ($F_{1,16} = 12.67$, $P = 0.0026$) but not if weevils were not present ($F_{1,16} = 2.44$, $P = 0.1378$). This means the biomass of MAM was lowered due to the native seed mix, but only when the seeds were in the presence of weevils.

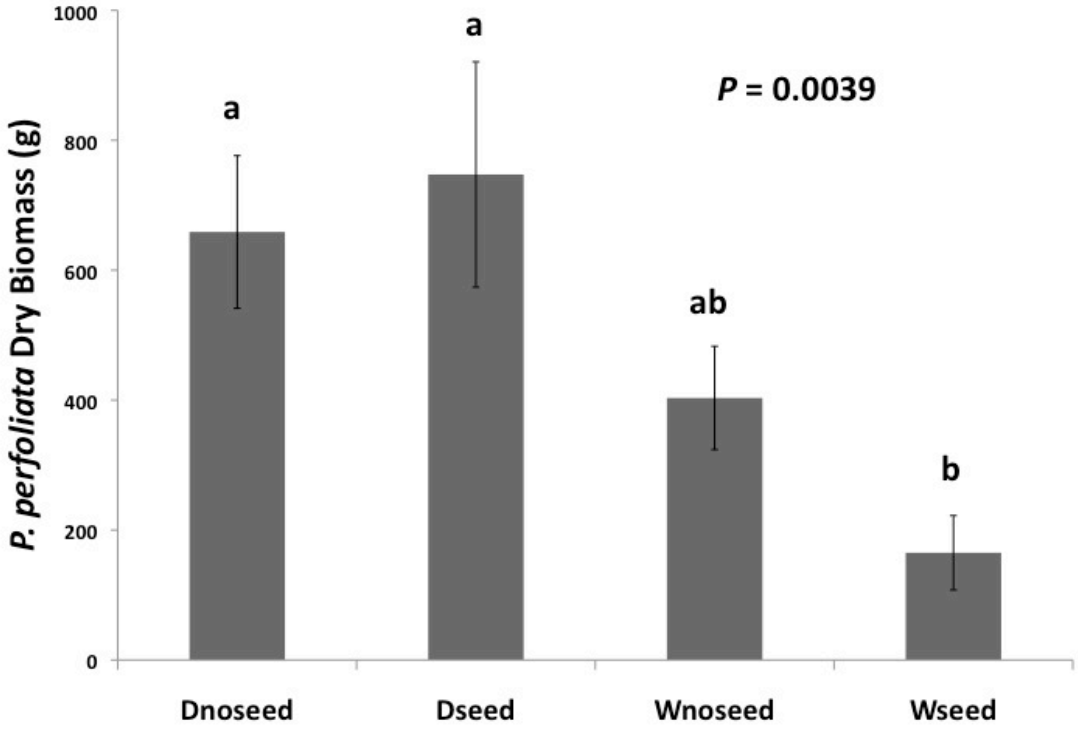

Fig. 4 Destructive harvest of *P. perfoliata* biomass, field experiment.

3.1.2 *Field Experiment Plant Community Response*

Both percent cover of and surviving numbers of native plugs planted in spring 2010 dwindled over the season. Of the 25 plugs planted in each plot, a mean of 21 survived in the Wseed treatment and only 17 in the Dseed treatment. Percent cover change over time was negligible, as both treatments started with a 5% cover measure and ended with a mean of 6% (Wseed) and 4% (Dseed). By mid-season 2010 it

became clear that the native seed mix distributed in 2009 was growing despite earlier observations to the contrary. Plant species richness surveys from 2010 showed Wseed plots consistently hosting the most species throughout the season, and the difference among treatments was significant in May ($F_{3,12} = 7.00$, $P = 0.0056$, Fig. 5) and July ($F_{3,12} = 4.72$, $P = 0.0212$; Fig. 5). In both months, factorial ANOVAs indicated the seed treatment main effect was significant (May: $F_{1,16} = 4.72$, $P = 0.0065$, July: $F_{1,16} = 5.81$, $P = 0.0284$) but the weevil treatment main effect was not (May: $F_{1,16} = 0.03$, $P = 0.8642$, July: $F_{1,16} = 0.90$, $P = 0.3565$).

