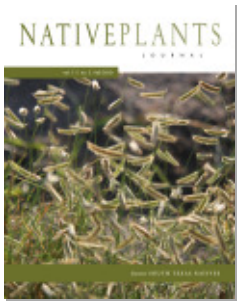
Sulfuric acid scarification of Callicarpa americana L. (Lamiaceae) seeds improves germination

Ryan N Contreras
John M Ruter



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ABSTRACT

An experiment was conducted to determine if sulfuric acid scarification improved seed germination of *Callicarpa americana* L. (Lamiaceae). Treatments included a control (0 min), 15-min, and 30-min soaks in concentrated (18N) sulfuric acid followed by a 15-min rinse in tap water. The 30-min treatment had the earliest germination with seedlings appearing 18 d after treatment (DAT). The 15-min treatment had seedlings emerge at 26 DAT while seedlings in the control did not begin to emerge until 60 DAT. After 60 d, seeds from the acid treatments had approximately 50% germination while the control had less than 10%. At the conclusion of the study, the control, 15-min, and 30-min acid treatments germinated at 8.9%, 57.8%, and 48.9%, respectively. The results of this study show the benefit of sulfuric acid scarification in the germination of *Callicarpa americana*. Recommendations should be amended to include a 15- to 30-min soak in concentrated sulfuric acid to promote rapid and more uniform germination for this species.

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KEY WORDS
beautyberry, propagation, sexual propagation

NOMENCLATURE
Cantino (1992)

Figure 1. Mature fruit of *Callicarpa americana* in early fall. Photo by John M Ruter

Callicarpa L. (Lamiaceae) (commonly called beautyberry) is a genus of approximately 150 species of shrubs and trees distributed throughout the world including warm-temperate and tropical America, Southeast Asia, Pacific Islands, and Australia (Harden 1992). Beautyberries are grown primarily for their handsome fruit, typically purple, which is produced in late summer to fall (Figure 1). *Callicarpa americana* L. is an attractive native shrub that is underutilized in the nursery and landscape industries. Selection, the sole means of improvement to date, has been primarily for pink or white-fruiting forms and for increased fruit production. To increase available diversity, a program has been initiated at the University of Georgia, Tifton Campus, with the goal of developing novel forms of beautyberry through interspecific hybridization and investigating inheritance of ornamental characters to assist in the breeding process. During this program, seeds were germinated after 60-d cold, moist stratification. Subsequent germination was observed to be slow, sporadic, and at low percentages.

The fruit of *Callicarpa* is a berry-like drupe with a fleshy exocarp and hard endocarp separated into 4 pyrenes, each containing a single seed (Moldenke 1936; Harley and others 2004). *Callicarpa americana* exhibits seedcoat dormancy, but this point has not been taken into account for previous recommendations on germinating beautyberry seeds (Connor 2004; Dirr and Heuser 2006). In nature, seed distribution is facilitated by a number of mammals (Connor 2004; Halls 1977) and birds, particularly quail (Halls 1977) and mockingbirds (Moldenke 1936). Fruits are consumed primarily during winter months when other food sources are scarce (Halls 1977). During passage through the gut of birds and mammals, the seeds become scarified and germinate the following spring when temperatures are not limiting. In addition to natural scarification by means of animal digestion, laboratory acid scarification has been used to improve germination of native species such as *Sophora secundiflora* (Ortega) Lag. ex DC. (Fabaceae) (Ruter and Ingram 1991). Germination of *C. americana* is reported to be slow but occurs without scarification or stratification treatment (Connor 2004). Dirr and Heuser (2006) report that seeds sown in the fall have excellent germination during the following spring. This delayed germination has also been observed in our program at the University of Georgia and has impeded the speed of research. Therefore, the objective of this study was to determine a more effective protocol for germinating *C. americana* seeds. It was hypothesized that scarification with sulfuric acid would aid in breaking seedcoat dormancy, thereby resulting in more rapid and uniform germination.

MATERIALS AND METHODS

Fruit resulting from open pollination was collected on 28 January 2009 from container-grown *Callicarpa americana* plants at the Coastal Plain Experiment Station, Tifton, Georgia. Parent plants were from a north Georgia provenance (USDA Hardiness Zone 7; USDA 1990). Plants were grown in isolation blocks to prevent pollination from other species of *Callicarpa*. Seeds were cleaned by hand and treatments were applied immediately after collection. The 3 treatments consisted of a control (direct sow) and either a 15-min or 30-min soak in concentrated sulfuric acid (18N H_2SO_4). During acid treatment, seeds were gently stirred periodically with a glass rod. Following acid treatment, seeds were rinsed in running tap water for 15 min. After rinsing, seeds were sown in 1.28-l (12.7 cm top diameter) containers filled with a mixture of 8 pine bark : 1 sand (by volume) amended with 1.2 kg dolomitic limestone and 0.59 kg Micromax® (The Scotts Company, Marysville, Ohio) per m^3 (2 lb/yd^3 and 1 lb/ yd^3, respectively). Seeds were covered lightly with substrate when sown. Containers were maintained in a glasshouse at 27 °C day/20 °C night temperatures (81 °F/68 °F) and hand-watered as needed. The experiment was completely randomized with 3 replications (15 seeds/replication). Seeds were considered germinated if cotyledons had emerged by the end of the study. The study was terminated 60 d after treatment (DAT). Data were analyzed using analysis of variance, and means were separated by comparing acid treatments with the control using Dunnett's procedure in SAS 9.1 (SAS Institute, Cary, North Carolina).

