

# Variable Seed Viability of Mile-a-Minute Weed (Devil's Tearthumb, *Persicaria perfoliata*)

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Mile-a-minute weed or devil's tearthumb (*Polygonum perfoliatum*, syn. = *Persicaria perfoliata*) is an invasive annual vine in the Mid-Atlantic and Northeastern United States that reproduces solely through seeds. Our study aimed to identify how mile-a-minute seed viability is affected by time of year and the maturity of the fruit surrounding the achene. Full-sized immature (green) and mature (blue) fruits were collected from five field sites every 2 wk over a 3 mo period, and seed viability was assessed using a triphenyl tetrazolium chloride (TZ) assay. At the onset of seed production in mid-August, 35% of seeds from immature fruits were viable. This percentage increased steadily, peaking at 84% in late September before declining at some sites around the time of the first frost. In contrast nearly all seeds with mature fruits (96%) were viable at all collection dates. Thus land managers who apply physical or chemical control methods for mile-a-minute weed should do so before the onset of any seed production and not simply before fruit maturation. If it is necessary to apply control methods after fruit set, it should be done as early in the season as possible.

**Nomenclature:** Mile-a-minute weed; devil's tearthumb; *Persicaria perfoliata* (L.) H. Gross; *Polygonum perfoliatum* L., POPE10.

**Key words:** Fruit maturity, intra-season variability, phenology, Polygonaceae, seed maturity.

Mile-a-minute weed or devil's tearthumb [*Polygonum perfoliatum* L., syn. = *Persicaria perfoliata* (L.) H. Gross (Freeman and Reveal 2005)], is an invasive weed in the eastern United States. It is native to China, Korea, Japan, and other parts of Asia (Cusick and Ortt 1987) and was likely accidentally introduced as seeds contaminating a shipment of holly seeds to a nursery near York, PA, in the 1930s (Moul 1948). After a lag period, the plant underwent rapid population expansion, and now occupies a range from Massachusetts to North Carolina, and westward to Ohio (EDDMapS 2013).

Mile-a-minute is an annual, herbaceous vine capable of growing up to 15 cm (6 in.) per day (Lehtonen 1994; Mountain 1989; Okay 1997). In its native range the plant is found primarily in riparian areas, but in its introduced range it colonizes disturbed sites, roadsides, utility rights-of-way,

and forest edges (Hough-Goldstein et al. 2008a; Wu et al. 2002). Mile-a-minute is easily identifiable because of its distinctive triangular leaves, circular ocreae, and recurved spines along its stems and petioles. As a result of its rapid growth rate and climbing habit, mile-a-minute can be a pest of tree plantations, orchards, and natural areas, where it overgrows and out-competes native vegetation (Hough-Goldstein et al. 2008a; Lehtonen 1994; McCormick and Hartwig 1995; Moul 1948; Okay 1997; Oliver 1996; Wu et al. 2002).

Mile-a-minute reproduces solely through seeds, and a single plant is capable of producing thousands of seeds (Hough-Goldstein et al. 2008b). Black shiny achenes are the true botanical "fruits" in mile-a-minute (i.e., the ripened ovary containing the seeds), but in this species these are surrounded by berry-like fleshy coverings derived from the perianth (Freeman and Reveal 2005); hereafter we refer to these structures as fruits, using the colloquial sense of the term. Mile-a-minute fruits develop in clusters of 4 to 24 (personal observation, JRS) and are initially green, but turn blue upon maturation (Hickman and Hickman 1977).

In the Mid-Atlantic region, mile-a-minute seeds are often produced beginning in late June, but the vast majority of seeds are produced later in the season, from August to October (Cutting and Hough-Goldstein 2013;

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## Management Implications

This study highlights two important implications for managing the invasive vine mile-a-minute weed (devil's tearthumb) using either chemical or physical control methods. First, mile-a-minute seeds surrounded by immature fruits were shown to be viable to some extent throughout the entire season of seed production. Therefore, physical and chemical controls should be applied before any full-sized immature (green) fruits are produced and not just before mature (blue) fruits are present. Second, the viability of seeds in green fruits increased throughout the season, peaking before the first frost. This indicates that if it is necessary to apply physical or chemical control methods during the fruiting period, these methods should be applied as early in the season as possible, when immature fruits are less likely to contain viable seeds.

