

**FACTORS AFFECTING THE REPRODUCTIVE POTENTIAL
OF THE INVASIVE VINE, *PERSICARIA PERFOLIATA***

by

Jeffrey R. Smith

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Science in Ecology with Distinction.

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ABSTRACT

Persicaria perfoliata, commonly known as mile-a-minute weed, is an aggressive invasive vine throughout the Mid-Atlantic and Northeastern United States. The plant has been shown to be highly problematic in both natural and agricultural areas, prompting extensive research into potential control methods. As an annual plant that reproduces primarily through seeds, specific attention has focused on ways to reduce the reproductive potential of *P. perfoliata*. In addition to a regimen of physical and mechanical controls, a classical biological control agent was identified and introduced in 2004. This insect, *Rhinoncomimus latipes*, is entirely host specific on *P. perfoliata* with the adults feeding primarily on leaves and the larvae boring into stems. Previous studies have shown that *R. latipes* reduces both the percent cover and number of seeds produced by *P. perfoliata*.

My first study aimed to identify how seed viability is affected by the maturity status of the fruit surrounding the seed and the time of year of seed collection. In order to do this, seeds were collected from five field sites in the Mid-Atlantic and returned to the lab where their viability was assessed using a chemical assay. The second study aimed to quantify the impact of *R. latipes* on the reproductive potential of *P. perfoliata*, both by determining the reduction in number of seeds produced and the proportion of resultant seeds which are viable. One experiment in this study solely investigated the effects of adult weevils confined to developing seed clusters while the other tested the effects of feeding by larvae and adults on the entirety of a *P. perfoliata* plant.

The first study revealed that seeds surrounded by immature fruits are viable, and the rate at which they are viable varies across the season. I found that at the onset of seed production, in mid-August, 35% of seeds with immature fruits were viable, but this rate increased to 84% in late September. As has been seen in previous studies, nearly all seeds with mature fruits (96%) were found to be viable. In the second study, I found that feeding by the entire lifecycle of weevils reduced the total number of seeds produced per plant by 32%. Further, the number of seeds per cluster was reduced with feeding strictly from adults on developing seed clusters (17% reduction) and with feeding by the entire weevil lifecycle on the whole plant (36% reduction). Feeding strictly by adults on developing clusters reduced seed viability by 24%, but I found no effect of feeding by adults and larvae across the entire plant on seed viability.

Our first study indicates to land managers that if they do want to apply physical or chemical control methods, they should do so prior to the onset of any seed production and not simply before fruit maturation. If they have to apply control methods after fruit set, our findings indicate they should do so as early in the season as possible. My second study demonstrates that any feeding activity by *R. latipes* will reduce reproductive potential by decreasing the number of seeds produced. However, only feeding by adults directly on clusters reduced seed viability, further lowering reproductive potential. Lastly, I showed that weevil activity delays the onset of fruit production and maturation, possibly limiting the capacity of the seed to spread via wildlife that are attracted to the mature fruits. This study outlines previously unknown ways in which *R. latipes* can help to stop to spread of a noxious invasive weed.

Chapter 1

PHENOLOGICAL VARIABILITY IN SEED VIABILITY

Introduction

Persicaria perfoliata (L.) H. Gross (Freeman and Reveal 2005), commonly known as mile-a-minute, is an invasive weed in the mid-Atlantic region of the United States. Mile-a-minute, which is native to China, Korea, Japan, and other parts of Asia, (Cusick and Ortt 1987) is believed to have been accidentally introduced to the United States in the form of seeds contaminating a shipment of holly. The plant was first observed in the eastern United States in the 1930's near York, Pennsylvania (Moul 1948). After a lag period in which the weed remained relatively in check, the plant underwent a rapid expansion and now occupies a range from New Hampshire in the north to North Carolina in the south, stretching westward to Ohio and West Virginia (EDDMapS 2013).

Persicaria perfoliata is an annual, herbaceous plant exhibiting a vine like growth habit (Mountain 1989; Okay 1997). In its native range the plant is primarily restricted to riparian areas, but in its introduced range it has the capacity to colonize disturbed sites, roadsides, utility rights of way, and forest edges (Hough-Goldstein et al. 2008a). Mile-a-minute grows very rapidly, up to six inches a day, which contributes to the invasive nature of this weed (Lehtonen 1994). The plant is easily identifiable due to its distinctive triangular leaves and light green coloration. An additional diagnostic feature is the presence of recurved thorns along the entirety of the stems, which allow the plant to overgrow other vegetation, degrading the habitat

for use by both humans and wildlife (Okay 1997; McCormick & Hartwig 1995; Oliver 1996; Wu et al. 2002). *P. perfoliata* has become problematic in both agricultural systems, specifically Christmas tree farms and orchards, and natural areas, where it overgrows and outcompetes native vegetation (Moul 1948; Lehtonen 1994; Hough-Goldstein et al. 2008a).

Persicaria perfoliata reproduces primarily by seeds, with a single plant being capable of producing thousands of seeds (Hough-Goldstein et al. 2008b). Seeds are surrounded by fleshy integuments and develop in clusters ranging from 4 to 24 seeds (personal observation). The fruits are initially the same color green as the foliage and stems, but turn blue upon maturation (Hickman and Hickman 1978). Early seed production is relatively small and occurs in late June in the mid-Atlantic region. The primary production of seeds occurs later in the season, from August to October (Mountain 1989; Cutting and Hough-Goldstein 2013). The timing of seed production varies, likely due to both climactic variations following latitudinal gradients as well as local habitat conditions such as moisture and light conditions (Sultan 2001). Seeds are primarily dispersed by wildlife, presumably attracted to their blue color, but are also buoyant and will disperse via water, especially in riparian habitats (Hill et al. 1981; Mountain 1989; McCormick and Hartwig 1995; Okay 1997). It has been shown that seeds require a period of cold stratification in order to become viable (Johnson 1996; Okay 1997; Colpetzer and Hough-Goldstein 2004). While the majority of these seeds will germinate the following year, seeds remain viable in the seed bank for up to seven years (Okay 1997; Hough-Goldstein et al. 2008a).