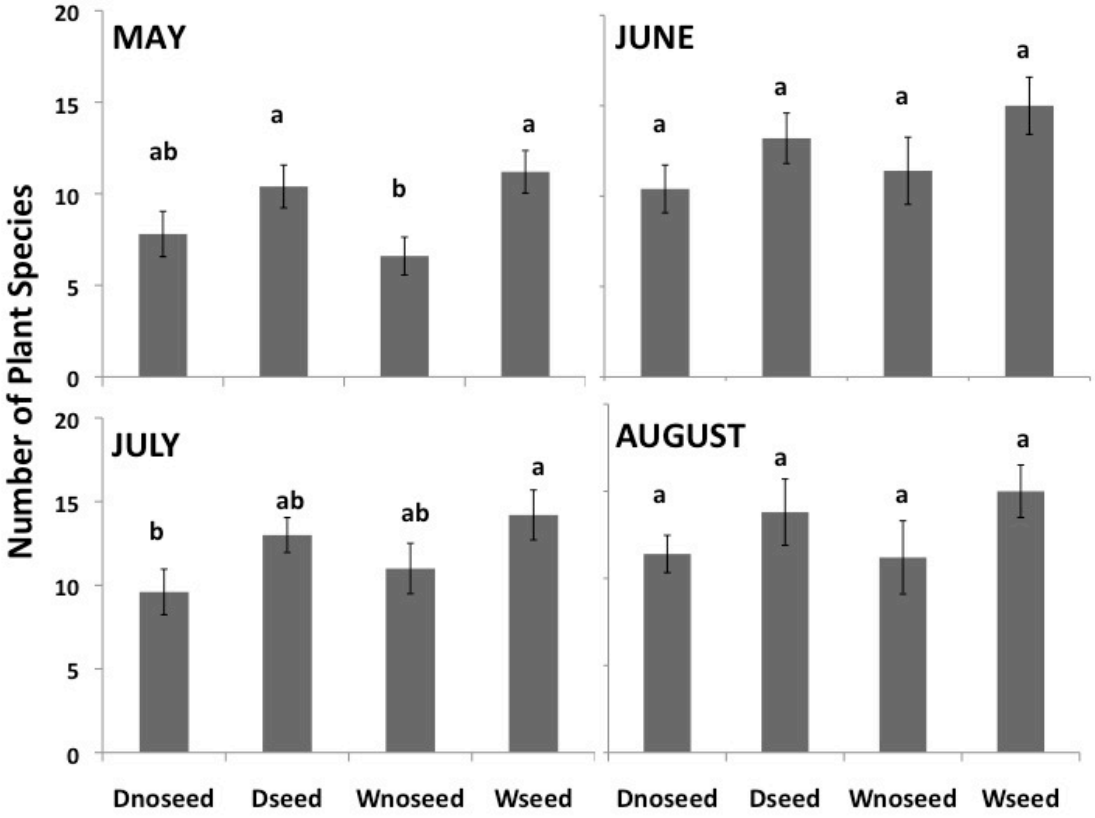

Fig. 5 Species richness in 2010, field experiment. Letters indicate significant differences within each month (two-way ANOVA, Tukey).

When analyzed by plant nativity, the Wseed treatment had significantly higher numbers of native species than the non-seed treatments every month (Fig. 6). Factorial ANOVAs showed this was again the effect of the seed treatment (May: $F_{1,16} = 30.39$, $P < 0.0001$, June: $F_{1,16} = 19.64$, $P = 0.0004$, July: $F_{1,16} = 33.56$, $P < 0.0001$, August: $F_{1,16} = 15.01$, $P = 0.0013$).

The Wseed treatment had the lowest average number of introduced species all season, significantly lower than the Wnoseed treatment in July ($F_{3,12} =$

4.80, $P = 0.0202$, Fig. 6). The factorial ANOVA for July revealed it was that effect of the seed treatment causing the difference ($F_{1,16} = 7.41$, $P = 0.0151$). The treatments without native seeding always had the lowest number of native species and highest number of introduced species (Fig.6).

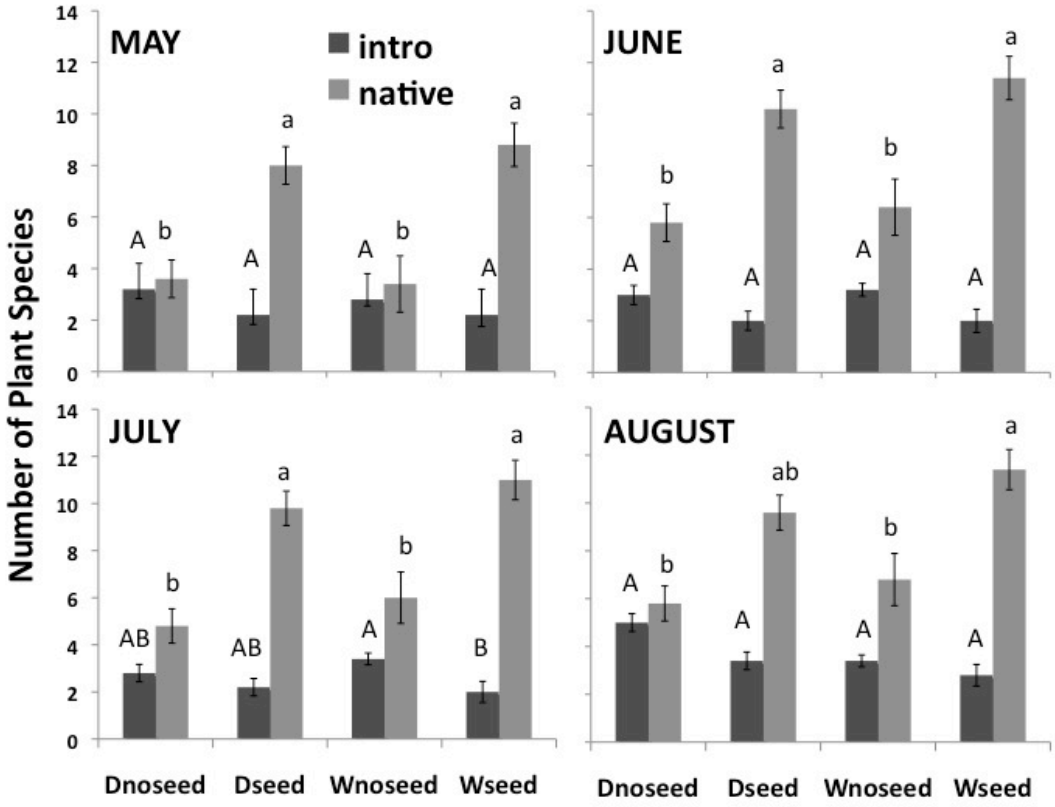

Fig. 6 Mean numbers of native and introduced plants in 2010, field experiment. Letters indicate significant differences within each month and within native (lowercase) and introduced (uppercase) species (two-way ANOVA, Tukey)

3.2 *Greenhouse Experiments*

3.2.1 *Greenhouse Main Experiment Results*

Number of adult *R. latipes* in the greenhouse did not increase much over the 2009 season, probably due to the large thermoregulation fans sucking weevils out of the greenhouse, as noted earlier. The highest number recorded on a flat was ten, and the average weevil number per flat over the season (combined weevil treatments) was only 3.7 (20.5 weevils/m^2). This is about half of that found in the field experiment, which was 39 weevils/m^2 over the season. When the cages were used in 2010 the weevil populations were able to grow more successfully, peaking at 44 on a single flat on one occasion. The mean number of *R. latipes* for the entire 2010 season (combined weevil treatments) was 12.5 (69.5 weevils/m^2), more than 3 times higher than in 2009.

