Scarification and Germination of *Amsonia tabernaemontana* (Walt.) Seeds[1]

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Abstract

Eastern bluestar [*Amsonia tabernaemontana* Walt. (Apocynaceae)] is a herbaceous perennial native to the Eastern United States, yet poor seed germination has inhibited its adoption into commercial product mixes. Germination of *A. tabernaemontana* seeds at a high percentage was demonstrated in this study. Using the best seed treatment protocol developed in this study, *A. tabernaemontana* germinated at 70.4% during a 28 day period. This protocol included mechanical scarification followed by imbibation for 48 hr at 24C (72F). Seeds receiving no scarification and no imbibition germinated at 0% during this same 28 day period. Seeds were dissected to determine the best method for seed coat scarification. These dissections showed embryos were arranged longitudinally within seeds and that mechanically removing 1–2 mm (0.04–0.08 in) of the seed coat terminus to expose the embryo was the best scarification method. Imbibition curves were developed for scarified and control (unscarified) seeds to explain dormancy mechanism(s) for *A. tabernaemontana* seeds. Water was imbibed at a similar rate (P = 0.5217) for all treatments and mechanically scarified seeds germinated at rates higher than unscarified seeds as shown in germination curves, indicating mechanical dormancy is the primary hindrance to germination of this species.

Index words: seed propagation, eastern bluestar, imbibition, mechanical barrier.

Species used in this study: *Amsonia tabernaemontana* Walt. [Apocynaceae] (Nomenclature: USDA NRCS, 2011).

Significance to the Nursery Industry

Eastern bluestar [*Amsonia tabernaemontana* Walt. (Apocynaceae)] is a showy herbaceous perennial that is native to the Eastern United States. Due to its tolerance of dry, non-irrigated landscapes, the popularity of this species is increasing. However, many plants sold in the nursery trade historically have been wild-dug, principally because little information is known regarding the seed propagation of this species beyond reports of slow and uneven germination. Due to increasing concerns over wild digging of plants, many growers continually attempt to utilize seed as a source for liner production, with minimal success. In production environments, untreated seed germination is typically extremely low and germination can take 6–12 months. For that reason, this study aimed to increase the germination percentage of this species while simultaneously shortening the germination period from months to an industry standard 28 days. Using the best seed treatment protocol developed in this study, *A. tabernaemontana* germinated at a rate of 70.4% during a 28 day period. This seed germination protocol should allow growers to produce *A. tabernaemontana* quicker while devoting less bench space and material resources to *A. tabernaemontana* seed germination. This protocol should also allow eliminate the need to wild dig plants and minimize the number of seed collected to establish a uniform crop of *A. tabernaemontana*.

Introduction

Eastern bluestar *(Amsonia tabernaemontana*), is an herbaceous perennial with a native range from southern New York to northern Florida to eastern Texas. The genus *Amsonia* is distributed worldwide with 16 recognized species and numerous subspecies occurring in North America (13). *A. tabernaemontana* is an attractive plant with interesting ornamental characteristics, including pastel blue flowers in the spring and yellow fall foliage. *A. tabernaemontana* experiences few pest and disease problems, making it an ideal addition to the landscape. *A. tabernaemontana* is propagated primarily by seed and are reported to be difficult to germinate in a production environment due to uneven germination time and low germination percentage (9, 12). Current production methods involve planting seed outdoors in fall (September and October), allowing seed to naturally scarify during winter months and germinate in late spring. In a commercial setting this method of natural scarification is inefficient in its requirement for long-term use of production facilities (bench space). Additionally, variable germination times result in non-uniform liner size that can lead to a lack of uniformity later in container production. Several popular publications (1, 4, 10) indicate that removing a portion of the seed coat increases germination percentage of *A. tabernaemontana*. The information provided by these sources is little more than anecdotal notes and provides no details as to the best method of seed scarification and if environmental conditions must be satisfied to facilitate germination. To date no scientific study specifying a technique for seed coat removal or stating a rate (percentage) that seed coat removal affects germination percentage has been published. A series of seed treatments, including the current commercial method of no scarification or imbibition, were developed to determine how scarification and imbibition affected *A. tabernaemontana* germination.

