

A rapid germination protocol for mile-a-minute weed, *Polygonum perfoliatum* L

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(Accepted October 2003)

Summary

A method for breaking dormancy and rapidly inducing germination of *Polygonum perfoliatum* L. achenes was developed through a series of laboratory and greenhouse experiments. *Polygonum perfoliatum* achenes were subjected to various treatments including mechanical scarification, chemical scarification, inundation, cold-wet stratification (4°C), warm-wet stratification ($\approx 23^\circ\text{C}$) and combinations of these treatments. After-ripened, stored achenes that were scarified for 60 minutes in 93% sulfuric acid and inundated with deionized water germinated following three to four weeks of stratification at 4°C. A subsequent study showed that post-harvest storage duration ("after-ripening") influenced the results of the germination protocol. Achenes germinated after one week of post-harvest storage at $\approx 23^\circ\text{C}$, but a high germination percentage ($85 \pm 4\%$) was not achieved until after five weeks of post-harvest storage. Achenes that were stratified in leachate water had lower germination percentages than achenes stratified in deionized water, but inhibition of germination was reduced over time. The results of these experiments suggest that *P. perfoliatum* achene dormancy is enforced by both coat-imposed and chemical-based mechanisms. Coat-imposed dormancy was overcome by scarifying achenes for 60 minutes in 93% sulfuric acid while chemical-based dormancy was overcome by storing freshly harvested achenes for at least one week at $\approx 23^\circ\text{C}$ and stratifying the seed in deionized water at 4°C for four weeks.

Introduction

Few studies have been published on the dormancy and germination requirements of mile-a-minute weed, *Polygonum perfoliatum* L., achenes. Wilbur Mountain, the Pennsylvania State botanist, conducted the earliest research into the reproductive biology of *P. perfoliatum* in the United States (Johnson 1996). His unpublished study showed that the achenes of *P. perfoliatum* required at least six weeks of cold-wet stratification to germinate, but maximum germination (80-89%) occurred after 18 weeks (Johnson 1996). Johnson (1996) demonstrated that 96% germination could be achieved with as few as nine weeks cold-wet stratification if achenes were first scarified with sulfuric acid and that similar germination percentages did not occur in non-treated achenes until after 18 weeks of cold-wet stratification. Johnson (1996) also showed that *P. perfoliatum* achenes were capable of germinating over a wide temperature range, from 2 to 20°C. Yang and Kim (1993) reported that cold-wet stratification was needed to break achene dormancy in a Korean population of *P. perfoliatum* and that light did not stimulate or

inhibit germination. Washitani and Masuda (1990) reported similar results for a Japanese population of *P. perfoliatum*. In 1997, Okay again demonstrated that the achenes of *P. perfoliatum* required a period of cold-wet stratification to germinate. More recently, Wu *et al.* (2000) showed that *P. perfoliatum* achenes stored at room temperature before stratification had higher germination percentages than achenes stored at low temperatures and that the amount of stratification needed to induce germination decreased with length of storage.

The objective of this investigation was to develop a protocol capable of achieving rapid and high germination percentages in *P. perfoliatum* achenes. Rapid germination was desired to permit continual production of young *P. perfoliatum* plants for evaluation of potential biological control agents in the United States, where the weed was introduced and has become noxious.

Materials and methods

Seed collection and pretreatment for first and second trials

Polygonum perfoliatum achenes were collected in September 2001 from plants growing near the Chambers Rock Parking Area, White Clay Creek State Park, Newark, DE (39°44'01''N 75°45'46''W). Achenes were dried for three weeks at room temperature ($\approx 23^{\circ}\text{C}$) because after-ripening is often a requirement for germination in *Polygonum* species (Fordham 1990, Timson 1965). Following after-ripening, perianths were removed from the achenes by hand and they were stored dry in a 26.8 × 27.9 cm self-sealing plastic bag (Glad Products Company, Oakland, CA) at $4 \pm 1^{\circ}\text{C}$ until used (eight weeks later for the first trial and 15 weeks later for the second trial).

First trial

The following treatments were each applied to 200 achenes: control (exposed to moisture only), mechanical scarification, inundation (submerged in water), acid scarification for various lengths of time with or without inundation and acetone scarification for various lengths of time. For the control, groups of 20 achenes were wrapped in paper towels, moistened with deionized water, placed in a 16.8 × 14.9 cm self-sealing plastic bag (Glad Products Company, Oakland, CA) and stored at $4 \pm 1^{\circ}\text{C}$.