Hough-Goldstein et al. 2008b; Mountain 1989). The timing of seed production varies by location, likely caused both by climatic variation following latitudinal gradients and by microclimatic conditions such as moisture levels and light availability (Sultan 2001; Sultan and Bazzaz 1993). For example, shaded *P. perfoliata* plants produce far fewer seeds than nearby plants located in full sun (Hough-Goldstein 2008; Hough-Goldstein and LaCoss 2012), but the impact of shade or other factors on phenology of seed production or viability is not known.

Mature mile-a-minute fruits are primarily dispersed by animals, including birds and deer, although frugivory is not required for germination (Hill et al. 1981; Hough-Goldstein et al. 2008a; McCormick and Hartwig 1995; Mountain 1989). The fruits are also buoyant and disperse via water in riparian and flood plain habitats (Okay 1997). Seeds require a period of cold stratification in order to germinate and, while most germinate the first or second year after production, some remain viable in the seed bank for up to 6 yr (Colpetzer and Hough-Goldstein 2004; Hough-Goldstein et al. 2008a; Johnson 1996; Okay 1997).

Traditional control of *P. perfoliata* has relied either on physical techniques, such as hand weeding or mowing, or chemical control, principally through broad-spectrum herbicides. Although there is a biological control program in place for mile-a-minute using the host-specific weevil *Rhinoncomimus latipes* Korotyaev (Hough-Goldstein et al. 2008a, 2012), physical and chemical control techniques are still frequently used. Understanding how seed viability varies based on the time of year the seed is produced is essential information for proper timing of chemical or physical controls to limit the reproductive potential of an invasive or aggressive plant (Benefield et al. 2001; McCaughey and Stephenson 2000; Thomsen et al. 1997). However, there has been relatively little research on the development of seed viability for invasive plants over the course of a single growing season (Benefield et al. 2001; Liao et al. 2000; McCaughey and Stephenson 2000).

In addition to varying across the growing season, seed viability may also vary among sites, depending on conditions such as light, temperature, and precipitation. Therefore, fruit color could serve as a better indicator of seed viability than time of year across a diverse array of habitats. Land managers seeking to control mile-a-minute may assume that seeds surrounded by full-sized green, immature fruits are not viable, but no empirical evidence currently exists to support this. Fruits dispersed by diurnal animals typically change color and become more conspicuous as they ripen. Ecologists view these changes as an evolved advertisement to potential dispersers that the fruit is now edible (Herrera 1982; Schaefer and Schaefer 2006; Schaefer et al. 2008), with the assumption that a fruit only matures when the seed within it is viable. However, in some cases seeds may be viable and capable of germinating before the surrounding fruit is fully mature (Bewley and Black 1994; Foster 1977; Hay and Smith 2004; Kermodé 1990; Miles et al. 1988).

Our study aimed to answer two primary questions. First, does the time of year seeds are produced affect their viability? Second, is there a difference in seed viability between seeds surrounded by full-sized green, immature fruits, and those surrounded by blue, mature fruits? By gaining more information on how the phenology of the plant influences the viability of its seeds we can better inform land managers about when to apply mechanical and chemical control methods.

## Materials and Methods

For this study, five field sites were selected in Delaware, Pennsylvania, and New Jersey (Table 1A). 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 sites also varied in light conditions. The two most northerly sites, Waterloo Mills (a light gap within a forest) and Pennypacker Park, were the most heavily shaded sites, followed by the most southern site, the Pennsville roadside. The Longwood Gardens and White Clay Creek sites were generally in full sun at mid-day.