Two imbibition temperatures were utilized; 24C (72F) and 34C (93F). Each temperature was paired with both non-scarified and scarified seed treatments. Using 24C (72F), room temperature, as the base temperature it was hoped that raising the imbibition temperature 10C (50F) to 34C (93F) would provide at Q_{10} effect (8) increasing embryo enzymatic activity and thereby decreasing germination time. Addition-

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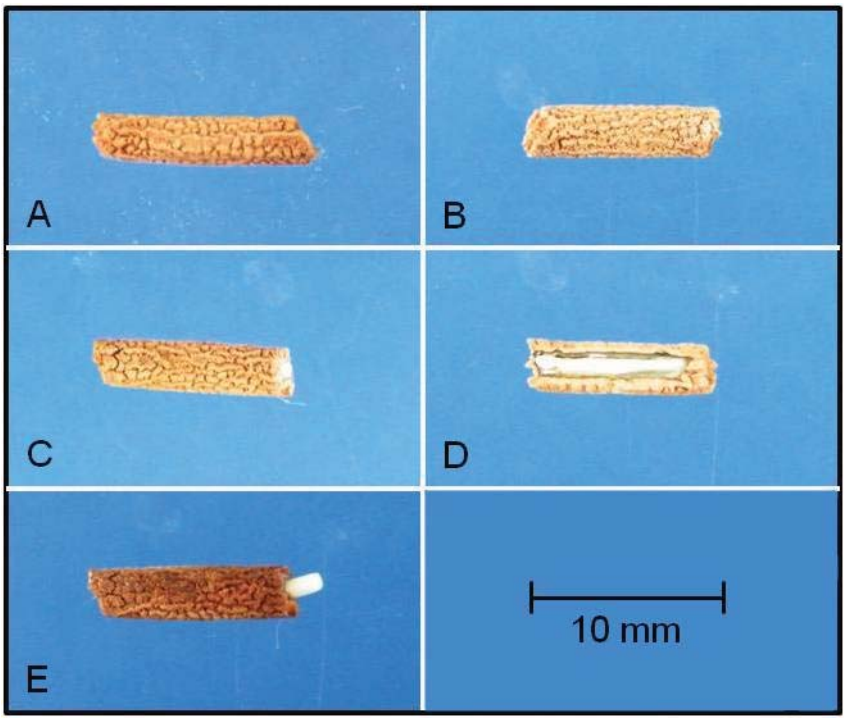

Fig. 1. Progression of *Amsonia tabernaemontana* seed scarification treatments. A, B. seeds with no scarification. C. Seed A with 2 mm of seed coat removed from end of seed (cut made on right side). D. Seed B cut longitudinally. E. Seed A imbibed for 48 hr at 24C (72F) after scarification, with partial embryo emergence.

ally, a scarified without imbibition treatment was included to discern the effect of imbibition on germination. The objectives of this study were to determine the best method for seed coat removal and develop an overall germination protocol for *A. tabernaemontana*.

Materials and Methods

Mature seeds were collected in August 2008 from wild populations of *A. tabernaemontana* in Oconee County, SC. After collecting, seeds were cleaned, sealed in polyethylene bags and stored at room temperature (24C; 72F) until the study began. To determine the best method for cutting (scarifying) *Amsonia* seed coats, 10 seeds were cut longitudinally to determine how embryos were positioned within seed (Fig. 1D, 1E). Additionally, seed length and width were measured for 60 seeds. Observations lead to the conclusion that carefully removing 1–2 mm (0.04–0.08 in) of either end of the seed coat with a surgical scalpel would not severely damage embryo. Using a method of removing thin slices of seed coat until embryo was exposed further minimized embryo damage; this methodology was used for treatments that had their seed scarified. Imbibition curves for control and scarified seeds (30 seeds in each group) were developed for seeds submerged in 24C (72F) water for 48 hr. Dry weight of each seed was taken at time 0 and reweighed at 1, 2, 3, 4, 6, 12, 24 and 48 hr intervals. After imbibition was initiated, seeds were blotted dry prior to weighing. Once a proper scarification technique had been developed, the following treatments were developed to simulate both natural and ideal germination situations.

