Propagation of the Endangered Legume *Amorpha nitens* and the Common Shrub *Amorpha fruticosa*©

Nadia E. Navarrete-Tindall
Department of Agronomy, University of Missouri-Columbia, Columbia, Missouri 65211 USA

J. W. Van Sambeek
USDA Forest Service, North Central Research Station, Columbia, Missouri 65211-7260 USA

Mark V. Coggeshall
Center for Agroforestry, University of Missouri, Columbia, Missouri 65211-7270 USA

Robert L. McGraw
Department of Agronomy, University of Missouri-Columbia, Columbia, Missouri 65211 USA

INTRODUCTION

Amorpha nitens Boynton (smooth wild indigo) is an attractive shrub found along streams or lakes from southern Illinois to Louisiana (Wilbur, 1975). This legume is listed as endangered in Illinois and Georgia. Taft (1994) suggests the reasons for its decline are habitat disturbance and low reproduction rates. *Amorpha nitens* is easily confused with the more common *A. fruticosa* L. (false wild indigo or indigobush). Although found in habitats similar to *A. nitens*, *A. fruticosa* has a much wider distribution range in the U.S.A. Both species are relatively shade tolerant and form symbiosis with nitrogen fixing bacteria making them candidate species for inclusion in several agroforestry practices and restoration of natural ecosystems (Navarrete-Tindall et al., 2000).

Members of the genus *Amorpha* are normally propagated from seed or cuttings; however, little is known about the propagation of *A. nitens*. Seed of most *Amorpha* species can be hulled, but are usually left inside the pods for field plantings (Vogel, 1981). To enhance germination, some growers stratify the seeds for 10 days at 5°C before planting. *Amorpha nitens* is easily propagated by seed (Taft, 1994; Navarrete-Tindall, 1998); however, much less is known about cutting propagation for this species. Early research on cutting propagation of *A. fruticosa* showed it is an easy-to-root species that does not require the addition of growth regulators (Ure, 1937). More recent research found that only forty-percent of soft-terminal cuttings rooted after treatment with Hormodin #3 (University of California, 1996). The objectives of our study were to compare response of *A. fruticosa* and *A. nitens* for (1) effects of hulling and stratification on seed germination, (2) effectiveness of talc-based auxins to promote rooting, and (3) impact of stock plant shading on rooting response of semihardwood cuttings.

MATERIALS AND METHODS

Seed of *A. nitens* were collected in October 2000 from six plants growing in three small populations in Hardin County, Illinois, and from two nursery-grown plants in Jackson County, Illinois. Seed of *A. fruticosa* were collected in 1999 from plants in Emmet County, Iowa; Boone County, Missouri; or purchased as a mixed seed lot from Prairie Moon Nursery (Winona, Minnesota). Seed of *A. nana* Nutt. (dwarf *Amorpha*) was also purchased as a mixed seed lot from Prairie Moon Nursery. Seed of *A. ouachitensis* Wilbur (Ouachita mountain indigo) was obtained from the Missouri Botanical Garden in St. Louis, Missouri. Seed had been collected in 1996 from a plant near the Glover River in Oklahoma (MoBot accession 1996-2606), in 1988 from a plant near the Ouachita River in Ouachita National Forest in Arkansas (MoBot accession 1988-1955-26), and in 1995 from a plant grown at Shaw Arboretum from accession 1988-1955-26 (MoBot accession 1995-2376-1).

In Experiment 1 for seed germination, one entry of *A. nitens* from the nursery plants and one entry of *A. fruticosa* from Minnesota were tested. One half of the seed was left in the pod and half was hulled. Between 10 and 30 hulled or unhulled seeds were sown on moist germination paper in Petri dishes for both species. Petri dishes were either kept in the dark at 25°C for 8 days or stratified at 4°C for 8 days and then kept at 25°C for 8 days before determining germination percentage. The experimental design was a completely random 2 × 2 × 2 factorial with five replications. Data was subjected to factorial ANOVA and the LSD was calculated for means or interactions significantly different at $P<0.05$ level.