Feeding damage was almost non-existent on no-weevil treatments due to use of dinotefuron and cages. Mean rated damage for weevil-treated flats throughout the 2009 season was 1.72 (on a 1-4 scale), while the following year it was 3.29 (on a 1-6 scale).

A repeated measures ANOVA showed the percentage cover of *P. perfoliata* was significantly different among treatments for 22 June – 28 July, 2009 ($F_{3,16} = 34.02$, $P < 0.0001$, Fig. 7A), but not for 21 June – 27 July, 2010 ($F_{3,16} = 1.54$, $P = 0.2424$, Fig. 7B).

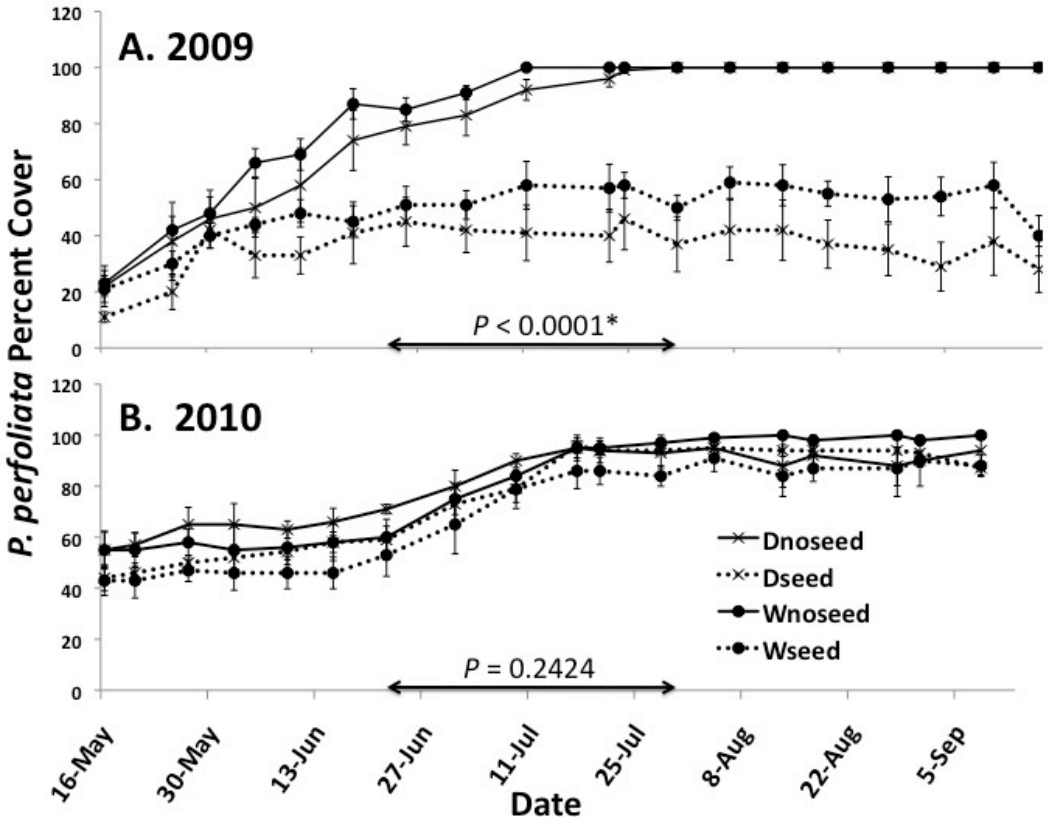

Fig. 7 Percent cover *P. perfoliata* in (A) 2009 and B (2010), University of Delaware greenhouse.

The number of immature *P. perfoliata* seed clusters was significantly different among treatments in the repeated measures ANOVA for 11 August – 14 September, 2009 ($F_{3,16} = 43.15$, $P < 0.0001$, Fig. 8A) and 16 August – 21 September, 2010 ($F_{3,16} = 9.14$, $P = 0.0009$, Fig. 8B). Mature seed clusters were also significantly different both years, 4 August – 8 September, 2009 ($F_{3,16} = 21.82$, $P < 0.0001$) and 16 August – 21 September, 2010 ($F_{3,16} = 13.32$, $P = 0.0001$).