Treatments were: 1) control (no scarification or imbibition), 2) seed coat scarified, no imbibition, 3) no scarification, imbibed for 48 hr at 24C (72F), 4) seed coat scarified, imbibed for 48 hr at 24C (72F), 5) intact seed coat, imbibed for 48 hr at 38C (100F) and 6) seed coat scarified, imbibed for 48 hr at 38C (100F). Seeds for treatments 3 through 6 were imbibed in 40 mL (1.35 oz) of deionized water using two Conviron CMP3244 plant growth chambers (Controlled Environments Limited, Winnipeg, Manitoba, Canada). Use of growth chambers ensured that treatment temperatures were maintained during the entire imbibition period. Each treatment consisted of 4 replications of 38 seeds (152 seeds per treatment), planted at a depth of 15 mm (0.59 in) in a 195 cm^3 (11.9 in^3) 38-cell seedling tray (Landmark Plastic Corp., Akron, OH) filled with Fafard 52 media mix (Conrad Fafard, Inc., Agawam, MA). After planting, seeds were placed under a mist bench to maximize germination potential. The mist system used Netafim Violet Vibro-Mist Misters (Netafim USA, Fresno, CA) spaced at 1 m (3.28 ft) intervals along the length of the bench and placed at a height of 0.75 m (2.46 ft) from the bench surface. The system operated from 7:00 am to 8:00 pm daily and activated every 6 min for 6 sec using a 1A Single Zone Controller (Phytotronics, Inc., Earth City, MO). The output of each misting head ranged from 650 to 700 mL (21.9 to 23.6 oz) per minute. Seed germination was measured daily for 28 day; a seed was considered germinated if the cotyledon had emerged from the soil surface. A 28 day germination period was selected to correspond with standard commercial propagation protocols for seed germination.

All data were analyzed using SAS 9.2 (SAS Institute Inc. Cary, NC) proc GLM. Seed imbibition data were analyzed using a student t-test to determine differences between imbibition for each treatment group. Germination percentage data were analyzed using a general liner model with Tukey's HSD for means separation. A significance of P = 0.05 was

Table 1. Mean days to 50% germination, final germination percentage, and final germination value for *Amsonia tabernaemontana* seeds after scarification and imbibition treatments.

Treatment	Days to 50%	Germination (%)	Germination value
Control (no scarification or imbibition)	*	0.0 ± 0d	0.0 ± 0c
Seed coat scarified, no imbibition	13.25a	57.2 ± 3.46b	13.04 ± 1.46b
No scarification, imbibed for 48 hr at 24C (75F)	*	4.61 ± 1.66d	0.14 ± 0.06c
Seed coat scarified, imbibed for 48 hr at 24C (75F)	13.50a	70.4 ± 3.29a	22.37 ± 2.62a
Intact seed coat, imbibed for 48 hr at 38C (100F)	*	3.29 ± 0.66d	0.06 ± 0.02c
Seed coat scarified, imbibed for 48 hr at 38C (100F)	*	36.2 ± 3.78c	5.65 ± 1.22c

Errors represent standard deviations, letters are means separation. * = treatment did not obtain 50% germination.

used for all testing. T50 values and germination values were calculated for all treatments.

Results and Discussion

Seeds of *A. tabernaemontana* used in this study were 9.92 ± 1.25 mm (0.39 ± 0.05 in) in length and 2.47 ± 0.25 mm (0.1 ± 0.01 in) in diameter. Dissection of *A. tabernaemontana* seeds showed the longitudinal arrangement (Fig. 1D) of embryos within seed. It was determined that a cross-sectional cut near the seed middle or a longitudinal cut through the seed would cause embryo mortality. Based on this observation, removing 1–2 mm of the seed coat end to expose the embryo (Fig. 1C) was the preferred seed scarification method. Ideally, this scarification cut would be made on cotyledon end of the seed but distinguishing one end of the seed from the other has proven impossible. This inability to distinguish ends caused concern that scarification cuts may damage root radicals, ultimately proving fatal to embryos. This concern proved unwarranted as seed scarified on radical end germinated similarly to seeds whose cotyledon ends were scarified (Table 1). Adventitious roots were observed on germinated seed where radicals had been damaged during scarification and no difference in seedling performance was observed.