Mechanical scarification was accomplished following the protocol of Parham (1999). Two hundred achenes were rubbed between sheets of 100-grit sandpaper for 10 minutes and then a test achene was soaked in water overnight at room temperature. The process was repeated each day until the test achene imbibed, which occurred after six attempts or a total of one hour of mechanical scarification. Achenes were then wrapped in moist paper towels, sealed in plastic bags and stored at $4 \pm 1^{\circ}\text{C}$.

For the inundation treatment, two groups of 100 achenes each were placed in separate plastic containers (5.5 cm diameter and 5.0 cm depth) and submerged in 100 ml of deionized water. Containers were covered with plastic lids, wrapped in parafilm and stored at $4 \pm 1^{\circ}\text{C}$.

For the acid scarification treatments, groups of 200 achenes were placed in 93% sulfuric acid for 10, 30, or 60 minutes. After soaking in sulfuric acid, achenes were rinsed

for five minutes under running tap water and then submerged three times for 10 minutes each in 500 ml of deionized water. Achenes were wrapped in moist paper towels in groups of 20, sealed in plastic bags and stored at $4 \pm 1^\circ\text{C}$.

For the combined acid scarification and inundation treatment, 200 achenes were placed in 93% sulfuric acid for 60 minutes. A 60-minute scarification removed most of the pericarp and allowed seeds to imbibe fully prior to inundation (indicated by splitting of the remaining pericarp into three plates during rinsing). Thus, any water-soluble germination inhibitors present in the testa, endosperm or embryo should leach quickly from the seed. Following scarification, achenes were rinsed using the method described above, placed in plastic containers in groups of 100, inundated with 100 ml of deionized water and stored at $4 \pm 1^\circ\text{C}$.

For the acetone scarification treatment, groups of 200 achenes were placed in pure acetone for 10, 30, or 60 minutes. Following scarification, achenes were rinsed as described above, wrapped in moist paper towels, sealed in plastic bags and stored at $4 \pm 1^\circ\text{C}$.

In December, after four and six weeks of stratification, 100 achenes from each treatment were split into five replicates and each replicate of 20 achenes was wrapped in a paper towel, moistened with deionized water and sealed in a separate plastic bag. Replicates were transferred to a greenhouse maintained at $20 \pm 2^\circ\text{C}$ and randomly placed on a centrally located table that was directly beneath a 1000 watt General Electric metal-halide lamp and bordered by four 215 watt Sylvania Gro-Lux lamps, which provided a 16-hour photophase and a minimum photosynthetic photon flux of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The bags of achenes were held in the greenhouse under the above conditions for four weeks, germination was checked weekly and bags were placed in the same location after checking germination. An achene was considered to have germinated when the radicle protruded through the pericarp.

Second trial

The following treatments were each applied to 500 achenes: control (exposed to moisture only), mechanical scarification, inundation (submerged in water) at 4°C , acid scarification with or without inundation at 4°C or at room temperature and acetone scarification with or without inundation at 4°C or at room temperature. For the control, groups of 20 achenes were wrapped in paper towels, moistened with deionized water, sealed in plastic bags and stored at $4 \pm 1^\circ\text{C}$.

For mechanical scarification, 500 achenes were rubbed for more than one hour between sheets of 100-grit sandpaper, wrapped in moist paper towels and sealed in plastic bags as described above. The duration of this treatment was established in the first trial and thorough scarification was ensured using the test method described by Parham (1999).

For the inundation treatment, five groups of 100 achenes each were placed in separate plastic containers (5.5 cm diameter and 5.0 cm depth) and inundated with 100 ml of deionized water that contained 100 ppmv Q-san (Chemstar Corporation, Lithia Springs, GA), a food grade quaternary sanitizer that inhibits fungal growth. Containers were covered with plastic lids, wrapped in parafilm and stored at $4 \pm 1^\circ\text{C}$.

