EFFECT OF SCARIFICATION TREATMENTS ON GERMINATION OF *SOPHORA SECUNDIFLORA* SEEDS

JOHN M. RUTER[1] AND DEWAYNE L. INGRAM

Department of Ornamental Horticulture, IFAS
University of Florida
Gainesville, Florida 32611

Abstract: Seeds of *Sophora secundiflora* (Ort) Lag ex DC. (mescal bean) were scarified with hot water and concentrated sulfuric acid to determine an optimal pretreatment for successful germination One-year-old seeds were successfully stored and germinated approximately two days before seed from the current year when both were given an acid pretreatment. Germination rate increased as acid pretreatment time increased from 30 to 120 min Soaking seeds in water at room temperature and in hot water (initially 93 ° C) for 24 hr. had no effect on germination

The genus *Sophora* (Papilionaceae) consists of approximately 30 species with world-wide distribution (1). *Sophora secundiflora* is an evergreen shrub or small tree native to western Texas, New Mexico, and northern Mexico. Other species found in the United States are *S. affinis,* native to Texas and Arkansas, *S. arizonica* from Arizona, and *S. tomentosa,* which grows in southern Florida. *Sophora secundiflora* is an excellent native plant for landscaping purposes in Texas because of its tolerance to alkaline soil conditions and moderate drought. The foliage is a glossy dark green and the plant produces fragrant, showy flowers in terminal racemes during early spring. The plant is hardy in USDA zones 8 to 10 and can be used for screening, hedges, and as a specimen tree.

Sophora secundiflora is considered difficult to root and is propagated primarily by seed (5, 8). The seeds are reported to be short-lived, and shipment of seed as soon as ripe without drying is recommended (9). Successful germination of *S. microphylla* was dependent upon time of collection and the prevention of seed coat hardening due to drying (4). Old seed requires acid scarification for germination, whereas fresh, mature seed of *S. secundiflora* germinate readily (8). While seed germination appears to be the accepted method for propagating this species, few protocols were found regarding pre-treatment to break seed-coat dormancy. Therefore, the objectives of this study were to: 1) test the viability of one-year-old seed as compared to fresh seed, and 2) determine an optimal pretreatment protocol for successful scarification and germination of *S. secundiflora* seed.

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[1] Former Graduate Assistant. Currently Assistant Professor, Coastal Plain Experiment Station, Department of Horticulture, P O Box 748, Tifton, GA 31793

MATERIALS AND METHODS

Seeds were collected from wild stands of *S. secundiflora* in Medina County, Texas. Seeds produced in 1987 were collected in March, 1988, while seeds produced in 1988 were collected in December, 1988. Both crops were sealed in polyethylene bags and stored at room temperature (20 C) until used. In March, 1989, seeds were manually extracted from pods (approximately 3 to 4 seeds per pod) and inspected to insure that no damage occurred before use.

Treatments included:

1) control (distilled H_2O soak for 24 hr.),
2) hot water soak,
3) sulfuric acid (30 min),
4) sulfuric acid (60 min), and
5) sulfuric acid (120 min).

For the hot water soak, seeds were placed in distilled water at 93° C and allowed to cool for 24 hr. Concentrated sulfuric acid (18 N H_2SO_4) was used for treatment of seeds. Seeds placed in acid were gently agitated periodically then rinsed for 2 hr. in distilled water.

After treatment, 30 (1987) and 50 (1988) seeds per treatment were rolled in moist paper towels (10 seeds/replication) and placed in plastic bags for germination. Seeds were germinated in a germination chamber with a day/night temperature of 30/30 ° C and no lighting. Distilled water was added to moisten the paper towels as needed. On days 0, 1, 3, 5, 7 , 9 , 11, 13, 17, 20, and 27, seeds were removed, and fresh weight, seed width, and length were determined. A seed was considered germinated if the radicle was visibly protruding through the seed coat. The experiment was terminated after 27 days.

Seed coat samples taken after each treatment for scanning electron microscopy (SEM) observation were oven-dried at 70 ° C for 24 hr before gold sputter-coating. Electron micrographs were taken using a Hitachi S-450 scanning electron microscope. Data were analyzed as a general linear model.