As a result of rapid growth rates, prolific seeding, and impact on both natural and agricultural systems, controlling *P. perfoliata* is of the utmost importance.

Traditional control of *P. perfoliata* has relied either on physical control, such as hand weeding or mowing, or chemical control, principally through broad spectrum herbicides. Land managers attempt to time these control efforts to coincide with times when mile-a-minute is not in seed. It may be assumed that seeds surrounded by green fruits are immature and not viable or less viable than seeds surrounded by blue fruits. However, there is not currently any empirical evidence to support this conclusion.

The experiment described below aims to answer two primary questions. First, is there a difference in seed viability between “immature seeds”, those surrounded by green fruit, and “mature seeds”, those surrounded by blue fruits? Second, is there an effect of the time of year the seeds were gathered from the field on their viability? By gaining more information on how the phenology of the plant influences the viability of its seeds we can better inform land managers on when and how to apply mechanical and chemical control methods.

Methods

For this study, five field sites were selected in Delaware, Pennsylvania, and New Jersey. The sites were well dispersed geographically and encompassed a diverse array of habitats including regularly disturbed open fields, forest edges, riparian zones, and roadsides. The five sites selected were White Clay Creek State Park, Newark, Delaware (39°43'37.27"N, 75°46'5.12"W); Longwood Gardens, Kennett Square, Pennsylvania (39°52'45.40"N, 75°40'16.52"W); Waterloo Mills Preserve, Easttown Township, Pennsylvania (40° 1'15.75"N, 75°24'57.42"W); Pennypacker Park, Haddonfield, New Jersey (39°54'13.94"N, 75° 1'21.49"W); and a roadside in Pennsville, New Jersey (39°37'7.81"N, 75°32'5.76"W). Collections of *P. perfoliata* seeds were made every two weeks at all five of these sites from mid-August to late

October. If possible, 50 seeds with immature fruits and 50 seeds with mature fruits were collected. However, during the beginning of the season it was not uncommon to find only seeds with immature fruits. A summary of the types of seeds collected is in Table 1.

Table 1 **Dates of collection and types of seeds collected at each field site** (Imm – surrounded by green fruit; Mat – surrounded by blue fruit)

Collection dates (All 2012)	White Clay Creek	Longwood Gardens	Waterloo Mills	Pennypacker Park	Pennsville roadside
8/13-8/15	Imm, Mat	Imm, Mat	Imm	Imm	Imm
8/27-8/30	Imm, Mat	Imm, Mat	Imm	Imm	Imm, Mat
9/13-9/15	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat
9/27	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat
10/11-10/13	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat
10/25		Imm, Mat		Imm, Mat	Imm, Mat

Seeds were returned to the lab and held at room temperature to desiccate. On November 15th, 2012, four weeks after the final seed collection, seeds were transferred to individual re-sealable plastic bags filled with moist peat moss and transferred to a refrigerator held at 4° C for four months.

After this four month period, seeds were removed from the peat moss and viability was assessed using a triphenyl tetrazolium chloride (TTC) assay (Peters 2000). The TTC solution was created by dissolving 1.00 g of TTC (Sigma-Aldrich Corporation, St. Louis, Missouri) into 100 mL of water. For each collection sample, 25 of the cold-stratified seeds were selected at random for inclusion in the assay. The chosen seeds were prepared by making a small incision into the achene using a scalpel. Approximately 5 ml of 1% TTC solution was then added to each sample of seeds, making sure to completely submerge the seeds in the solution. Each container

with seeds and TTC was closed with a fitted lid and covered with aluminum foil. After 24 hours in the TTC solution, seeds were removed from the solution and rinsed with water. Seeds were then bisected using a scalpel. Under this test, viability is indicated by a red staining of the embryo. The number of seeds showing red embryos was enumerated as a measure of viability.

Data Analysis

The proportion of seeds viable was calculated as the ratio of the viable seeds to the number of seeds tested. The proportions of immature and mature seeds viable were first analyzed separately, using a two-way analysis of variance by site and time of collection. The late October sample was excluded from this analysis as seeds were only found at three sites. A linear regression was fit to the number of immature seeds viable at each site for the mid-August through mid-October samples (excluding the Waterloo Mills sample from mid-October because the air temperature fell below 0 °C causing early plant senescence [wunderground.com 2012]). For each biweekly sample, a paired Student's T-Test was performed between the proportion of mature and immature seeds viable at each site. The level of significance for all tests was defined to be $P < 0.05$. All statistical analyses were performed using R Statistical Software (R Core Development Team 2012).

Results

There did appear to be an effect of local conditions in influencing the phenology of *P. perfoliata* seed production. I found differences of up to one month in the onset of fruit maturation. Similarly, the timing of senescence, indicated by the end of seed production, varied among field sites (Table 1).

The proportion of mature seeds viable averaged across all six collection samples was 0.96 ± 0.0087 . There was no significant difference in the proportion of mature seeds viable by either site or time of collection ($F \leq 0.100$; $df = 4, 19$; $P \geq 0.114$).