Imbibition curves (Fig. 2) of control and scarified seeds show that water uptake was not influenced by scarification, as there was no difference between values for the two treatments throughout imbibition period (P = 0.5217). After 48 hr, non-scarified seeds had increased their mass by 88% and scarified seeds had increased their mass by 90%. This indicates seed

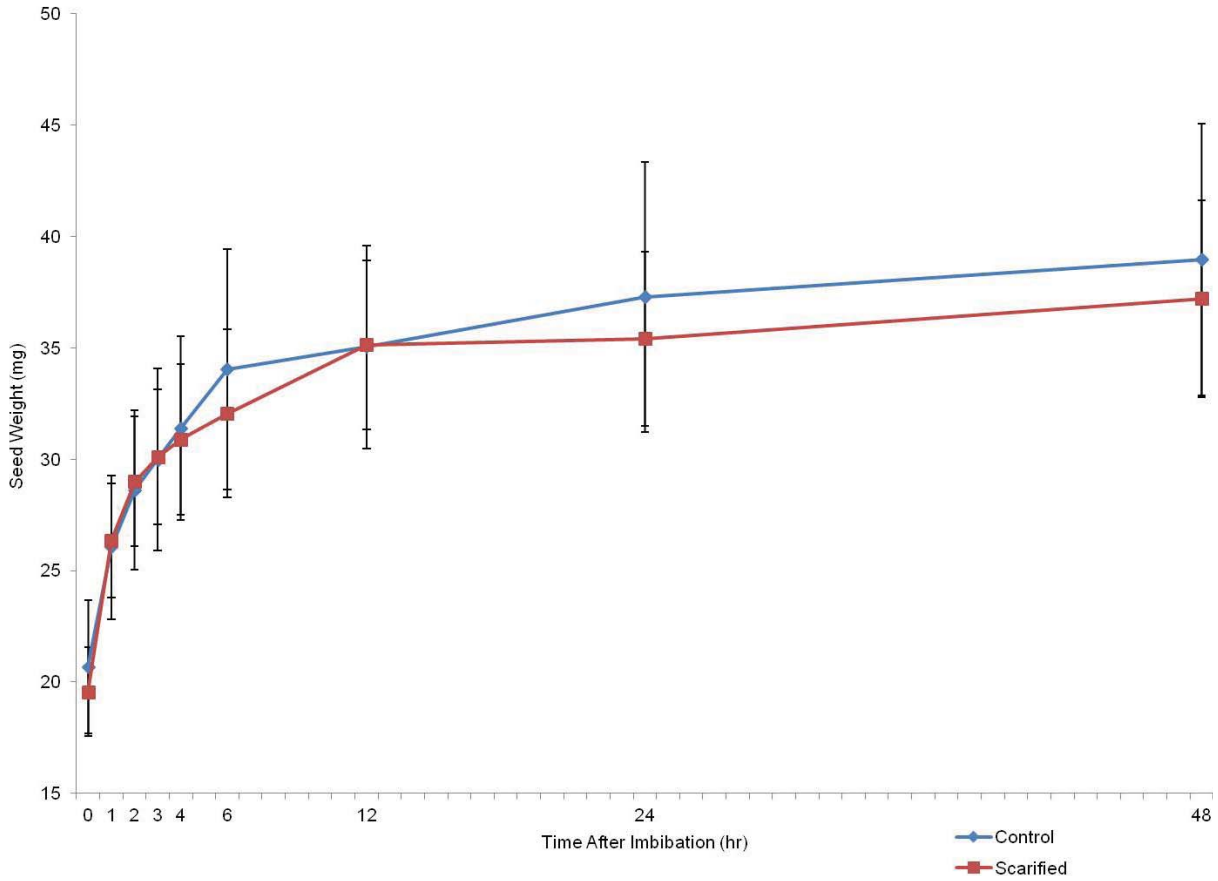

Fig. 2. Imbibitio curves for control and scarified *Amsonia tabernaemontana* seeds. Error bars are based on standard deviations at each measurement.