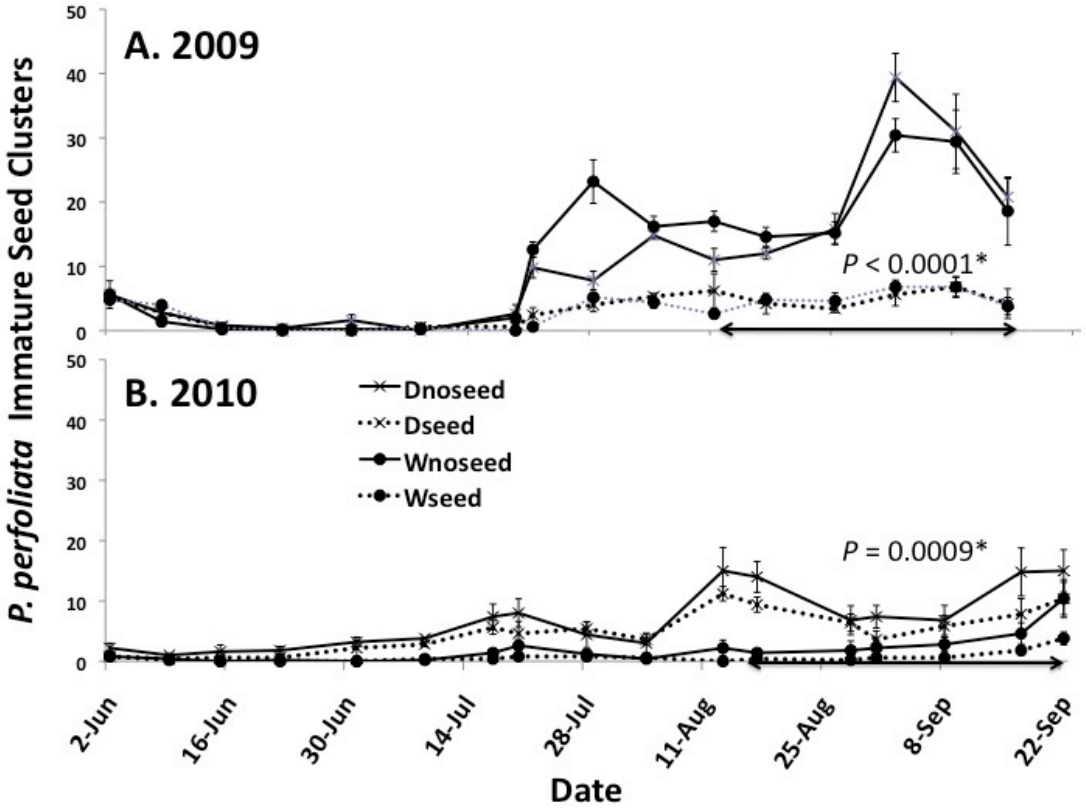

Fig. 8 Number of seed clusters in (A) 2009 and (B) 2010, University of Delaware greenhouse.

The destructive harvests for each year contrasted strongly. In 2009 a two-way ANOVA showed there was a significant difference in MAM biomass among treatments ($F_{3,12} = 5.47$, $P = 0.0133$, Fig. 9A). The Dseed and Wseed treatments both weighed least (both under 50 g) while the other two treatments much higher (both over 175 g). Following a factorial ANOVA, the slice procedure in LSMeans for 2009 indicated significant effects of the native seed mix treatment in weevil plots ($F_{1,16} = 5.25$, $P = 0.0358$) and no-weevil plots ($F_{1,16} = 10.22$, $P = 0.0056$). In 2010 a two-way ANOVA of biomass produced marginally significant results ($F_{3,12} = 3.20$, $P = 0.0620$, Fig. 9B). A factorial ANOVA followed by the slice procedure found a significant seed

effect in this data only when weevils were present ($F_{1,16} = 6.98$, $P = 0.0177$); thus in 2010 the native seed mix only reduced MAM biomass when in the presence of weevils.

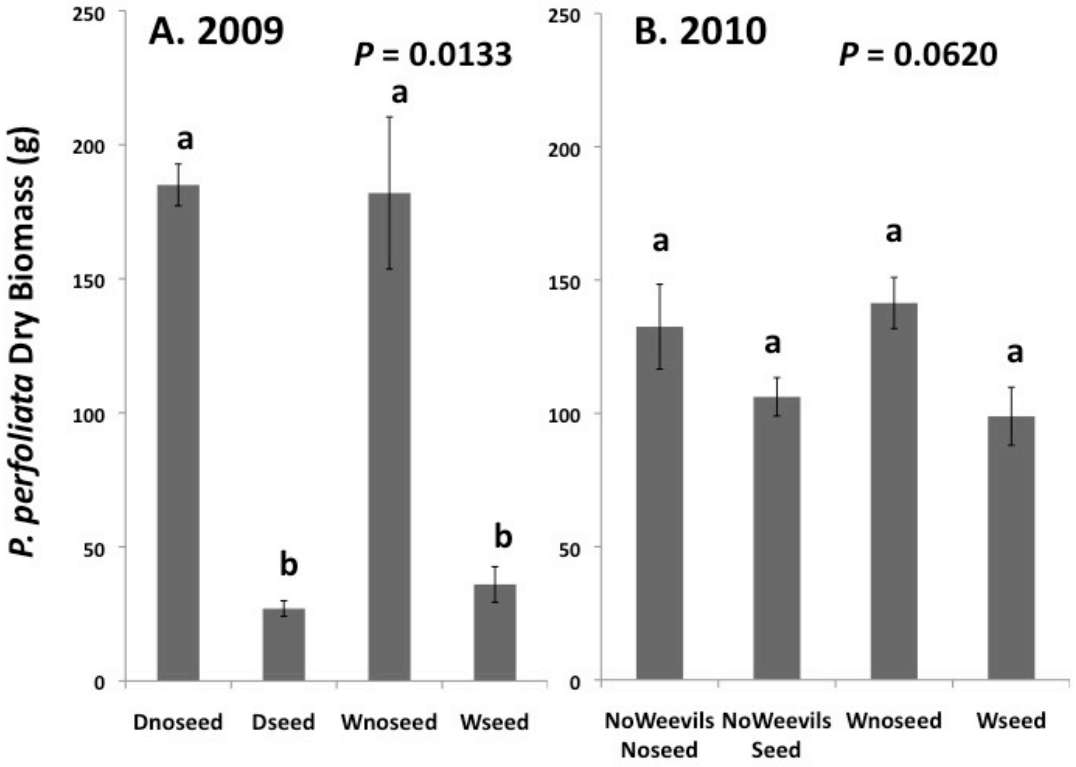

Fig. 9 *P. perfoliata* biomass in (A) 2009 and (B) 2010, University of Delaware greenhouse. Letters indicate significant differences (two-way ANOVA, Tukey).

When native biomass was compared via two-way ANOVA, the grasses had significantly greater mass only in 2009 ($F_{1,4} = 80.21$, $P = 0.0009$). In 2010 the grasses once again had greater biomass, but not significantly so ($F_{1,4} = 4.93$, $P =$

0.0906). Total native biomass compared between Dseed and Wseed treatments was not significantly different ($F_{1,4} = 0.10$, $P = 0.7712$).

3.2.2 *Greenhouse Allelopathy Experiment Results*

A two-way ANOVA revealed that natives grown alone had a significantly greater biomass than those growing with MAM ($F_{1,4} = 35.71$, $P = 0.0039$, Fig. 10). Their average mass was over twenty times greater than native plants grown with MAM.