There was an effect of the time of collection on the proportion of immature seeds viable ($F = 306.621$; $df = 4, 25$; $P < 0.001$), as well as an effect of site ($F = 10.626$; $df = 4, 25$; $P < 0.001$), and a significant interaction between site and time of collection ($F = 4.129$; $df = 4, 25$; $P = 0.020$). Of the seeds collected during mid-August, the proportion of immature seeds viable was found to be 0.35 ± 0.06 . The proportion of immature seeds viable increased over the season, peaking during the late September collection, with the proportion of seeds viable being 0.84 ± 0.03 . In the final collection the proportion of immature seeds viable dropped to 0.63 ± 0.22 (Fig. 1F). There was a strong positive correlation between the number of immature seeds viable and the time of collection up through the fifth date (excluding Waterloo Mills; $y = 0.1502x + 0.1863$, $r^2 = 0.8077$, $F = 92.38$, $df = 1, 22$; $P < 0.001$; Fig. 2). During the fifth and sixth samples there was substantial variation in the number of immature seeds viable between sites (Fig. 1A – 1E).

There was a significant difference between the proportion of immature and mature seeds viable in samples taken from mid-August ($t = 8.88$; $df = 2$; $P = 0.013$), late August, mid-September, and late September ($t \geq 3.16$; $df = 4$; $P \leq 0.034$; Fig. 1F).

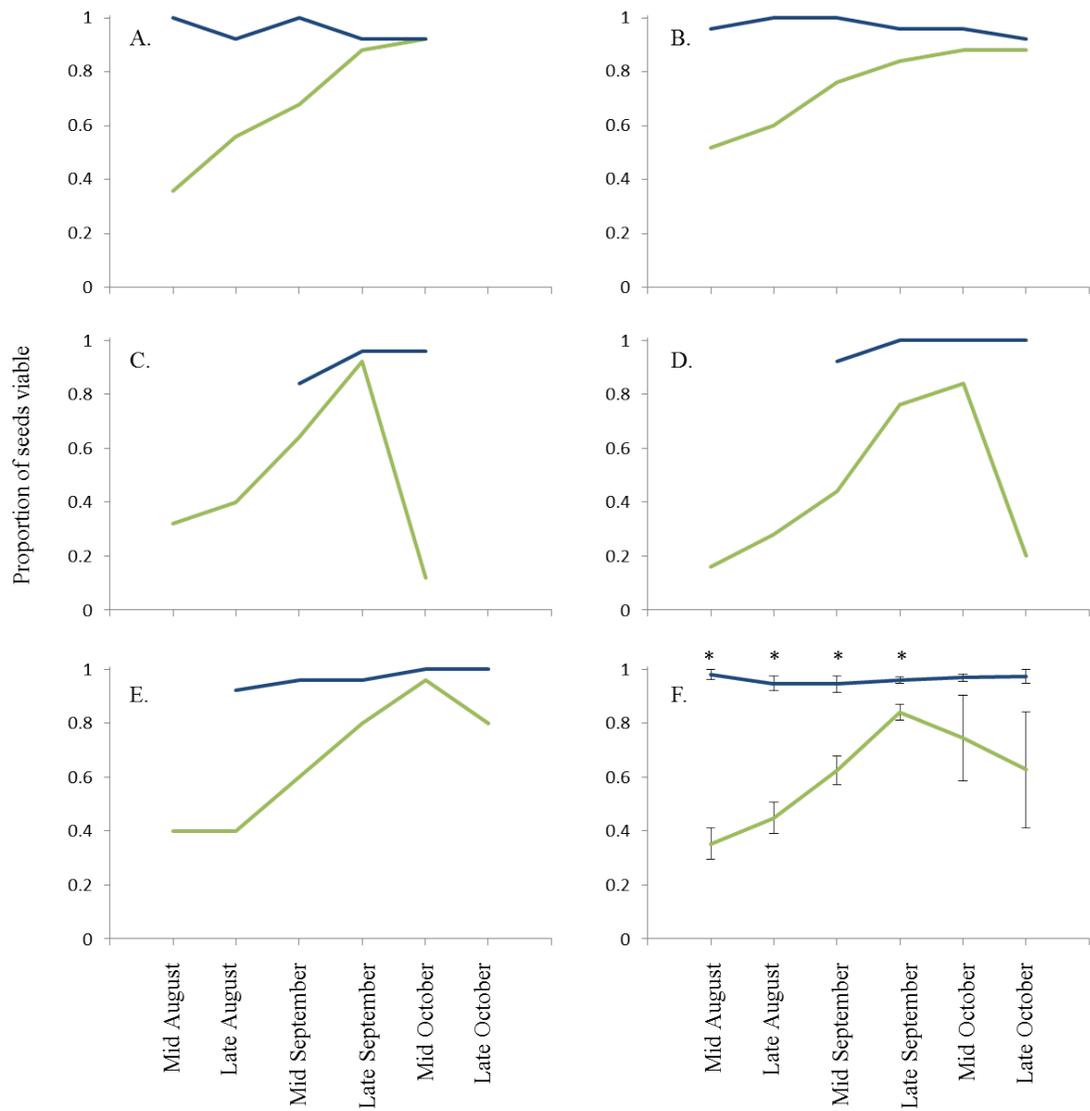


Figure 1 **Viability of field gathered *Persicaria pefoliata* seeds** - Proportion of immature (green line) and mature seeds (blue line) shown to be viable from (A) White Clay Creek, (B) Longwood Gardens, (C) Waterloo Mills, (D) Pennypacker Park, (E), a Pennsville roadside, and (F) the average (\pm SEM) across all five sites. * indicates significance at $P < 0.05$, $N = 5$ for samples from Mid-August to Mid-October, $N = 3$ for the sample in Late October, Unpaired Student's t-test.