In Experiment 2 for seed germination, eight entries of *A. nitens*, two entries of *A. fruticosa*, one entry of *A. nana*, and three entries of *A. ouachitensis* were tested. Hulled seed were sterilized in 95% ethanol for 1 minute followed by 3 minutes in 3% hydrogen peroxide (H_2O_2) and then stored in distilled water overnight. Depending on availability of seed, 10 to 100 nonstratified seeds were sown in a greenhouse without replication in plastic 1020 flats filled with Metro Mix®. Percent seed germination was determined 21 days after sowing.

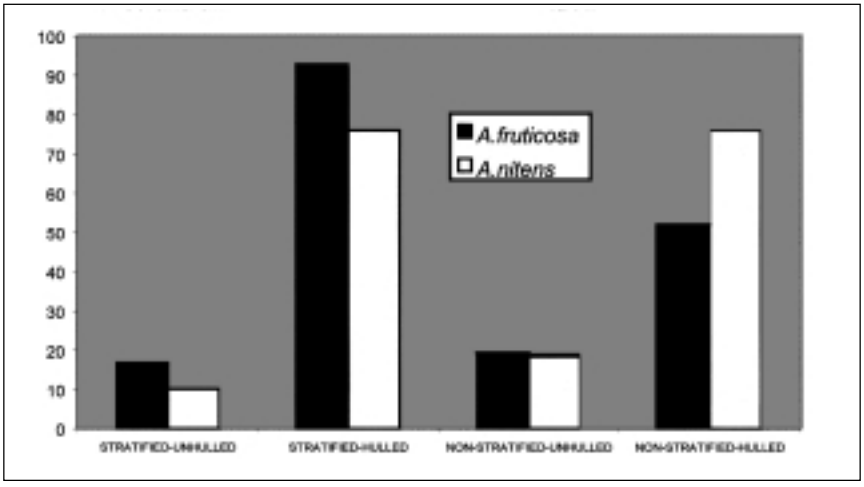

Figure 1. Percent germination for stratified and nonstratified seed (hulled and unhulled) of *Amorpha fruticosa* and *A. nitens*.

For the rooting experiments, stock plants of *A. fruticosa* and *A. nitens* were grown under 20%, 45%, or 100% of full sunlight. Plants were grown in the outdoor shade tolerance laboratory at theHorticulture and Agroforestry Research Center inside 5 m × 15 m × 2.5 m high rectangular frames covered with shade cloth. Stock plants were grown in white pots filled with horticultural potting medium and watered daily by drip irrigation. Stock plants were coppiced in early May, and stump sprouts (60 to 100 cm long) were harvested in late July. Stump sprouts were cut early in the morning and refrigerated. To prepare cuttings, the leaves were removed, stems cut into 15- to 18-cm long semihardwood cuttings, and soft shoot tips removed. Two cuts, approximately 1 cm long, were made at the base of each cutting with a razor blade. Cuttings were bundled according to species and stock plant shade regime, set in water, and refrigerated overnight.

The following morning, the basal 1.2 to 1.3 cm of each cutting was dipped in talc-base growth regulators and immediately planted in 6 cm square by 13 cm tall Anderson® plant bands containing moist horticultural grade perlite. Cuttings were randomly distributed among five auxin treatments: an untreated control, Hormex 1® (0.1% IBA), Hormex 2 (0.3% IBA), Hormex 8 (0.8% IBA), and Rootone® (0.1% IBA + 0.03% NAA). Cuttings were inserted vertically 6 to 7 cm deep in pre-formed holes. Cuttings were subirrigated as described by Regan and Henderson (1999) where Anderson trays containing 36 cuttings were set in 5-cm deep plastic trays and continuously flooded with 2 to 3 cm of water. Subirrigated trays were kept in a climate-controlled laboratory at 25°C under fluorescent light (16 to 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR). After 30 days, we checked the 300 cuttings for survival, number of adventi-

Table 1. Effect of shading stock plant on rooting of cuttings of *Amorpha fruticosa* and *A. nitens*.