Collections of *P. perfoliata* fruits were made at all five sites every 2 wk from mid-August to late October (Table 1B). If possible, 50 immature fruits and 50 mature fruits were collected. Immature fruits were defined as green fruits of approximately the same size as mature (blue) fruits, which did not rupture when mild pressure was applied, indicating the presence of an achene within the fruit. Several sites had only immature fruits present at the first two collection dates, and two sites had no fruits present at the last collection date because of plant senescence (Table 1).

Fruits were returned to the laboratory and held at room temperature to desiccate. On November 15, 3 wk after the

Table 1. A. Field site locations (from north to south), and B. dates of collection (2012) and types of fruits collected.

	Waterloo Mills	Pennypacker Park	Longwood Gardens	White Clay Creek	Pennsville roadside
<b>A. Locations</b>					
City or Township, State	Easttown Township, PA	Haddonfield, NJ	Kennett Square, PA	Newark, DE	Pennsville, NJ
Latitude (N)	40°1'15.75"	39°54'13.94"	39°52'45.40"	39°43'37.27"	39°37'7.81"
Longitude (W)	75°24'57.42"	75°1'21.49"	75°40'16.52"	75°46'5.12"	75°32'5.76"
<b>B. Collection dates</b>					
Aug. 13–15	Imm <sup>a</sup>	Imm	Imm, Mat	Imm, Mat	Imm
Aug. 27–30	Imm	Imm	Imm, Mat	Imm, Mat	Imm, Mat
Sept. 13–15	Imm, Mat <sup>b</sup>	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat
Sept. 27	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat
Oct. 11–13	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat	Imm, Mat
Oct. 25	None found	Imm, Mat	Imm, Mat	None found	Imm, Mat

<sup>a</sup>Imm: immature, full-sized green fruit.

<sup>b</sup>Mat: mature, blue fruit.

final collection, seed samples were placed in individual resealable plastic bags filled with moist peat moss and held in a refrigerator at  $4 \pm 1$  C ( $39 \pm 2$  F) for 4 mo. Achenes were then removed from the peat moss and viability was assessed using a triphenyl tetrazolium chloride (TZ) assay (Peters 2000). For each sample, 25 of the achenes were selected at random and a small incision was made in each using a scalpel. The achenes were then soaked in distilled water for 24 h after which each group of achenes was submerged in a 1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride (Sigma-Aldrich Chemical Supply Company, St. Louis, MO). Containers with the solution were closed with fitted lids and covered with aluminum foil. After 24 h achenes were rinsed with distilled water, bisected using a scalpel, and examined under a microscope. With this test, viability is assessed based on red staining of the embryo, a result of reduction of 2,3,5-triphenyl-2H-tetrazolium chloride indicating the activity of dehydrogenase enzymes that are present in living tissue. TZ assay results generally agree closely with those of germination tests, but include viable seeds that might not germinate in a given year because of dormancy (Roberts 1972).

**Data Analysis.** We analyzed the proportion of seeds viable using a linear model by site, time of collection, and the maturity of the surrounding fruits. We transformed the proportion of seeds viable using an arcsine-square-root transformation to improve normality and homoscedasticity for the statistical analysis, but untransformed data are presented in Figure 1. The samples from mid-October and late October were excluded from the models, because on the morning of October 13, 2012, temperatures throughout the sampling region were either slightly above or slightly below the freezing point (DEOS 2013), likely causing plant senescence at some of the study sites. All

statistical analyses were performed using R Statistical Software (R Core Development Team 2012).

## Results

Differences were observed among sites in both the time of fruit maturation and the time of plant senescence (Table 1B). Mature fruit had already developed at Longwood Gardens and White Clay Creek at the beginning of the experiment in mid-August. Mature fruit appeared at the Pennsville roadside site 2 wk into the experiment and at the Waterloo Mills and Pennypacker Park sites 4 wk into the experiment (Table 1). At the end of the season, plants at Waterloo Mills and White Clay Creek senesced at least 2 wk before the other sites (Table 1B). Further, there was a significant effect of site on seed viability from the first four samples ( $F_{4, 15} = 14.0$ ,  $P < 0.001$ ) and a significant