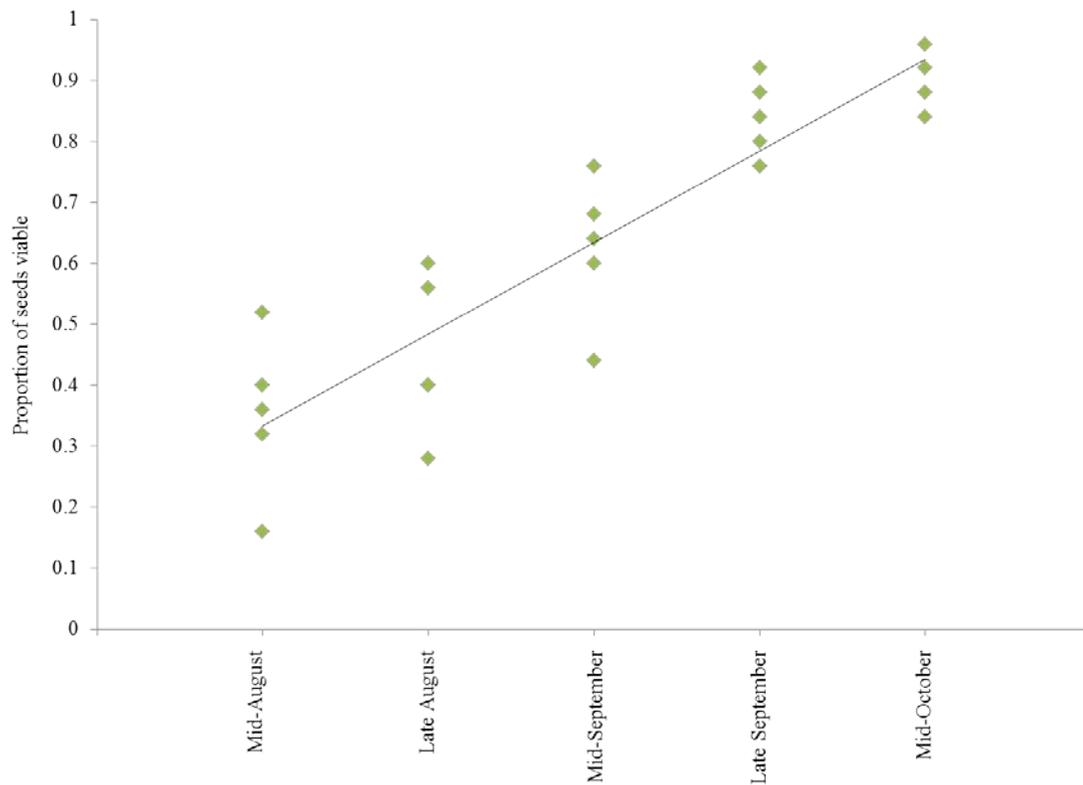


Figure 2 **Proportion of seeds surrounded by immature fruits viable –** Proportion of field collected seeds with immature fruits shown to be viable from the first five samples, with one site excluded during the fifth sample, fit with a linear regression.

Discussion

This study outlines three important and previously unknown characteristics of the reproductive strategy of *P. perfoliata*. First, some of the seeds surrounded by immature fruits are in fact viable and therefore need to be treated as such. I hypothesize that this is due to a lag of fruit maturation behind seed maturation. Although from the data presented I cannot present a precise timetable, based on observations made in the field, there is approximately three weeks between fruit set

and the maturation of the fruit. At some point during this three week period the seed matures, but the fruit remains green, giving rise to viable seeds surrounded by immature fruits. The amount of time between fruit set and seed maturation cannot be determined from this study, but is something that warrants further investigation. This would provide information to land managers which would greatly enhance their ability to time herbicide sprays or mechanical control methods for the period of time after fruit set, but before seed maturation.

The second major finding is the variability in the proportion of seeds with immature fruits viable. The strong upward trend in the proportion of seeds viable across the season could be explained in a few ways. It is possible that as the season progressed, seeds had also developed further and even though they were surrounded by an immature fruit, they were more likely to be viable. While this is one possible explanation, I do not think that it is the most likely. A more reasonable alternative is that as the season progressed, plants allocated a greater portion of their available energy from vegetative growth to reproductive growth. This would accelerate the rate of seed maturation, while fruit maturation remained constant, resulting in a higher proportion of seeds with green fruits being viable. Regardless of the physiological reason, this finding demonstrates that if land managers wish to control *P. perfoliata* while it is in seed, they should do so as early in the fruiting period as possible.

The final noteworthy finding to come from this phenology study is the drop off in the viability of seeds with immature fruits late in the season, but the maintenance of relatively stable levels of seed viability for seeds surrounded by mature fruits. The fact that seeds produced late in the season suffer with changing environmental conditions, but those produced a few weeks prior do not, indicates that the change in seed viability

is likely derived from a change in plant health at time of seed production rather than weather or other abiotic factors. The most likely explanation is that the plant does not have enough resources at the end of the year to invest in maturing seeds. Conversely, seeds that have already matured have acquired sufficient resources and face no negative consequences of either dropping temperatures or shortening day length.