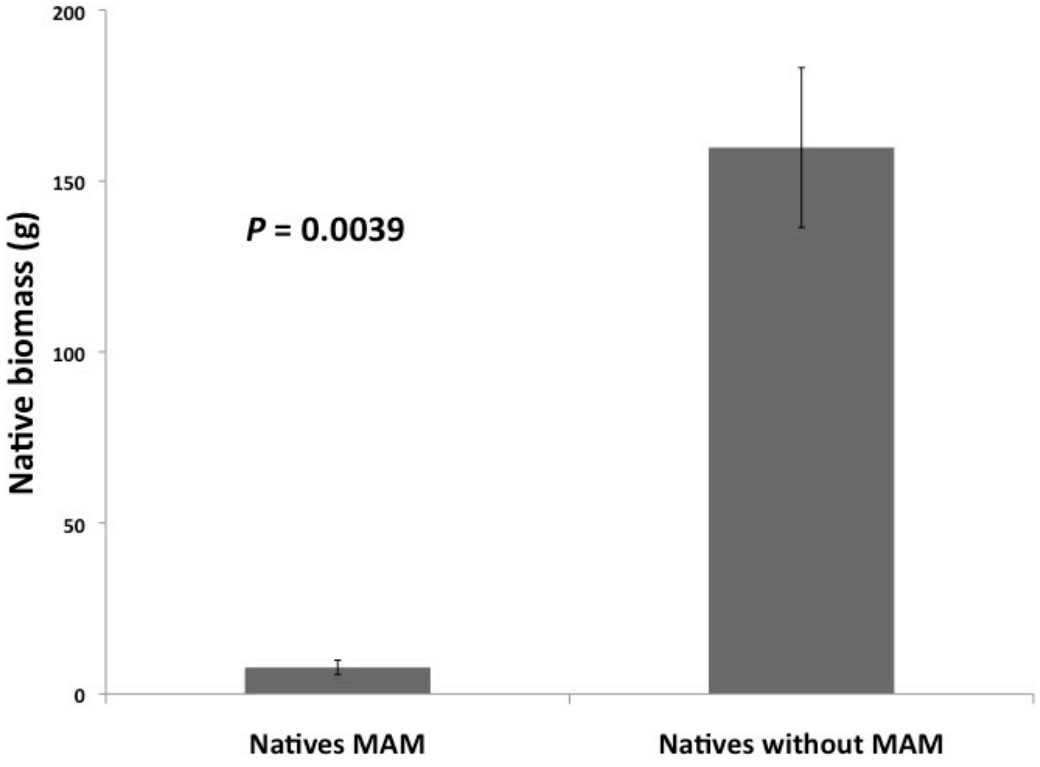

Fig. 10 Native plant biomass grown with and without MAM weed.

3.2.3 *Greenhouse Disturbance Experiment Results*

Undisturbed treatments containing established native plants had a significantly lower MAM germination rate than disturbed ones ($F_{1,24} = 19.47$, $P = 0.0002$). The undisturbed flats with established native plants had a mean MAM germination rate of 6.5 seedlings, compared to almost three times as many (18.7) in the disturbed flats. The percent cover MAM in disturbed flats reached and maintained 100%, while percent cover of MAM never went above 10% in the undisturbed treatments. By five weeks into the experiment, all MAM in the undisturbed treatments had died.

Chapter 4

DISCUSSION

4.1 *Field Experiment*

Results from the field experiment showed some interesting variations from year to year. The large increase in *P. perfoliata* seedlings from year one to year two is of note (Table 2). It is plausible that planting plugs in April 2010 disturbed the MAM seed bank. If this were the case though, there should be greater seedling emergence in plug-treated plots only, which is not so (Table 2). Instead, we attribute such high germination to the unusually warm, early spring of 2010. Germination is temperature-sensitive in respect to stratification (Hough-Goldstein et al. 2008a), so it would not be surprising if temperatures determined the proportion of the seed bank to germinate.

In 2009 coarse wood shavings were mixed with the native seed mix and distributed on seed-treated plots. While a seeding/growth medium was needed for optimal seed distribution and establishment (Dr. S. Barton, University of Delaware, personal comm. 2008), it has been noted that woody detritus can cause carbon enrichment of the soil that boosts soil microbe nitrogen uptake and growth (Redente et

al. 1992; Hunt et al. 1988). This can result in reduction of available inorganic nitrogen, ultimately limiting some plant growth (Spielberger et al. 2009), especially annual seral species (Morghan & Seastedt 1999) such as MAM. It has been suggested that such species' nutrient cycling is rapid and soil dynamics such as this should be considered in restoration attempts (Kulmatiski et al. 2006). In retrospect, this experiment would have been improved if a finer sawdust medium was used, and on all plots, in order to control for any effects it may have had. As it is, we assume, based on other experiments (Morghan & Seasdtedt 1999; Spielberger et al. 2009) that the comparably small, one-time addition of wood shavings over two years made no major difference in soil nutrient cycles or the results of our experiment.

Weevils were supplemented in early spring each year because immediate heavy feeding pressure on seedlings results in altered seed phenology later on, expressed as lowered seed production and quality (Hough-Goldstein et al. 2008b). A spring release also allowed the supplemented weevils to reproduce to their full capacity of three to four generations over the full season (Lake et al. 2011, in press).