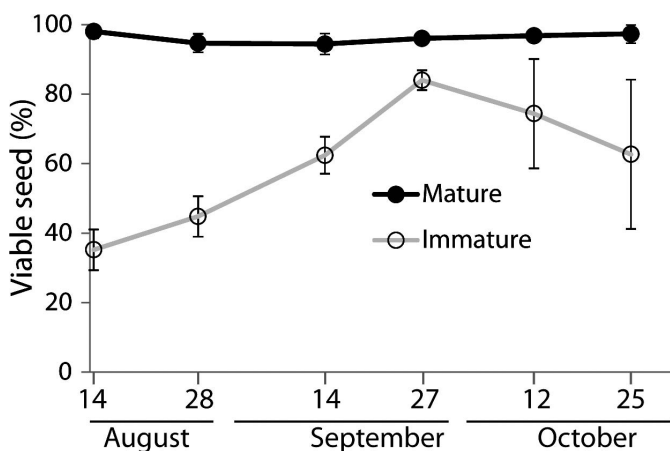


Figure 1. Mean ( $\pm$  SEM) percentage of viable seeds from mature (blue) and immature (green) fruits from five sites.

interaction between time of collection and site ( $F_{4, 15} = 4.0$ ,  $P = 0.020$ ).

The percentage of seeds viable in the first four collection dates was affected by the time of collection ( $F_{1, 15} = 81.3$ ,  $P < 0.001$ ), with a strong positive correlation between time of year and total seed viability ( $r^2 = 0.909$ ,  $F_{19, 15} = 18.9$ ,  $P < 0.001$ ,  $y = 0.123x + 0.668$ ). The proportion of seeds viable was significantly higher for seeds surrounded by mature fruits than for those surrounded by immature fruits ( $F_{4, 15} = 175.2$ ,  $P < 0.001$ ).

There was also a significant interaction between time of collection and maturity ( $F_{1, 15} = 17.6$ ,  $P < 0.001$ ), indicating that the seasonal trend for seed viability followed distinctly different trends based on fruit maturity. On average, 35% of the seeds from immature fruits collected in mid-August were viable, and this percentage increased steadily until late September, when 84% were viable (Figure 1). There was considerable variation in percent viability in seeds from green fruit collected on the last two sample dates (Figure 1). This was primarily caused by low viability of seeds from immature fruit collected at Waterloo Mills in mid-October (12% viable) and at Pennypacker Park in late October (20% viable); seeds from immature fruits collected at the other sites on these two collection dates ranged from 80 to 96% viable. In contrast, most seeds from mature fruits were viable at all six collection dates, with viability averaging 96% (Figure 1).

## Discussion

Several previously unknown characteristics of the reproductive strategy of mile-a-minute weed were elucidated in this study. We determined that a portion of the seeds surrounded by immature fruits are viable and that proportion increases throughout the season. In contrast, an average of 96% of seeds within mature fruits were viable on all collection dates. Both of these findings have important implications for the timing of physical and chemical control techniques to maximize their effectiveness.

The upward trend in seed viability across the season is similar to trends observed in seeds of various agricultural crops (e.g. Kermodé 1990; Miles et al. 1988), and the invasive weeds purple loosestrife, *Lythrum salicaria* L. (McCaughy and Stephenson 2000), and yellow starthistle, *Centaurea solstitialis* L. (Benefield et al. 2001). However, these studies looked at total seed viability and did not consider such trends with respect to the maturity of the fruit. In our study we found that a very high proportion of seeds in mature fruits were viable throughout the season, but viability of seeds from immature fruits increased steadily until plant senescence. We hypothesize that as the season progresses, *P. perfoliata* allocates a greater portion of its available energy from vegetative growth to reproductive growth (Bazzaz et al. 1987), including seed maturation,

resulting in a reduction in the length of the delay between seed maturation and fruit maturation.