It is important to note that there was shown to be dramatic variation between sites in the onset of both fruit set and fruit maturation, with site and the interactive effect of site and time of collection both showing significant differences. This is not unexpected, given the variability shown in other seeds based on climactic factors (Sultan 2001), but it is information that needs to be disseminated to landowners to reinforce that the timing any of physical or chemical control methods needs to be specific to a given site and will vary from year to year. Readily available models for degree day accumulation, both heating and cooling days, may provide an index which land managers could use to account for variations between sites and years in the timing of seed production. Future work should test if degree days can in fact be used as an estimate of the timing of seed production.

In conclusion, this study outlines two important findings that should be passed along to land managers in order to better use physical and chemical controls against *P. perfoliata*. First, if possible, land managers should try to apply control methods prior to the appearance of any seeds, not simply before the onset of mature fruits. However, if control methods do need to be applied while *P. perfoliata* is in seed, it is essential to do so as early as possible in the season. Second, the phenological progression of this invasive plant is likely to show drastic variation among sites and even between

different years at the same site. Therefore, it is imperative to institute control methods on an as-needed basis rather than as a calendar spray.

Chapter 2

EFFECT OF BIOLOGICAL CONTROL ON REPRODUCTIVE POTENTIAL

Introduction

Though physical and chemical controls are a valuable tool in management of *Persicaria perfoliata*, they lack specificity, are highly resource intensive, and have not been shown to be highly effective on a large scale (Mountain 1989). Biological control presented the potential to solve these issues, and therefore in 1996 the United States Forest Service initiated a search for a classical biological control agent throughout the native range of the plant (Wu et al. 2002). After years of host-specificity testing under quarantine conditions in China and the United States, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) (Korotyaev 1997) was identified as a potential biological control agent (Jianqing et al. 2000; Ding et al. 2004). *Rhinoncomimus latipes*, now commonly known as the mile-a-minute weevil, was found to feed exclusively on *P. perfoliata* when presented with a choice between the target and congeners or other closely related non-targets (Colpetzer et al. 2004a). In no-choice feeding tests there was found to be minimal feeding on non-target plants, but no successful reproduction on any host other than mile-a-minute (Colpetzer et al. 2004a). Due to this very high level of host specificity in feeding trials and complete reproductive specialization, in 2004 a permit was obtained from the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) for the release of *R. latipes* as a biological control agent in North America (USDA/APHIS-PPQ 2004; Hough-Goldstein et al. 2008a). This host specificity was

further confirmed in field testing as a post-release screening measure (Frye et al. 2010).

Rhinoncomimus latipes is a small weevil, measuring approximately 2 mm as an adult (Korotyaev 2006). The adults are primarily folivores and the larvae are stem borers in *P. perfoliata* (Price et al. 2003). The lifecycle of the weevil takes approximately 26 days to complete in the laboratory, allowing three to four overlapping generations per year in the mid-Atlantic region (Colpetzer et al. 2004b; Lake et al. 2011). Newly emerged adults are black, but take on an orange coloration after feeding. This change is hypothesized to be from chemicals released from the plant during feeding (Price et al. 2003). A single female lays on average 130 eggs over the course of her lifetime, on the stems, leaves, and other parts of *P. perfoliata* (Price et al. 2003). Small, yellow larvae emerge from these eggs and bore into the stem (Hough-Goldstein et al. 2012). After maturing, these larvae exit the stem, dropping to the ground to pupate. After about one week, adults emerge from the ground and disperse to host plants either through flight or by walking (Price et al. 2003; Colpetzer et al. 2004b). In the fall, adult weevils enter diapause and overwinter, a process that is likely triggered by a combination of photoperiod, temperature, and declining host plant quality (Lake et al. 2011).

Rhinoncomimus latipes has been shown to reduce both the percent cover of mile-a-minute and the number of seed clusters produced by the invasive plant (Lake et al. 2011; Cutting and Hough-Goldstein 2013). Although it was quantified by Hough-Goldstein et al. (2008b) that weevil activity did reduce the total number of seeds produced per plant, the number of seeds per cluster has never been quantified. This is essential information needed to interpret studies which relied on seed clusters as an

index of total number of seeds (Lake et al. 2011; Cutting and Hough-Goldstein 2008). Further, to this point there have been no studies on the effect of *R. latipes* on seed viability. Together, the number of seeds produced and the proportion of those seeds that are viable constitute the total reproductive potential of *P. perfoliata*. Since a single *P. perfoliata* plant can produce thousands of seeds it is likely that even though *R. latipes* decreases the number of seeds produced, this reduction is not sufficient to account for long-term reduction in *P. perfoliata* populations. In addition to decreasing the number of seeds produced, *R. latipes* may also decrease the viability of *P. perfoliata* seeds. A reduction in seed viability has been shown in other plants as a result of curculionid feeding (Buntin et al. 1995; Follett and Gabbard 2000).

The experiments described below investigated the impact of *R. latipes* on *P. perfoliata* reproductive potential. The field study investigated the impacts on seed production and viability of no-choice feeding by adults directly on seed clusters. While *R. latipes* adults are primarily foliage feeders, evidence suggest that adults will also feed on developing seed clusters (Colpetzer et al. 2004b, Hough-Goldstein et al. 2008a). This evidence, combined with the large number of curculionids which feed primarily on seeds (Triplehorn and Johnson 2005), gives reason to believe that adults may feed directly on the fruits or seeds, which may in turn influence reproductive potential. The greenhouse study more broadly considered the effect of feeding by both larvae and adults on the stems, leaves, and reproductive structures of *P. perfoliata*. Unlike the field study, this experiment aimed to investigate the indirect effects of *R. latipes* feeding on other parts of the plant, chiefly adults feeding on foliage and larvae boring in stems, on seed production and viability rates. Together these experiments

will help to quantify the impact of *R. latipes* on *P. perfoliata* reproductive potential and allow for more accurate modeling of *P. perfoliata* populations from year to year.