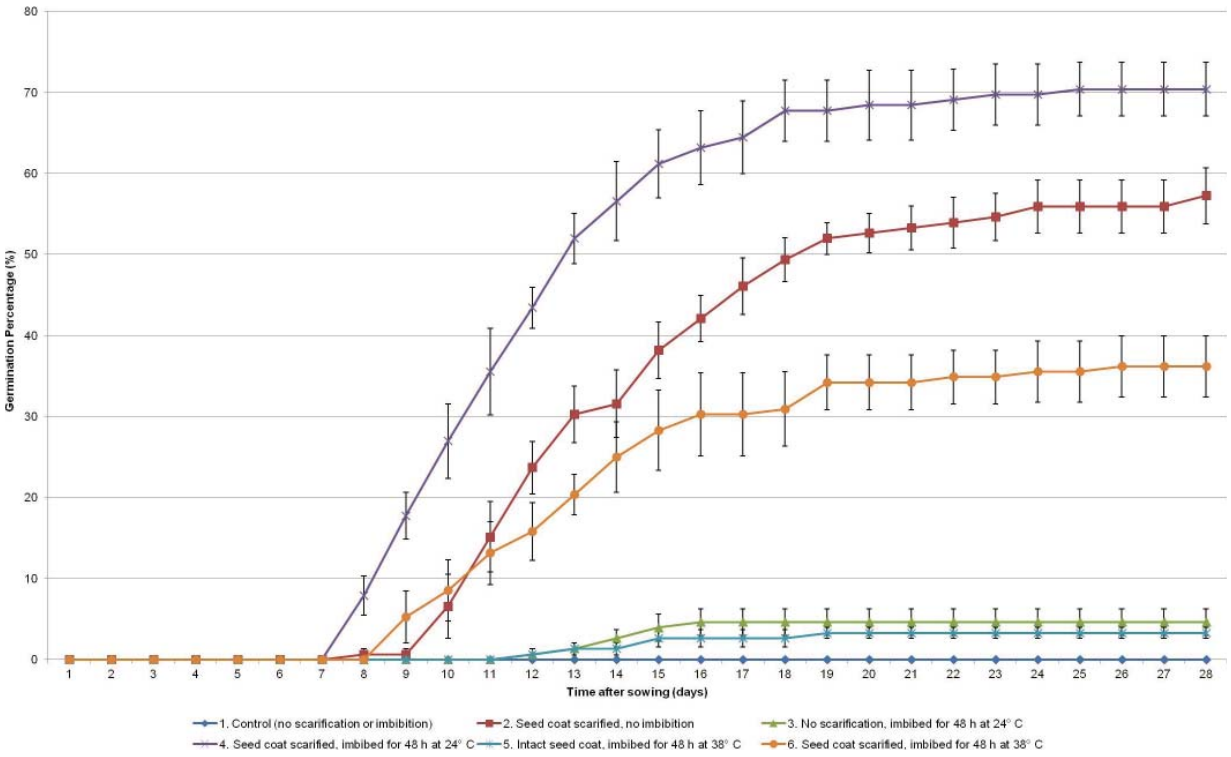

Fig. 3. Germination curves for control and combinations of imbibition and scarification treatments of *Amsonia tabernaemontana* seeds. Error bars are based on standard deviations at each measurement.

