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Scarification Technique Affects Germination of Stylisma pickeringii (Patterson bindweed), an Illinois Endangered Plant

ABSTRACT.—The seed coat of *Stylisma pickeringii* (Torr.) Gray var. *pattersoni* (Patterson bindweed), an endangered species of Illinois sand prairies, inhibits water uptake and seed germination. The purpose of this research was to find an effective and efficient way to scarify seeds of *S. pickeringii* to aid reintroduction into its natural habitat. Seeds were collected from sandy areas close to the Illinois River near Snicarte (Mason Co.), Illinois during the summers of 1998 and 1999. Experiments were conducted to determine the best scarification techniques (basal cut, sandpaper shakes, sulfuric acid, sand shakes and sonication). Initially, each technique was evaluated by scarifying the seeds for different times (except for the basal cut). The optimal time for each scarification technique then was compared. Scarified seeds were germinated in petri dishes at 25 C, 16 h photoperiod, with a mean light intensity of 51 μ mol $m^{-2}s^{-1}$. The basal cut, 48 h sandpaper shake, 120 min acid soak and 72 h sand shake techniques did not differ significantly in germination (96, 92, 84 and 84%, respectively). The sonicator technique and the unscarified control yielded only 4 and 0% germination, respectively. For scarification of *S. pickeringii* seeds the 48 h sandpaper shake and 120 min acid soak were very effective and efficient relative to other techniques. Of these two techniques, the sandpaper shake is safer than the acid soak, although when scarifying large numbers of seed, the sandpaper shake would require a large shaker. The techniques have potential applicability to other threatened and endangered species whose seed coat also inhibits germination.

INTRODUCTION

Stylisma pickeringii (Torr.) Gray var. *pattersoni* (Patterson bindweed) is a state-endangered species of sand prairies along the Illinois and Mississippi Rivers in three Illinois counties—Cass, Henderson and Mason. Currently in two of these counties, populations of *S. pickeringii* are located on privately owned land. Typically, *S. pickeringii*, a perennial prostrate or diffusely spreading herb of the Convolvulaceae Family, grows in the south-central United States (Herkert, 1991; USDA, 2001).

Only recently has information pertaining to the plant and its seed been reported. In *S. pickeringii*, the seed coat inhibits germination (Heisler *et al.*, 1999). Preliminary studies show that nonscarified seeds increased in fresh weight by 29.5% after 24 h imbibition compared to 78.7% increase in fresh weight of scarified seeds. Although nonscarified seeds imbibed some water, they did not germinate based on our previous studies. Frequently, inhibition from seed coats can be alleviated by scarification, a process that breaks the seed coat and allows exchange of materials. Scarification techniques used for other species include cutting the seed coat with a blade, manually rubbing the seed on sandpaper, acid treatment or sonication (Baskin and Baskin, 1998). Although these techniques are effective, they are time consuming and hazardous, making them undesirable to use for large numbers of seeds. When seeds of *S. pickeringii* were scarified by cutting the basal end of the seed coat, seed germination was 55% as compared to 0% for seed that was not scarified (Heisler *et al.*, 1999).

The purpose of this experiment was to find a scarification technique that would yield a high seed germination percentage with a more efficient method than the basal cut for scarification of large numbers of seed. Scarification techniques included sandpaper shakes, acid, sand shakes and sonication.

MATERIALS AND METHODS

Fruits of *Stylisma pickeringii* (Torr.) Gray var. *pattersoni* were collected during September 1998 and also in August and September 1999 near Snicarte (Mason Co.), Illinois. Since seeds of different colors differ in germination, seeds were removed from fruits and sorted by color (yellow, tan or maroon) (Heisler *et al.*, 1999). Yellow seeds were scarified using various techniques. Tan and maroon seeds were not used because they germinated poorly or not at all in a previous study (Heisler *et al.*, 1999). Fol-

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TABLE 1.—Percent germination of *Stylisma pickeringii* (fall 1999 seed) with five scarification techniques. Duncan's Multiple Range Test at 5% level. Means followed by a different letter are significantly different, n = 20

Scarification technique	Germination (%)
Basal cut	96 a
Sandpaper shake (48 h)	92 a
Sulfuric acid (120 min)	84 a
Sandpaper shake (72 h)	84 a
Sonicator (2 h)	4 b
Control	0 c

lowing scarification, 5 seeds/dish, 4 dishes/treatment were dusted with the fungicide Thiram (tetramethylthiuram disulfide, 50%), placed on two sheets of filter paper (Whatman #1) in 90 mm glass petri plates with 5 ml distilled water and placed in plastic tubs (to reduce evaporation) in an environmental control chamber at 25 C, 16 h photoperiod with a mean light intensity of 51 μ mole $m^{-2}s^{-1}$. Seeds were considered germinated when the radicle emerged from the seed at least 2 mm. Final results were recorded 10 d after the seeds were placed in the chamber. Data were analyzed with one-way analysis of variance followed by mean separations using Duncan's Multiple Range Test at 5% level.