Methods

Experiment 1 – Field Study

This experiment was conducted at White Clay Creek State Park, Newark, Delaware (39°43'40.69"N, 75°46'12.19"W). A mile-a-minute patch of sufficient size located away from any areas of heavy human traffic was selected as the study site. The experiment was conducted for a period of six weeks from August 20th, 2012 to October 1st, 2012.

At the onset of the trial, 100 mile-a-minute developing seed clusters of similar phenology were selected to be used in the experiment. The clusters were enclosed with a small (approximately 15 cm x 15cm), transparent, white, fine mesh bag closed with a twist tie. Fifty of the bagged clusters were enclosed with five weevils each while the other fifty bags were enclosed with no weevils. The weevils used for this experiment were obtained from the Phillip Alampi Beneficial Insects Lab in Trenton, New Jersey. The bagged clusters remained on the plants for six weeks. After this time, 42 treatment bags and 38 control bags were found, separated from the plant, and collected. The number of seeds per cluster was counted, the cluster was weighed, and the average weight per berry was calculated. Seeds were then left at room temperature for 4 weeks to desiccate.

After this four week period seeds were again weighed to collect the dry biomass of each cluster, from which the average dry biomass per berry was calculated. All of the seeds from a given cluster were then placed into a re-sealable plastic bag

combined with moist peat moss. Bags were transferred to a refrigerator held at 4° C where they remained for a period of four months.

After this four month period, seeds were removed from the peat moss and viability was assessed using a triphenyl tetrazolium chloride (TTC) assay as described in the previous chapter. A total of 30 treatment clusters and 30 control clusters were tested. For each cluster tested, 5 of the cold-stratified seeds were selected at random for inclusion in the assay.

Experiment 2 – Greenhouse Study

This experiment was conducted in a greenhouse located on the University of Delaware Newark campus from August 3rd to November 19th, 2012. Five weeks prior to the start of the trial mile-a-minute plants were started using vegetative propagation. The mother plant used for the cuttings was obtained from the Phillip Alampi Beneficial Insects Lab in Trenton, New Jersey. Cuttings were dipped into rooting hormone and planted into 7.5 cm pots filled with vermiculate. After 2 weeks, plants were transferred into heavy-duty flats (Kardon Corporation, now owned by Buckhorn Incorporated, Milford Ohio), 35.6 cm wide x 50.8 cm long x 15.3 cm deep, at a density of 2 plants per flat. Plants were watered daily and fertilized twice during this time.

On August 3rd the flats were moved into fine mesh, white, tent-like cages measuring 61 x 61 x 61 cm (BugDorm 3, Bioquip Products, Rancho Dominguez, CA). Four beige ceramic tiles were used to create a floor for each cage. The trial began with 20 enclosed flats each with two mile-a-minute plants. Flats were laid out in two rows along a single greenhouse bench in an east-west direction. Half of the flats were treated with 20 *R. latipes* individuals while the other half were left with no insects as a

control. These densities used for this experiment are comparable to those seen throughout the invaded range (Hough-Goldstein 2008a). The weevils used for this experiment were obtained from the Phillip Alampi Beneficial Insects Lab in Trenton, New Jersey. Initially the treatment and control flats were laid out in a checkerboard pattern. Each north/south pair of cages was considered to be a single block, giving a total of ten blocks. However, one of the treatment flats had to be excluded due to the death of all weevils and one of the control flats was excluded as it became highly infested with armyworms. The two blocks including these flats were therefore excluded, resulting in 8 viable blocks for data collection and analysis.

Every week during the experiment flats were monitored for the following factors: adult feeding damage assessed on a scale of 0-5 (0 = less than 1% damaged, 1 = 1 to 10% damaged, 2 = 10 to 20% damaged, 3 = 20 to 40% damaged, 4 = 40 to 60% damaged, 5 = greater than 60% damaged), number of immature seed clusters (clusters with at least three full-sized immature [green] fruits and no mature [blue] fruits), and number of mature seed clusters (clusters with at least three fruits, one of which had matured). Feeding damage was used to ensure continued weevil presence, and the number of seed clusters was used as an index of energy investment in reproduction.

A destructive harvest was performed for the eight blocks from November 17th to November 20th. At this time I removed seed clusters from each plant and counted the number of immature and mature seeds on each cluster. In addition, any loose seeds, either those that had already fallen off the plant or were resting in the ocrea, were counted separately. The total number of seeds for each flat was calculated by summing the total number of seeds in clusters and loose seeds. At this point, seeds were weighed and fresh biomass recorded. Seeds were then left at room temperature

for three weeks to desiccate. These seeds were transferred to moist peat moss in re-sealable bags and placed in the refrigerator on December 14th, 2012.

During the destructive harvest, above-ground plant biomass was harvested and transferred to large brown paper bags. These bags were then transferred to a drying oven held at 60° C for one week. Following this period plants were weighed and dry biomass recorded.

After the seeds had remained in the refrigerator for three months, seeds were removed from the peat moss and viability was assessed using a TTC assay as described in the previous experiment. Five randomly selected blocks were assessed for seed viability using this method. For each flat tested for viability, 25 immature and 25 mature, were selected at random for inclusion in the assay.