Herbivore population growth, and consequently, foliar feeding damage, most likely differed by year due to weather conditions, as has been found in other studies (Bacher & Schwab 2000). Spring 2009 was unusually cool and rainy, limiting weevil metabolism and hence reproduction capacity. In contrast, the 2010 season brought early heat and later, severe drought, which appeared to favor weevil population growth. Increased insect population growth and feeding pressure in warm temperatures is supported by findings of Bacher & Schwab (2000) in augmentative

biocontrol work done with *Cassida rubiginosa* Müller (Coleoptera, Chrysomelidae] (shield beetles). They found that this biocontrol agent had a significant impact on its host, creeping thistle (Cirsium arvense [L.] Scop. [Asteraceae]), also known as Canada thistle, but only in the hotter season, when insect metabolism was fastest. In addtition to heat, *P. perfoliata* faced water shortages in 2010, combined with strong herbivory pressure, which appeared to tax the extent of plant tolerance. This supported our prediction and was consistent with the conclusions of Sun et al. (2010) after observing lowered herbivore tolerance under water stress in *Alternanthera sessilis* (L.) R. Br. ex DC. (sessile joyweed). Supplemental watering was applied to aid plant survival in 2010 only. The newly planted native plugs also struggled for survival that year, but the more established seeded natives from 2009 showed good survival and a competitive advantage. In the case of the seeded grasses this was probably due to established fibrous root systems.

A dinotefuron drench was our selected means of insect control because of its systemic nature and long-lived efficacy (two to three months). Dinotefuron ensured minimal feeding on treated plants. Any stimulatory effects of this insecticide on plant growth are unlikely, as two separate greenhouse experiments using dinotefuron in a similar manner failed to show any impacts on growth of MAM or other plants (see end of section 4.2.2 and Appendix A). If dinotefuron had an effect on plant growth it would most likely be reflected in plant biomass. However, neither the MAM nor the total native biomass comparisons between dinotefuron and non-dinotefuron treatments

in the greenhouse indicated that there was stimulatory growth effect of the insecticide since there was no significant difference in biomass.

As the field season progressed it became evident that the insecticide used to exclude weevils from the no-weevil plots may have been causing a population sink. We concluded this because dead and dying weevils were found on dinotefuron-treated MAM each visit. This was not surprising because these weevils are fairly mobile (Lake et al. 2011, in press) and probably moved among plots to feed, including herbicide-treated plots. To counteract the population sink, the 2010 summer supplement of weevils occurred earlier than in 2009 and with more weevils.

In those plots where dinotefuron was applied all plants were affected, hence becoming invulnerable to feeding by all insect species. Plants other than MAM were given the opportunity to re-allocate resources away from herbivory defense and instead to growth and reproduction. This enemy release may have caused MAM's plant neighbors to do better than they otherwise would have, competing unusually well with the MAM. Even so, results show a highly significant greater MAM biomass in plots free of weevils (Fig. 6). This suggests that *P. perfoliata* may be a vigorous competitor even when the whole community lacks top-down regulation by insects.

Plants free of dinotefuron were vulnerable to insect feeding of many types. Japanese beetles have been noted as sole primary herbivores on MAM since its introduction (Moul 1948), but another study suggests that other species of generalist herbivores have been known to feed on MAM (Wheeler & Mengel 1984), though it is unlikely they cause strong effects on growth or spread (Mountain 1989). Indeed, it

appears that MAM is not capable of supporting native generalist Lepidoptera to much extent when compared with their native hosts (Burghardt et al. 2010). As a result of these considerations, we were concerned only about Japanese beetles and hung the traps to reduce any source of herbivory on MAM weed besides that of *R. latipes*.

4.1.1 *Field Experiment MAM Weed Response*

Results indicate that the integration of weevils and native seeding successfully reduced both percent cover (Fig. 2) and immature seed production (Fig. 3) of MAM weed significantly by two years post-treatment. Although mature seed clusters were counted weekly, those data were not analyzed due to heavy deer browse (Hough-Goldstein et al. 2008a; K. Cutting, personal observation, 2009). Birds and rodents are also known as MAM seed dispersers (Mountain 1989; personal observation, K. Fryberger), suggesting that mature seed counts may be a poor metric of *P. perfoliata* reproduction.

The destructive harvest at Longwood supported the percent cover and seed count data. Dry biomass of MAM from the Wseed treatment was significantly lower than the plots that had no weevils (Fig. 4). When comparing between seeded treatments we see that MAM from the Wseed treatment had half the weight of the Wnoseed treatment (Fig. 4). The slice procedure showed that MAM was reduced in plots with seed mix only when weevils were present. We conclude from this that

weevils are required to suppress the weed, hence opening a niche (Elton 1958), and exposing the plant to normal competition from neighbors. This in turn could maximize species richness, returning the community to a new stable state, but of a much higher caliber than the original state (Hacker & Dethier 2009; Perry & Galatowitsch 2003).

4.1.2 *Field Experiment Plant Community Response*

Monthly plant species richness surveys allowed for a temporal perspective on the community through the summer of 2010, a year after the various treatments were applied. The Wseed treatment consistently hosted the greatest numbers of species and it is interesting to note that once again, the seed treatment was the main effect causing significance (Fig. 5). This result is not unlike that of Perry & Galatowitsch (2003) who found that a mix of four perennial and annual native cover crops suppressed growth of annual introduced species.

While species richness is important, the main concern is that those new appearances are native plants (Burghardt et al. 2010). The most diverse treatment, the Wseed treatment, did in fact host the greatest number of natives all season (Fig. 6). This was due in part to the fact that this treatment included native species that were planted. We suspect that additional native species observed were both released from the seedbank and colonized from nearby. The high number of natives contrasted with the low numbers of introduced species in this treatment. Comparing the Wseed and

Wnoseed treatments exemplifies what can happen if the biocontrol agent is used alone, depending on what the present competition consists of. In our results, weevil-only plots are comprised of 50% introduced species, with the lowest overall richness.

These data suggest that plots with weevils, but not seed, were prone to higher introduced species invasion. It also confirms the hypothesis that the underlying mechanism is weevil-mediated creation of disturbance, opening a niche, which when not purposefully re-vegetated, can promote new invasion. In such instances species richness may increase, but this metric does not necessarily indicate a diverse native community. In our study, in the presence of weevils introduced plant species numbers were negatively correlated with native seeding.