RESULTS AND DISCUSSION

Germination of *S. secundiflora* seed was influenced by collection date and sulfuric acid treatment. All seeds treated with sulfuric acid germinated in 27 days, whereas only 1 control seed and only 4 hot-water-treated seeds germinated from the two seed lots. Seeds collected from the 1987 crop germinated an average of 2 days earlier (5.3 days) than seeds collected in 1988 (7.3 days). Mean number of days to germination decreased linearly (R^2 = 0.86) as acid treatment period increased from 30 to 120 min. Seed treated

with sulfuric acid for 120 min germinated in an average of 4.9 days compared to 6.2 days (60 min) and 8.3 days (30 min). After 27 days, remaining seeds from the control and hot water treatments were treated with sulfuric acid for 60 min. Over 90% of the seeds germinated within 5 days, indicating continued seed viability.

Initial seed size was not correlated with initial seed weight. Final seed length (1.97 cm) and seed width (1.37 cm) increased for the sulfuric acid treatments whereas the control and the hot water treatment showed no increase in size. Germination was not correlated ($r < 0.4$) with initial or final seed size or weight change due to imbibition.

Anatomical features of papilionaceous legume seed coats include three layers known as the cuticle, the epidermal, and the hypodermal layer (6, 7). The cuticle is made of cutin, a waxy, fatty hemicellulose, or pectinaceous outer layer of the seed coat. The epidermal layer consists of macrosclereid cells often known as the Malpighian cell layer. Malpighian cells are usually elongated, thick-walled, and approximately hexagonal when viewed in transection. The hypodermal layer consists of irregularly shaped osteosclereid cells, which are usually separated by intracellular spaces.

The cuticle was determined to be approximately 3μm in thickness. The epidermal and hypodermal layers were approximately 350μm and 230μm thick, respectively. The cuticular surface of legume seeds often appears smooth at low magnifications with textural patterns becoming visible in SEM micrographs above 50 $\times$ (6). At a magnification of 100 $\times$, the cuticle of *S. secundiflora* seeds soaked in water for 24 hr. appeared to be smooth with numerous nearly circular small patterns.

In some species of the legume family, the waxy seed cuticle may interfere with the uptake of water, thereby limiting germination due to a coat-imposed dormancy (2). Seeds treated with hot water showed some loss of cuticular material. Research with Penngift crownvetch seed showed that 1 min. in boiling water caused enough thermal expansion to rupture the seed coat and separate the Malpighian cells which allowed water to penetrate into the seed (3). No such rupturing of the epidermal layer was seen in this study due to the hot water soak.

Treatment of seeds for 30 min. with sulfuric acid resulted in removal of the cuticular layer and visibility of the Malphigian layer in a majority of the seed. Cracks in the outer portion of the epidermal layers were evident at high magnification (1800x). When seeds were treated with acid for 60 or 120 min., the cuticular layer was completely removed. The 60- and 120-min acid treatments resulted in localized deep etching of the epidermal layer. These same treatments dissolved the tops of the Malphigian cells and caused cracks in the epidermal layer.

Previous research suggested that impermeability of crownvetch seed was due to Malphigian cell layer caps (3). The cuticular and the epidermal layer have also been implicated in the interference of water uptake in leguminous seed (2). Scanning electron micrographs showed that partial removal of the cuticular layer in *S. secundiflora* seed was necessary before germination occurred. The hot-water soak resulted in poor germination because the treatment did not remove the cuticular layer. When seeds were treated with sulfuric acid, some or all of the cuticular layer was removed. Following the 30-min. sulfuric acid treatment cracks in the epidermal layer were evident, and 100% germination resulted. Therefore, it is evident that the cuticular layer in *S. secundiflora* can prevent germination by preventing inhibition of water. However, it could not be determined from this study whether scarification of the hypodermal layer was required for germination of *S. secundiflora* seed.

Seed stored for four months became sufficiently hard to prevent germination unless the seed was acid scarified. Germination rate increased as acid treatment time increased. Fastest germination occurred using one-year-old seed and acid scarification for 120 min. Soaking seed at 93 °C for longer periods of time may remove enough cuticular layer to enhance germination. While germination can be accomplished with fresh, mature seed that have not hardened (8), *S. secundiflora* seed can be successfully stored and germinated if treated with acid scarification.

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