Data Analysis

All data from Experiment 1, the field study, was analyzed using unpaired Student's t-tests. All data from Experiment 2 was analyzed using paired Student's t-tests with east-west position on the bench determining the blocking of flats. All statistical analyses were performed using R statistical software (R Core Team 2012).

Results

Experiment 1 – Field Study

After the six week feeding period, the majority of seeds had fallen from the clusters and rested in the bags. The berries collected no longer had the characteristic blue, fleshy fruit of a mature seed, but had instead begun to discolor and desiccate. Adult weevils were recovered from the bags containing weevils, but were not counted.

Bagging adult weevils on developing mile-a-minute clusters reduced the number of seeds per cluster from 8.5 ± 0.5 to 7.1 ± 0.4 seeds per cluster ($t = 2.43$; $df = 37$; $P = 0.0118$). Further, adult weevil activity decreased the average fresh biomass per berry from 29 ± 3.7 mg to 24 ± 0.9 mg, a 17.0% reduction ($t = 4.83$; $df = 37$; $P < 0.001$). Similarly, weevil activity decreased the average dry biomass from 21 ± 0.4 mg to 18 ± 0.1 mg, a 15.3% reduction ($t = 4.16$; $df = 37$; $P < 0.001$).

The presence of weevils on developing clusters also significantly reduced the proportion of seeds viable, from 98.7% ($\pm 0.9\%$) to 75.3% ($\pm 3.7\%$) ($t = 6.34$; $df = 29$; $P < 0.001$).

Experiment 2 – Greenhouse Study

Although each flat containing weevils initially had only twenty weevils, by the end of the experiment observations of both adult weevil abundance and feeding damage indicated that weevil populations were likely greater than one hundred weevils per flat. However, the actual number of weevils per flat was not enumerated at any point during the experiment. Of the eight blocks included in harvest data analysis, all control flats consistently had adult feeding damage scores of 0 on the 0-5 feeding damage scale, while treatment flats had values ranging from 1 to 5 with values escalating as the experiment proceeded.

The number of immature seed clusters was higher on control flats lacking weevils than on treatment flats with weevils each week from week 4 through week 8 ($t \geq 4.67$; $df = 7$; $P \leq 0.0023$; Fig. 1A). Similarly, there were more mature seed clusters in control flats than treatment flats from week 6 through week 12 ($t \geq 2.68$; $df = 7$; $P \leq 0.0314$; Fig. 1B). The total number of seed clusters per flat was greater in control flats than treatment flats from week 4 through 11 ($t \geq 3.43$; $df = 7$; $P \leq 0.011$, Fig. 1C).

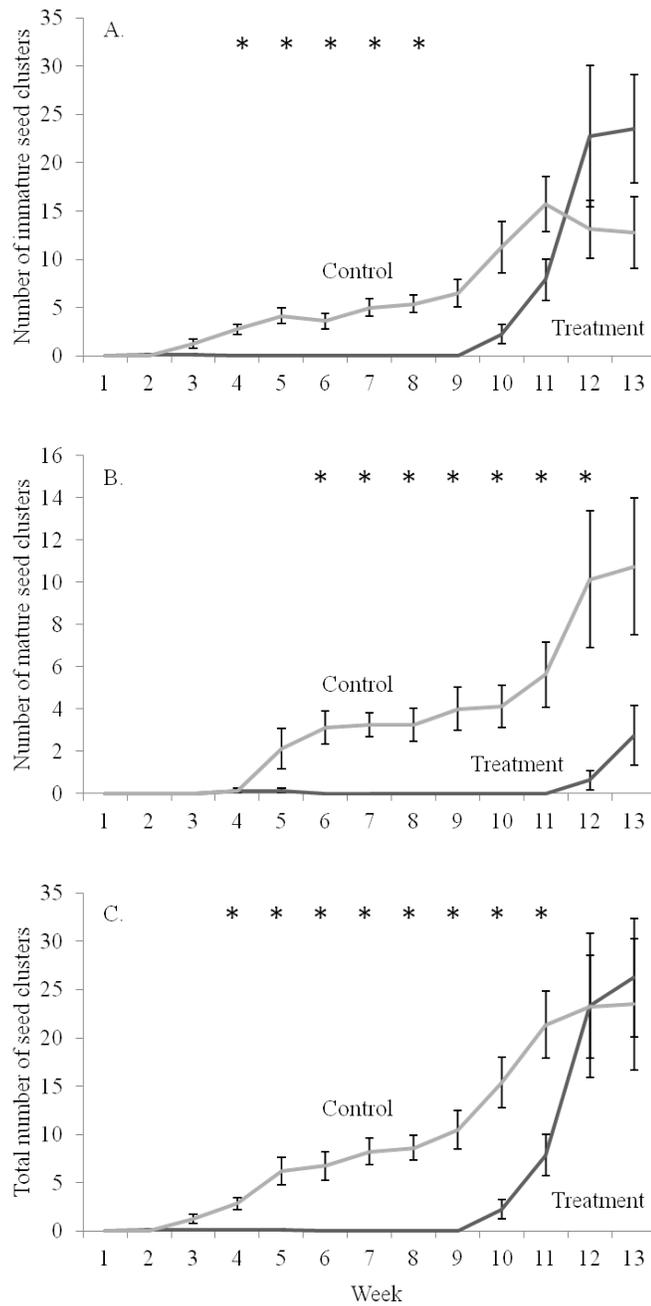


Figure 3 **Weekly *Persicaria perfoliata* seed cluster production** - Mean (\pm SEM) number of (A) immature, (B), mature, and (C) total seed clusters per flat per week starting with either 20 weevils (treatment) or no weevils (control). Asterisks indicate significant differences in number of clusters between the treatment and control flats ($P < 0.05$, Student's paired t-test, $N=8$).