For the acid scarification treatments, 1500 achenes were placed in 93% sulfuric acid for 60 minutes. A 60-minute scarification was used because in the first trial, it yielded a higher germination percentage than 10 or 30 minutes, following six weeks of cold-wet stratification. After soaking in sulfuric acid, achenes were rinsed as described above. Five hundred achenes were wrapped in moist paper towels in groups of 20 and sealed in plastic bags as above. The remaining 1000 achenes were placed in separate plastic containers in groups of 100 as above, inundated with 100 ml of deionized water containing 100 ppmv Q-san, covered with plastic lids and wrapped in parafilm. Five of these containers were stored at $4 \pm 1^\circ\text{C}$ and the rest were placed in a desk drawer at room temperature ($\approx 23^\circ\text{C}$).

For the acetone scarification treatments, 1500 achenes were placed in pure acetone for 30 minutes. A 30-minute scarification was used because in the first trial, it yielded a higher germination percentage than 10 or 60 minutes, following six weeks of cold-wet stratification. The achenes were rinsed as described above, sorted into plastic bags and containers as above, half were stored at $4 \pm 1^\circ\text{C}$ and the rest were stored in a desk drawer at room temperature.

In January and February, after 2, 3, 4, 5 and 6 weeks of stratification, 100 achenes from each treatment were split into five replicates and each replicate was wrapped in a paper towel, moistened with deionized water and sealed in a plastic bag. Replicates were randomly placed on a centrally located table in the greenhouse as above. The bags of achenes were held in the greenhouse for one week following stratification and then checked for germination. Replicates were kept in the greenhouse for only one week because achenes from the first trial that failed to germinate within one week of being placed in the greenhouse did not germinate.

Effects of post-harvest storage and seed leachate

Three thousand *P. perfoliatum* achenes were collected on 11 October 2002 from plants growing near the Chambers Rock Parking Area, White Clay Creek State Park, Newark, DE. Perianths were removed from the achenes by hand and the achenes were scarified for 60 minutes in 93% sulfuric acid as described above to remove their pericarps. Following scarification, groups of 1000 seeds were placed in beakers, inundated with 500 ml of deionized water, covered with parafilm and stored at $4 \pm 1^\circ\text{C}$. This procedure was designed to leach any water-soluble germination-inhibitors from the testa, endosperm or embryo. Such compounds could be present at high levels immediately following harvest. One week later, on 18 October 2002, more achenes were collected from the same area of White Clay Creek State Park. These achenes were stored in an open plastic container at room temperature until used. Each week for 13 weeks perianths were removed from 200 of these achenes and they were scarified for 60 minutes as described above to assess the effects of achene storage on germination. Following scarification, groups of 100 seeds were placed in separate plastic containers (5.5 cm diameter and 5.0 cm depth) and inundated with either 100 ml of deionized water or 100 ml of the water used to leach seeds scarified on 11 October 2002. This water had turned yellow and contained a white precipitate by 18 October 2002. Plastic containers containing inundated seeds were covered with lids, wrapped in parafilm and stored at $4 \pm 1^\circ\text{C}$ for four weeks. After four

weeks of stratification, five replicates of 20 seeds from each treatment were wrapped in separate paper towels, moistened with deionized water, sealed in separate plastic bags and transferred to a greenhouse as described above. The bags of seeds were held in the greenhouse for one week and then checked for germination.

Statistical analysis

For all experiments, percent germination data were arcsin square root transformed and analyzed using a two-way ANOVA by treatment and duration of stratification or storage. A one-way ANOVA was then performed on data for each trial with each treatment/ time combination considered separately. Tukey's range test was used for mean separation.

Results

First trial

After four weeks of cold-wet stratification, germination was observed only in the combined acid scarification/ inundation treatment, with 90% germinating (table 1). After six weeks of stratification, 91% of the achenes subjected to this treatment germinated and 6 to 10% of the achenes that were treated with acid alone, inundation alone, or 10 and 30 minutes of acetone scarification germinated (table 1). Achenes that were not treated, mechanically scarified, or scarified for 60 minutes in acetone failed to germinate.

Table 1. Effects of scarification and four or six weeks of cold-wet stratification at 4°C on *Polygonum perfoliatum* achene germination when subsequently kept at 20°C.