coats of *A. tabernaemontana* are permeable. Seeds that were scarified imbibed water and had embryo emergence from seed as seen in Fig. 1E. Given that control seed showed no sign of embryo emergence but imbibed similar quantities of water, it is probable that the seed coat acts as a mechanical barrier to germination (mechanical dormancy). This differs from physical dormancy, as defined by Baskin and Baskin (3) and Finch-Savage and Leubner-Metzger (7), who define physical dormancy as the prevention of water uptake by the seed coat or endosperm. This mechanical dormancy may explain poor and uneven germination of seeds allowed to naturally scarify during winter months. Unscarified seed would require a combination of natural abrasion or microbial action to weaken seed coat and an increase of embryo turgor pressure to break through the seed coat so germination could occur. Washitani and Masuda (14) conducted germination studies on *Amsonia elliptica* [(Thunberg ex Murray) Roemer & Schultes], an Asian *Amsonia* species, utilizing increasing or decreasing temperature regimes (stratification). Washitani and Masuda (14) found an increasing temperature regime following 'a long moist chilling' yielded a study high of 10% germination for *A. elliptica*. This study did not report on the imbibition of water prior to germination or the effect of mechanical scarification on germination, so it is impossible to discern if there is a physical barrier to water uptake or physiologic barrier to germination in *A. elliptica*; or simply a mechanical barrier as described in this study with *A. tabernaemontana*. Nonetheless, Baskin and Baskin (2) interpreted Washitani and Masuda's (14) results to be a form of physiological dormancy, yet noted this classification was inferred. It is possible that the dormancy exhibited by *A. tabernaemontana* in this study is a form of nondeep physiological dormancy that is met after a short period in storage and that storage of seeds at room temperature (24C; 72F) met requirements to break this dormancy. Regardless, this work has demonstrated that the dominant form of seed dormancy in *A. tabernaemontana* is mechanical dormancy imposed by the seed coat. From a commercial producer's prospective, this mechanical dormancy is the most difficult to overcome and hence should be the focus of any seed treatment in *A. tabernaemontana*.

Germination curves (Fig. 3) showed mechanically scarified seeds germinated at rates higher than seeds that were not mechanically scarified. Germination curves also showed that imbibition and imbibition temperatures influenced germination rates of scarified seed. Treatments whereby seeds were not mechanically scarified germinated at rates between 0 and 4.61 ± 1.66% (Table 1). These low rates of germination were likely due to mechanical dormancy caused by the seed coat. Scocco and others (12) found that untreated seeds germinated at rates between 80 and 82.7% for seeds collected previous growing season and germinated in Petri dishes during a 30 day period. This implies a lack of mechanical dormancy and is in direct contradiction to findings of present study (Table 1) and the low germination percentage observed anecdotally by commercial growers and many trusted resources (1, 4, 10). Additionally, Scocco et al. (12) found that seeds soaked for 24 hr in 10 mM GA_3 germinated at rates between 96.0–98.7%. Differences in germinations rates between the two studies could be explained by differing seed morphology, specifically seed coat properties as related to seed provenience or population.

Scarified seeds imbibed at 38C (100F) germinated at lower rate (36.18 ± 3.78%) than scarified seeds that received no imbibition or were imbibed at 24C (72F). This lower germination rate is possibly due to high temperature embryo damage or increased respiration rates exhausting endosperm reserves prior to germination. Scarified seeds that received no imbibition germinated at a high rate, 57.24 ± 3.46%. This moderate germination rate is due to imbibition of soil moisture once seed were planted. While this rate is lower than a 24C (72F) imbibition treatment, it is note-worthy that scarified seed, when provided with adequate soil moisture (germination conditions) will germinate at a rate near commercially acceptable levels. Further, imbibition of scarified seeds may act as a primer, increasing germination rates and possible decreasing time to maximum germination rates. Based on these results, the following protocol is recommended for *Amsonia tabernaemontana* seed germination: scarification of one end of the seed and imbibition for 48 hr at 24C (72F).

T_{50} values indicate *Amsonia tabernaemontana* seed scarified and imbibed for 48 hr at 24C and seed that were scarified but with no imbibition had similar germination rates. For the two stratification treatments, seed scarified and imbibed for 48 hr at 24C (72F) and seed scarified but with no imbibition, stratification decreased the number of days to 50% germination. It is likely that the high imbibition temperatures (38C/100F) of the third stratification treatment significantly reduced the germination of seeds in this treatment. All unscarified treatments as well as seed scarified and imbibed at 38C (100F) did not obtain a T_{50} value during the course of this 28 day study (Table 1). Furthermore, germination value, a combined index of speed and completeness of germination (11) based on peak germination percent and mean daily germination (5, 6) was calculated. Germination values indicate that *A. tabernaemontana* seed that were scarified and imbibed at 24C (72F) germinated at a rate nearly twice that of the next closest treatment of seed that were scarified with no imbibation. Germination values of unscarified treatments were much lower than those of scarified treatments. A direct comparison of unscarified and scarified seeds both imbibed at 24C (72F) showed that scarification increases germination values by 160 times, demonstrating the importance of mechanical scarification to germination of *A. tabernaemontana* seeds.

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