The addition of weevils to the flat reduced the average number of mature seeds produced per plant from 1632 ± 317 seeds per flat to 1100 ± 333 seeds per flat, a reduction of 32.3% ($t = 3.28$; $df = 7$; $P = 0.0135$). Although there was no difference at the end of the experiment in the number of clusters per flat ($t = 1.13$; $df = 7$; $P = 0.2975$), weevil activity reduced the number of seeds per cluster from 11.0 ± 1.5 to 7.0 ± 1.7 seeds per cluster ($t = 4.58$; $df = 7$; $P = 0.0025$).

The addition of weevils to a flat of mile-a-minute reduced the above ground dry biomass (not including seed weight) from $227.2 \text{ g} \pm 13.9 \text{ g}$ to $188.9 \text{ g} \pm 15.8 \text{ g}$ ($t = 3.04$; $df = 7$; $P = 0.0118$), a 16.8% reduction. However, there was no effect of weevil presence on the per-seed biomass of immature or mature berries, either fresh or dry ($t \leq 1.00$; $df = 7$; $P \geq 0.3499$).

There was no effect of weevil activity on the percentage of seeds viable for immature ($80.0\% \pm 3.0\%$) or mature ($98.0\% \pm 1.0\%$) seeds ($t \leq 1.63$; $df = 4$; $P \geq 0.178$).

Discussion

These findings provide important quantitative justification for previous claims made by Lake et al. (2011) and Cutting and Hough-Goldstein (2013) stating that weevils reduced seed production using seed clusters as an index of reproductive potential. This is the first study to demonstrate that weevil activity, both feeding directly by adults on developing seeds and feeding by all life stages on the entire plant, reduced the number of seeds per cluster. This not only verifies the claims of the previous authors, but also suggests that the impact of weevil feeding in these experiments on the reproductive potential of *P. perfoliata* is greater than initially expected.

A second way in which weevil feeding was demonstrated to affect the reproductive potential of *P. perfoliata* is by shifting of the onset of fruit production and maturation later in the season. This confirms and expands upon the findings of Hough-Goldstein et al. (2008b). My greenhouse study described above clearly shows that weevil activity delays both the onset of seed production and the onset of seeds with mature fruits. Shifting the reproductive phase of the plant later in the growing season reduces the amount of time between the initiation of seed production and the time of plant senescence. This reduces not only the total number of seeds produced, but also, and more important, the number of seeds surrounded by mature fruits, which, as shown in the previous chapter, have a higher viability rate than immature seeds. Further, since seeds surrounded by mature fruits are more attractive to wildlife and capable of floating, the weevil also limits the ability of *P. perfoliata* to disperse to new areas either via water or ingestion by wildlife. This is one possible way in which the weevil may slow the spread of *P. perfoliata*, but as there is no way to determine how the invasive plant would be spreading in the absence of the weevil there is no way to confirm this hypothesis.

The most novel information that can be drawn from this study, however, addressed both adult weevil feeding preference and the effect of weevil activity on seed viability. The field study, which confined feeding by adult weevils to developing seed clusters, showed that adult weevils will in fact feed on developing seeds as was suggested by Colpetzer et al. (2004b). The most direct evidence for this claim is provided in the reduction in weight per berry by weevils, likely showing that adult weevils fed directly on the berry. Secondary evidence is provided by the observation of live weevils six weeks after their confinement to the cluster, likely showing they

were able to obtain nutrition and water from the cluster. Interestingly, however, when adult weevils were allowed to choose where to feed over the entirety of the plant, there was not found to be a reduction in seed biomass, indicating that when given a choice between foliage and seeds, adult weevils preferentially feed on foliage. This is further supported by the finding that above ground plant biomass decreased in treatments with weevils.

The proportion of seeds viable correlated very closely with adult weevil feeding preference. When adult weevils were forced to feed on developing seeds, the proportion of viable seeds decreased. Conversely, when weevils were allowed to feed on the entirety of the plant there was no effect on the proportion of viable seeds. This suggests that if weevils reach very high densities and are forced to feed on seed clusters due to intraspecific competition, the reduction in seed viability may further reduce the reproductive potential of *P. perfoliata*. It is important to remember that even when weevils were allowed to feed on the entire plant, the total number of seeds was still reduced and there was a delay in the onset of seed production, and that this drop in viability is just another way in which reproductive potential may be lowered in some cases.

Preliminary population modeling by Hyatt and Araki (2006) indicated that reductions in plant germination, survival, and fecundity are the most likely way in which the spread of *P. perfoliata* can be controlled. Their model does not differentiate between these three variables, but as an annual plant (in temperate regions where this study was conducted) the population dynamics of *P. perfoliata* are driven mainly by the number of seeds produced, the number of seeds germinating in a given year, and the number of seedlings surviving. While percent cover is an easily measurable index

for plant success and has been used both for *P. perfoliata* and other invasive annual plants, by changing the focus of experiments to the reproductive phase of the plant we will be able to acquire information which will allow us to better predict population trends.

However, despite all these avenues for reduction in *P. perfoliata* reproductive potential, there is still likely a large overabundance of seed production. Therefore, it is probable that competition amongst seedlings for resources and space the following spring serves as a major mechanism for reducing the number of *P. perfoliata* seedlings as was suggested by Colpetzer et al. (2004b). This remains the largest unanswered component of how *P. perfoliata* populations are likely to fluctuate from year to year, and warrants further study.

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