Treatment	Percent Germination (Mean ± SEM)	
	Weeks of Stratification	
	4	6
No Treatment	0 c	0 c
Mechanical Scarification	0 c	0 c
Inundation	0 c	6 ± 1 b
H ₂ SO ₄ 10 min	0 c	6 ± 1 b
H ₂ SO ₄ 30 min	0 c	7 ± 1 b
H ₂ SO ₄ 60 min	0 c	10 ± 2 b
H ₂ SO ₄ 60 min + Inundation	90 ± 2 a	91 ± 2 a
Acetone 10 min	0 c	6 ± 1 b
Acetone 30 min	0 c	9 ± 1 b
Acetone 60 min	0 c	0 c

Overall treatments (mean ± SEM) and weeks of stratification differed significantly ($P < 0.0001$ and $P < 0.0001$, respectively). Means followed by the same letter are not significantly different (Tukey's range test applied to arcsin square root transformed data; the average of the untransformed values are shown). Percent germination was calculated from five replicates of 20 seeds in each treatment/ stratification group.

Second trial

No germination was observed in any of the treatments after two weeks of cold or warm stratification (table 2). The acid scarification/ inundation plus 4°C stratification treatment produced 26% germination after three weeks of stratification and 85 and 96% germination after four and six weeks of stratification, respectively (table 2), results similar to those of the first trial (table 1). After six weeks of stratification, germination was observed in all treatments except for the control (no treatment), mechanically scarified and treatments stratified at room temperature (table 2).

Table 2. Effects of scarification and stratification for two to six weeks at 4°C or 23°C on *Polygonum perfoliatum* achene germination when subsequently kept at 20°C.

Treatment	Stratification Temperature, °C	Percent Germination (Mean ± SEM)				
		Weeks of Stratification				
		2	3	4	5	6
No Treatment	4	0 c	0 c	0 c	0 c	0 c
Mechanical Scarification	4	0 c	0 c	0 c	0 c	0 c
Inundation	4	0 c	0 c	0 c	0 c	6 ± 1 c
H ₂ SO ₄ 60 min	4	0 c	0 c	0 c	0 c	19 ± 4 b
H ₂ SO ₄ 60 min + Inundation	4	0 c	26 ± 5 b	85 ± 5 a	91 ± 2 a	96 ± 2 a
H ₂ SO ₄ 60 min + Inundation	23	0 c	0 c	0 c	0 c	0 c
Acetone 30 min	4	0 c	1 ± 1 c	2 ± 1 c	6 ± 2 c	21 ± 5 b
Acetone 30 min + Inundation	4	0 c	0 c	0 c	0 c	1 ± 1 c
Acetone 30 min + Inundation	23	0 c	0 c	0 c	0 c	0 c

Overall treatments (mean ± SEM) and weeks of stratification differed significantly ($P < 0.0001$ and $P < 0.0001$, respectively). Means followed by the same letter are not significantly different (Tukey's range test applied to arcsin square root transformed data; the average of the untransformed values are shown). Percent germination was calculated from five replicates of 20 seeds in each treatment/ stratification group.

Effects of post-harvest storage and seed leachate

Fewer than 10% of achenes stored at room temperature for one or two weeks before stratification germinated, but germination increased to 38% after three weeks, 74% after four weeks and 85% after five weeks of storage for seeds stratified in deionized water (figure 1). Seeds stratified in deionized water had consistently higher germination percentages than those stratified in water that was previously used to leach *P. perfoliatum* seed and this difference was significant between three and seven weeks of post-harvest storage (figure 1).