In the early stages of seed development following fertilization there is a period of rapid cell division and differentiation followed by a maturation phase, when storage reserves are laid down. The attainment of maximum dry weight at “mass maturity” is thought to coincide with formation of an abscission layer between the seed and the parent plant (Hay and Smith 2004; Hong et al. 2000; Probert et al. 2007; Vidigal et al. 2011). Seeds also acquire desiccation tolerance, or the ability to survive an extended dry period without losing germinability, during the maturation period. In our experiment, all seeds were air-dried for at least 3 wk and then cold stratified for 4 mo, as required for germination (Colpetzer and Hough-Goldstein 2004), before being assayed for viability. The timing of mass maturity relative to the acquisition of germinability and desiccation tolerance varies among species, populations, and even among individuals from the same population (Hay and Smith 2004; Hay et al. 2010; Probert et al. 2007).

Fruit color in agricultural crops is often associated with seed maturation (e.g. Rao et al. 2005; Vidigal et al. 2011), and the same is true of most wild plants that have been studied, though with considerable variation (Hay and Smith 2004). One study that considered seed viability for immature and mature fruits separately was conducted by Foster (1977), who reported that seeds taken from immature and mature *Ardisia revoluta* Humboldt, Bonpland, and Kunth germinated with almost equal frequency, but seeds from mature fruit germinated more quickly than those from immature fruit. Foster (1977) also determined that seeds taken from fruits of different ripeness were similar in weight, while exocarp and mesocarp weights were generally lower in immature and ripening fruit than in mature fruit, suggesting that seeds develop and mature more rapidly than other fruit tissues. In mile-a-minute, where the “fruit” is derived from the perianth after flowering, the weight of immature fruits is similarly generally lower than that of mature fruits (unpublished data), suggesting that its seeds also develop and mature more quickly than fruit tissues.

Late in the season, around the time of the first frost, we found very low viability of seeds in immature fruits at the two most northerly and shaded sites, Waterloo Mills and Pennypacker Park, while seeds from mature fruits at these sites remained highly viable. The fact that seeds produced late in the season at these sites failed to mature, but those that matured a few weeks prior were not impacted, indicates that the change in seed viability is likely derived from a change in plant health at the time of seed production, here probably caused by freezing. The most likely explanation is that seeds in mature fruits have acquired sufficient resources and face no negative consequences from plant senescence, while senescing plants do not have enough resources at the end of the year to invest in

continuing the development of immature seeds. Variation in germination and viability in crop seeds have been shown to be dependent on weather conditions both before and at harvest, with susceptibility dependent on the state of maturity of the seeds (Austin 1972; Woltz et al. 2006).

Plants in shady sites may have had even fewer resources remaining to invest in maturing seeds than those in sunny sites. This is consistent with other studies that have shown reduced seed production and plant biomass for mile-a-minute plants grown in the shade compared to plants grown in the sun (Hough-Goldstein 2008; Hough-Goldstein and LaCoss 2012; Smith and Hough-Goldstein 2013) as well as studies on other plant species that showed a decrease in seed viability of seeds produced in the shade (Cantagallo et al. 2004; DiTommaso et al. 2005; Smith et al. 2006). We hypothesize that the combination of shade and declining temperatures contributed to the decrease in seed viability for seeds surrounded by immature fruits at Waterloo Mills and Pennypacker Park.

The timing of both fruit set and fruit maturation varied among the five study sites. Further, there was a significant effect of site, time of collection, and their interaction on the percentage of seeds viable. This is not unexpected, given the variability shown in other seeds based on climatic factors (Hierro et al 2009; Sultan 2001; Sultan and Bazzaz 1993), but it is information that needs to be disseminated to land managers to reinforce that the timing of any 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 that land managers could use to account for variations between sites and years in the timing of seed production and viability. Future work should test whether degree days can in fact be used to estimate the timing of viable seed production. However, the differences found here did not vary in an obvious way from north to south, suggesting that site microclimate characteristics, including extent of shade present, may be as important as general temperature and photoperiod regimes in determining the timing of seed production and maturation.

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