Fall 1998 seed.—In the spring of 1999 a series of experiments was conducted to determine the optimal length of time for acid scarification. Seeds of *Stylisma pickeringii* were soaked in concentrated sulfuric acid (H_2SO_4) for 0, 15, 30, 45, 60, 120 or 180 min. After the acid soak seeds were rinsed in distilled water for approximately 2 min and germinated as previously described.

Fall 1999 seed.—Experiments were conducted to determine lengths of time that should be used for scarification with sandpaper, sand and sonication. Five seeds were placed in each of four 118 ml jars (6 cm diameter × 7 cm height) either lined with coarse sandpaper (60 grit) or filled with 20 ml coarse sand. The jars then were shaken for 18, 48 or 72 h at 500 rpm. In addition, an experiment was conducted to determine the number of seeds that should be used for sandpaper scarification. Either 5 or 50 seeds were placed in the sandpaper lined jars and were shaken for 48 h at 500 rpm. Scarified seeds were germinated as previously described. The sonication process involved sonicating 20 seeds in 230 ml distilled water in a 300 ml sonicator for 2 or 4 h. Seeds then were blotted with paper towels to remove excess surface water and germinated as previously described.

To determine the best scarification technique, a final germination experiment was conducted using the times determined as optimal for each scarification technique, *i.e.*, 120 min sulfuric acid, 48 h sandpaper shake, 72 h sand shake, 4 h sonication and basal cut (nicking the blunt end of the seed coat with a razor blade). These times were used since each yielded the highest germination within each technique. Seeds were scarified with each of these techniques and placed in the environmentally controlled growth chamber to assess germination as before.

RESULTS

Sulfuric acid scarifications for 0, 15, 30, 45, 60 or 180 min did not differ significantly in germination (0–5%). The 120 min acid scarification had significantly higher (80%) germination than the other times. The 72 h sand shake had a germination of 55%, which was significantly higher than that of the 0, 18 or 48 h sand shakes with 0–5% germination. Seeds shaken in a jar lined with coarse sandpaper for 48 or 72 h had significantly higher germination with 85 and 75%, respectively, than seeds shaken for 18 h (25%), which were significantly higher than those not shaken (0%). However, 5 seeds/jar had significantly higher germination than 50 seeds/jar (85 and 45%, respectively). With the 0 and 2 h sonication, germination was 0%, and with 4 h sonication germination was significantly higher, but still only 10%.

The highest germinations were attained using the basal cut, 120 min acid, 48 h sandpaper shake or 72 h sand shake, with similar germination (84–96%). The sonicator technique had only 4% germination whereas the control had no germination (Table 1).

DISCUSSION

Basal cut.—The basal cut had high germination. Although this method is a relatively rapid technique per seed, it is labor intensive and requires skilled technicians to scarify each seed. Thus, it is not an efficient method when scarifying large numbers of seed. This method also imposes a safety risk, as it is easy to cut oneself when trying to scarify seeds.

Sand and sandpaper.—It is possible that a longer sand shake would provide higher germination than that of the 72 h shake, but it is less desirable than the sandpaper shake, which provided as high a germination percentage in less time. Also, it is more difficult to sort the seeds out of the sand than the sandpaper-lined jars. The 48 h sandpaper shake is easily the most efficient and effective method of the various shake techniques as it works well and is very safe. However, an experiment comparing the number of seeds/jar showed that 5 seeds/jar had significantly higher germination than 50 seeds/jar, suggesting this technique should be limited to less than 50 seeds/jar. Many jars however can be shaken simultaneously on a single shaker to scarify a large quantity of seeds.

Acid.—The 120 min sulfuric acid soak was the best of the acid scarifications. The shorter time periods (0, 15, 30, 45 and 60 min) probably did not break the seed coat enough to allow water to enter the seed. The 180 min acid soak possibly broke the seed coat to the point that the sulfuric acid entered the seed, killing the embryo. Although this method is very efficient and fast, it is more dangerous due to the safety aspect of handling concentrated sulfuric acid (H_2SO_4).

Sonication.—Sonication for 4 h produced higher germination than 2 h, but is still not adequate when compared to other techniques. Although a longer sonication may increase germination, it imposes problems when the scarification is complete. The seeds already have been soaked in water and then may not store well when the process is completed (although this aspect was not investigated). Thus, if scarification by sonication is used, the seeds might need to be scarified just before planting. Therefore, not only is low germination a factor in disregarding this technique as a preferred technique, but sonication also imposes problems when the scarification is finished.

Based upon all of the scarification methods tested, the 48 h sandpaper shake is recommended for scarifying seeds. This method is safe, very effective and more efficient than other techniques when scarifying large numbers of seed. The number of seeds per jar during the sandpaper scarification affected germination. Thus, it is best to use fewer seeds per jar, although large quantities of seeds can be shaken simultaneously. Acid scarification also is a satisfactory method to scarify large numbers of seeds provided that proper safety precautions are taken. Scarification of large numbers of *Stylisma pickeringii* seeds should be a useful technique for re- introduction of this plant into its natural habitat.

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