4.2 *Greenhouse Experiments*

The main greenhouse experiment bolstered data from the field by looking at the same four factorial treatments in more controlled conditions. As with the field sites, it facilitated observations of plant interactions between the MAM and native seeds under *R. latipes* impact. The other greenhouse experiments addressed questions regarding MAM growth in various circumstances and potential effects on plant neighbors.

4.2.1 *Greenhouse Main Experiment*

Mature and immature seed cluster numbers in both years of greenhouse research were significantly lowest in the Wseed treatment (immature seeds shown only, Fig. 8). Although there was a marked difference in percent cover during 2009, we see in 2010 that percent cover remained constant between treatments (Fig. 7). It was only seed production that dropped, and only in association with weevils. It is not too surprising that a biocontrol agent would alter seed production (Hough-Goldstein et al. 2008b; Lonsdale & Farrell 1998), but our finding indicates that MAM weed reproduction may be limited by biocontrol agents before vegetative growth is. This is potentially contrasting with Mountain (1989), who notes that stress appears to trigger seed production in *P. perfoliata*, something the authors have also observed. This dynamic could be valuable to investigate further, as it points to a plastic response in MAM reproductive strategy. Is it reduced growth of the vine or lowered seed set that reduce the overall fitness and lower competition thresholds? Hyatt & Araki (2006) propose that a 50% reduction of MAM plant survival and 60% reduction of seed survival in the novel range may be necessary to keep MAM from spreading. They add the caveat that the best life-stage to combat differs with demographic response to control efforts and environmental changes. In the end, seed production itself may be largely irrelevant, as MAM can be a prolific seed-producer (Moul 1948; Hough-Goldstein et al. 2008b) depending on circumstances (Hyatt & Araki 2006). Other studies agree with this notion, suggesting that 'r' selected introduced species would

have to undergo severe seed reduction to impact recruitment (Lonsdale & Farrell 1998). It is probable that many successfully germinated seeds undergo rigorous intraspecific competition hence limiting the number of seeds reaching final recruitment to adulthood.

Problems with greenhouse pests and extremely high temperatures complicated year two of greenhouse work. This is evident in percent cover (Fig. 7) for 2010, which was a very hot year. The greenhouse thermoregulation system was cooling at its maximal level many days but temperatures were still over 38° C (100° F), leading to even higher temperatures in the cages. The flats were kept on concrete benches, often hot to the touch, and likely conducting heat into the flats and soil itself. We surmise that these temperatures caused some mortality in the weevils, as they were prevented from searching out a cooler haven to retreat to as they might in the field. Dead weevils were found on occasion.

Pests probably played a role in reducing competition from the native seeded plants. In the beginning of the season slugs impacted the black eyed susans, despite attempts to reduce their populations through use of beer-bait dishes, which attracted and drowned them. Later on white flies and mealy bugs infested plants and we made attempts to prevent confounding of treatment effects by removing them. Unlike the natives, MAM appeared to be unaffected by pest infestation. Unfortunately it is likely these pests hampered native plant competitive ability, giving MAM the advantage and negating this aspect of the 2010 experiment.

Further possible explanation of unexpected 2010 results centers around delay in weevil application. This occurred ten days after native seeding because cages were back ordered and arrived later than anticipated. This set-back may have caused the MAM in the weevil-treated flats to get a better start than it would have, as it was weevil-free for the first two weeks after germination, unlike in 2009. We believe that this in combination with heat-induced weevil mortality and pest-suppressed native competition explains the similarities in percent cover among treatments in 2010.

P. perfoliata biomass in year one of the greenhouse experiment was remarkably low in the Dseed and Wseed treatments because of the native seed mix (Fig. 9A). We probably would have seen a greater impact of the weevils in year one if they had not been unintentionally and artificially removed by greenhouse fans. Most likely they could have reduced the Wseed treatment to the lowest MAM biomass. In year two (Fig. 9B) we did see the Wseed treatment hosting lowest MAM biomass, but due to pests and temperatures, only with marginal significance. The slice procedure for this year supports the concept that native seeding may only be beneficial when a biocontrol agent first suppresses the target plant. Other studies have found that removal of the problematic vegetation is critical to successful establishment of planted species (Barton et al. 2009).

When comparing biomass of grasses and forbs grown in these flats with MAM we see that grasses may be the most competitive. This is not surprising, given the aggressive nature and robust sod masses of the selected grass species. The small experimental flats used here heightened competition for soil nutrients and water, a

disadvantage to the small, short MAM roots. We must also keep in mind that the grasses comprised 60% of the seed mix, giving them a greater chance of higher biomass.

In conclusion, the greenhouse biomass results suggest that use of biocontrol agents to weaken MAM can promote native plant competition, especially under severe resource limitations. This can ultimately lead to a significant reduction in MAM growth and reproduction, and increased native plant growth.

4.2.2 *Greenhouse Allelopathy Experiment*

Based on our preliminary investigations, it is apparent that MAM may be allelopathic. We observed severe growth retardation in native plants grown with MAM when compared to those grown alone (Fig. 10). These results are similar to those found by studies of other invasive introduced species (Grant et al. 2003; Kato-Noguchi et al. 2010; Wixted & McGraw 2010) and warrant further investigation. A future step in this direction may be to test the effect of *P. perfoliata* root exudates on one of the native species used in this study, with the plants spatially separate (see Saxena 2000). This could help verify allelopathy and eliminate the possibility of spatial or nutrient competition as causation. Kulmatiski et al. (2006) suggest that some introduced plants may boost growth through relationships with beneficial fungi. The idea that either allelopathy (Perry & Galatowitsch 2003) or mycorrhizae (Schwartz et

al. 2006) play roles in introduced plant interactions is nothing new. Regardless of the exact mechanisms, happenings underground may be an important element to address in restoration after introduced species invasions (Heneghan et al. 2008), such as those of MAM weed.