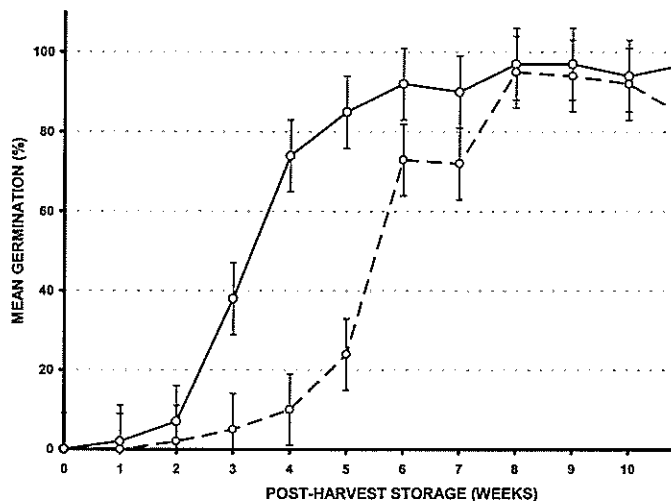


Figure 1. The effect of after-ripening (drying) *Polygonum perfoliatum* achenes at $\approx 23^{\circ}\text{C}$ on germination following acid scarification and 4 weeks of stratification at 4°C . The average of the untransformed mean \pm the detransformed minimum significant difference are shown (Tukey's range test applied to arcsin square root transformed data). Overall treatments and weeks of post-harvest storage differed significantly ($P < 0.0001$ and $P < 0.0001$, respectively). Means were calculated from five replicates of 20 seeds in each treatment/ weeks of post-harvest storage group. —○— inundated with deionized water during stratification. -○- inundated with water previously used to leach *P. perfoliatum* seeds during stratification.

Discussion

This study demonstrated that dormancy of *P. perfoliatum* achenes can be broken and rapid germination can occur if the achenes are sequentially after-ripened for several weeks at $\approx 23^{\circ}\text{C}$, scarified for 60 minutes in 93% sulfuric acid and inundated with deionized water during four weeks of stratification at 4°C . A moderate germination percentage ($38 \pm 3\%$) was achieved by storing freshly harvested achenes at $\approx 23^{\circ}\text{C}$ for three weeks, followed by acid scarification and four weeks of 4°C stratification. A high germination percentage ($85 \pm 4\%$) was achieved using this protocol and extending the post-harvest storage to five weeks. The high germination percentage obtained in this study after four weeks of cold-wet stratification is similar to values obtained by other researchers after 18 weeks of cold-wet stratification when pericarps were left intact (Johnson 1996) and exposed seeds were not inundated during stratification.

Primary dormancy of *P. perfoliatum* achenes appears to be enforced by both coat-imposed and chemical-based mechanisms. The pericarp undoubtedly acts as a mechanical barrier to radicle growth, but the results of this experiment suggested that the pericarp primarily enforces dormancy by being impermeable. If primary dormancy were enforced solely by the pericarp restricting radicle growth, then acetone scarification should not enhance germination because acetone scarification maintains pericarp integrity but permits gas and water exchange (Timson 1965). In the first and second trials, 10 or 30 minutes of acetone scarification enhanced germination. If dormancy were solely

coat-based, then mechanical scarification also should have enhanced germination by weakening the pericarp. However, mechanical scarification did not enhance germination in either the first or second trial. Metzger (1992) noted a number of researchers who concluded that primary dormancy in Polygonaceae achenes was due to the hard pericarp, but he recognized that the radicle emerges from a natural split in the pericarp without ever actually piercing the pericarp.

In addition to coat-imposed dormancy, dormancy of *P. perfoliatum* achenes appears to be enforced by germination-inhibitors. The water used to leach *P. perfoliatum* achenes, which had their pericarps removed by soaking in 93% sulfuric acid for 60 minutes, turned yellow and contained a white precipitate within one week of seed submersion leaching materials from the seeds. Seed inundated with this leachate water had consistently lower germination percentages than those inundated with deionized water (figure 1), indicating an inhibitory nature of the solute(s). Leaching of germination-inhibitors may be the primary reason why both after-ripening and cold-wet stratification were necessary to break dormancy of *P. perfoliatum* achenes. Although after-ripening is not fully understood, intact seeds that have not been dried prior to introduction into a liquid do not leak solutes, while after-ripened (dry) seeds do leak solutes when introduced into a liquid (Bewley and Black 1994). Furthermore, it has been hypothesized that cold stratification increases the concentration of unsaturated fatty acids in cell membranes and this change may increase membrane permeability (Bewley and Black 1994). Thus, after-ripened and cold stratified seeds may lose germination-inhibitors more readily than freshly harvested seeds.

The results of these experiments suggest that *P. perfoliatum* achene dormancy is enforced by both coat-imposed and chemical-based mechanisms. In addition, a rapid germination protocol was developed that allows production of young *P. perfoliatum* plants following three weeks of after-ripening and four weeks of cold-wet stratification, an improvement over other protocols that was exploited in our testing of biological control agents.

Acknowledgements

This research was funded by the USDA, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV. We thank Dr. Wallace Pill and Dr. Hugh Frick for reviewing and making comments on earlier drafts of this manuscript. Published as Paper No. 1728 in the Journal Series of the Delaware Agricultural Experiment Station.

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