RESULTS AND DISCUSSION

Seeds of *C. americana* germinated more quickly after treatment with sulfuric acid. The 30-min treatment had seedlings emerging 18 DAT, and the 15-min treatment had seedlings emerging 26 DAT, while the control had no germination until 60 DAT. The 30-min treatment reached maximum germination of 49% 50 DAT and showed no further germination at 60 DAT, at which time the experiment was concluded (Table 1). The 15-min treatment exceeded 50% germination 50 DAT and reached 58% at 60 DAT (Table 1). The control had the lowest germination rate, only 9% at 60 DAT. Both acid treatments were statistically different from the control from 40 DAT until the end of the study (Table 1). No distorted or abnormal growth was observed among seedlings in any of the treatments.

These results show the benefit of acid scarification in germinating *C. americana* seeds. Overall germination may ultimately be similar between control and acid-treated seeds; however, it could take 6 mo for untreated or stratified seeds to reach 50% germination. In a previous study on 8 species of *Callicarpa*, seeds were collected 28 November 2007 and cold-moist stratified for 60 d prior to sowing. The study was concluded 28 May 2008. Germination ranged from 6 to 45% with a mean of 27% (data not shown). Bonner (2008) reported that a sample of *C. americana* seeds stratified for 30 d resulted in 22% germination after 90 d. In the current study, the seeds were collected in January, which exposed them to at least some degree of stratification while still on the plant. This *in vivo* stratification is unlikely to have had an affect on the results. Haywood (1994) reported that *C. americana* seeds are unaffected by stratification and found that germination of seeds planted in a forest site increased over 5 y, reaching 100% at y 5. The ability of *C. americana* to remain viable for a number of years and to increase germination rate is likely due to its hard seedcoat slowly breaking down over time to become permeable to water.

For production scheduling, it may be desirable to delay germination until tender seedlings can survive outside of heated glasshouses. Therefore, growers may not necessarily benefit from rapid

germination. For a research and breeding program, however, it is desirable to decrease time from seeds to seeds; that is, the time it takes from germination of a seedling to crossing and collection of the next generation's seeds. Currently, it is possible to obtain one generation per year, but with acid scarification it may be possible to collect seeds in August, scarify and sow, and have the next generation flowering in a glasshouse by early spring. This could allow our program to obtain 2 generations per year as compared with the current scheduling, which is only slightly more rapid than in a natural setting, even with the use of heated glasshouses.

The results of this study show the benefit of acid scarification of seeds of *C. americana*. Because of the limited treatments applied, the relatively small number of seeds, the single seed source, and the short duration of the study, further work should be conducted to optimize treatments. *Callicarpa americana* has a native range from Maryland to Texas in the US. Our study was conducted using a north Georgia seed provenance, but further work with other germplasm over the extent of its range may be necessary to optimize germination protocols for this species. Previous attempts to germinate *C. americana* under conditions similar to those described by Baskin and Baskin (unpublished results; see McDonald and Kwong 2005) resulted in sporadic emergence and low percentages even though they obtained germination as high as 90%, possibly providing evidence for differences in germination requirements of different seed sources. Even with the small scale of this study, however, significant differences were observed between treatments and the control. Therefore, we predict these findings will extend to other species of *Callicarpa* as well. The 15-min and 30-min scarification treatments were both superior to the control; therefore, germination of *Callicarpa* seeds can be improved by including a 15- to 30-min scarification treatment with concentrated sulfuric acid followed by rinsing and direct sowing.

TABLE 1

Germination percentage of Callicarpa americana *seeds for each treatment (n = 3; 15 seeds/replication).*

Acid scarification treatment (minutes)	Days after treatment (DAT) 18	28	40	50	60
0	0.0	0.0	0.0	0.0	8.9
15	0.0	20.0	37.8*	53.3*	57.8*
30	4.7	31.1*	46.7*	48.9*	48.9*

*Means significantly different from control at $\alpha = 0.05$ using Dunnett's procedure for mean separation.

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AUTHOR INFORMATION

Ryan N Contreras
Graduate Research Assistant

John M Ruter
Professor
ruter@uga.edu

Department of Horticulture
University of Georgia, Coastal Plain Experiment Station
4604 Research Way
Tifton, GA 31793