4.2.3 *Greenhouse Disturbance Experiment*

It is already known that disturbance promotes invasion (Harper 1965; Mack 1989). This experiment verifies that the habitats where we find MAM operate as other systems do in regard to this dynamic. Our data emphasize the need to reduce disturbance in wildland areas where MAM weed could gain a foothold. If disturbance is unavoidable, prompt re-vegetation with native plants to reduce the opened niche is paramount (Tilman 1999; Lonsdale & Farrell 1998).

4.3 *Conclusion*

Results from the Longwood Gardens field experiment support the approach of integrated management as a successful strategy for combating introduced plant invasion while restoring native flora. This conclusion is in agreement with previous work done with similar designs and objectives (Lym 2005; Benz et al. 1999;

Davies et al. 2005; Simmons 2005; McEvoy 1993). Use of a biocontrol agent suppressed the target plant while native seeding promoted plant competition and significantly increased native plant species richness. The same methods may not work identically in all habitats or with other introduced plant species invasions (Hilderbrand et al. 2005). Further research is needed to understand the complexities of restoration in a variety of systems. There is hope for the future of invaded areas as the discipline of restoration ecology grows, aimed at re-interpreting the human footprint on nature.

Appendix A

NEONICOTINOID INSECTICIDE PILOT STUDY

A.1 *Introduction*

A greenhouse study was conducted to determine effects of two insecticides on the weevils *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) and on their host plant mile-a-minute (*Persicaria perfoliata* (L.) H. Gross). The focus of the study was two-fold: first, two neonicotinoid insecticides were applied to determine which was more effective in eliminating weevil populations on mile-a-minute (MAM). Second, the study provided an opportunity to observe whether the insecticides promote plant growth in MAM. Growth stimulated by the insecticide could lead to incorrect conclusions about weevil versus no-weevil impact.

Imidaclopird, one of the neonicotinoid systematic insecticides tested, is produced by Bayer Crop Science, known also by the marketing names Gaucho®, Admire®, and Condifor®. The second neonicotinoid was dinotefuron (also known by marketing names of Venom® and Safari®), and is a more recent product of Valent Biosciences Corporation. Both insecticides are systematic in nature and were applied as a drench to potted plants in the greenhouse. Imidacloprid is believed to have growth

enhancing properties aside from its intended insecticidal purpose (Dr. Casey Sclar, Longwood Gardens, personal comm. 2008; Hundley 2004; Dr. Brian Kunkel, University of Delaware, personal comm. 2008). There is less industry experience with dinotefuron due to its recent appearance on the market, but it may produce the same growth inducing effect as imidacloprid since it is chemically similar. An advantage of dinotefuren over imidacloprid is the speed of uptake due to its high solubility (Ali & Caldwell 2010). Quick uptake is necessary to prevent weevils from impacting plots early in the season.

A.2 *Materials and Methods*

Potted MAM plants and weevils were provided by the Phillip Alampi Beneficial Insects Laboratory (PABIL), Trenton, NJ. The treatments were applied in a fully factorial design, combining weevils, no weevils, dinotefuron, no dinoteuron, and imidacloprid, no imidacloprid.

The objectives of this study were to observe how quickly and thoroughly the insecticides eliminated weevils, and what growth response occurred in the plants. There were three replicates of each treatment, eighteen pots used in total. The plants were started as cuttings the week of September 14th, 2008 at PABIL and were grown there until they were picked up for the study on November 12th, 2008.

A weevil density of 5 mating pairs per plant was suggested (Amy Diercks, PABIL, personal comm. 2008). This number of weevils allowed the effects of the insecticides to show before the weevils could decimate the weevil-only treatments. All weevils were collected from PABIL between November 4th, 2008 – November 7th, 2008 (hence they emerged between November 1st – November 7th). The weevils were immediately introduced to the plants in the University of Delaware greenhouse, where they were allowed to acclimate for a period of 5 days before application of insecticides. The light and temperature conditions were controlled so the weevils were actively feeding and reproducing throughout the experiment. Light was set to a 16 hours light, 8 dark, and the temperature varied between 75°-80° F. The potted plants were grouped by replicate on three separate tables in the same room. Each plant was held in a clear ventilated plastic box

The plants were checked every other day to ascertain water needs and to visually monitor weevil numbers for insecticide-induced mortality. After one month (11/17/08 – 12/19/08) all replicates were destructively sampled to count dead and living weevils and rated on feeding damage. The plants were cut at the base and dried at room temperature in paper bags for 10 days to compare any differences in dry matter biomass.

A.3 *Results*

Dinotefuron caused 100% weevil mortality in a short time period, whereas imidacloprid had one weevil remaining unaffected at the end of the trial and generally took longer to cause mortality . Dinotefuron eliminated weevil populations almost a week before imidacloprid did. In addition, while imidacloprid caused lethargy and prolonged death, dinotefuron acted faster once taken up by the weevils.

Table 3 Mean plant weights and standard errors for each treatment.

Treatment	Mean Plant Biomass ± SEM	Mean Weevils ± SEM
Control – No weevils	2.78 (± 0.16)	N/A
Control - Weevils	2.63 (± 0.30)	20.67 (± 6.23)
Dinotefuron – No Weevils	2.98 (± 0.20)	N/A
Dinotefuron - Weevils	2.80 (± 0.29)	0
Imidacloprid – No Weevils	2.93 (± 0.13)	N/A
Imidacloprid - Weevils	3.19 (± 0.09)	0.33 (± 0.33)

There was no apparent growth enhancement of the insecticides. No significant difference in plant biomass was found by block ($F_{2,17} = 0.08$, $P = 0.922$) or treatment ($F_{5,17} = 0.70$, $P = 0.6359$, Table 1). There was no significant difference between treatments without weevils ($F_{2,17} = 0.33$, $P = 0.737$).

There was no significant difference in weevil mortality by block ($F_{2,17} = 0.95$, $P = 0.418$) but there was between treatments ($F_{5,17} = 10.83$, $P = 0.0009$).

These results indicate that dinotefuron does not alter plant growth. In addition, it eliminated weevil populations faster and completely when compared to imidacloprid.

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