Species	Light levels	Percent rooting (%)	Primary roots (no.)	Longest primary root (mm)	Secondary roots (no.)
Amorpha fruticosa	20%	66	2.7	74	46
	45%	83	3.9	52	26
	100%	60	2.2	52	36
Amorpha nitens	20%	51	2.6	40	17
	45%	58	1.5	14	6
	100%	66	2.1	13	23
Significance:	Species	NS	*	*	*
Significance:	Shade	NS	NS	*	NS
Significance:	Sp × Sh	NS	*	NS	NS
Least Significant Difference (Sp × Sh):		20	1.4	17	15

NS and * are nonsignificant or significant at $P<0.05$ level, respectively.

Table 2. Auxin effect on number of roots of cuttings of *Amorpha fruticosa* and *A. nitens*.

Species	No auxin	Hormex 1	Hormex 3	Hormex 8	Rootone
Amorpha fruticosa	1.9 a	2.9 a	2.0 a	3.9 a	4.1 a
Amorpha nitens	1.4 b	2.1 b	3.6 a	1.9 b	1.3 b

Means within rows followed by different letters are significantly different at the $P<0.05$ level.

tious (primary) roots, length of longest primary root, and number of secondary roots. Treatment means by species, stock plant shading, and auxin were calculated for percent survival, percent of live cuttings that rooted, average number of primary roots and secondary roots, and length of longest primary root on rooted cuttings. The experimental design was $2 \times 3 \times 5$ factorial with three blocks. Treatment means were subjected to factorial ANOVA and the LSD was calculated for means or interactions significantly different at $P<0.05$ level.

RESULTS

In Experiment 1 for seed germination, germination percentage showed a three-way interaction between *A. fruticosa* and *A. nitens*, with and without cold stratification, and unhulled and hulled seed (fig. 1). The interaction was primarily because hulled *A. fruticosa* seed with stratification had 92% germination while seed without stratification had 52% germination when there were no differences between nonstratified and stratified hulled seed of *A. nitens* (76% for both) or for unhulled seed of either species (10% to 18%).

In Experiment 2 for seed germination, germination of hulled seed harvested from the six *A. nitens* plants in natural stands ranged from 0% to 40% compared to 74% and 84% germination for seed collected from two nursery-grown plants. Germination of hulled seed from an *A. fruticosa* plant in a natural stand as well as a mix seed lot from Minnesota averaged 45%. Seed germination of *A. nana* averaged 92% for seed from a mixed seed lot of unknown origin. Seed germination of another endangered legume, *A. ouachitensis*, averaged 24% and 58% for stored seed from plants in natural stands in Oklahoma and Arkansas, respectively, and 29% seed from a cultivated plant.

Few of the semihardwood cuttings for either *A. fruticosa* or *A. nitens* died (6% and 3%, respectively) when stuck in moist perlite maintained with subirrigation without the use of mist to raise the relative humidity. Between 50% and 80% of the cuttings rooted within 30 days with no differences among species or stock plant shade treatments (Table 1). Cuttings of *A. fruticosa* initiated more adventitious roots than did cuttings of *A. nitens* (2.9 vs. 2.1 roots per rooted cutting, respectively). Significant differences between species for cuttings harvested from stock plants grown under 45% full sunlight were observed due to unknown reasons. An auxin treatment with Hormex 8 resulted in more adventitious roots on cuttings of *A. nitens* than were found on the untreated control and other auxin treatments (Table 2). In contrast, no

differences were found among the auxin treatments for the number of adventitious roots on *A. fruticosa* cuttings.

The length of the longest adventitious root on cuttings of *A. fruticosa* was nearly three times the length of the longest adventitious root on rooted cuttings of *A. nitens* (59 mm vs. 22 mm) (Table 1). In addition, the length of the longest adventitious root on cuttings harvested from stock plants grown under 20% full sunlight were longer than for cuttings harvested from plants grown under 45% or full sunlight. Cuttings of *A. fruticosa* had initiated more than twice as many secondary roots from the adventitious roots as did the rooted cuttings of *A. nitens* (35 vs. 15 secondary roots, respectively).

Rooted cuttings of both species survived transplanting to potting medium under shade and initiated shoot growth before going dormant in late fall. Potted cuttings were overwintered with minimal protection from freezing; however, only the *A. fruticosa* cuttings survived. Survival differences may be related to origin of the seed for the stock plants. Seed for stock plants of *A. nitens* were harvested from the northern boundary of its distribution range in southern Illinois while seed of *A. fruticosa* stock plants originated from Iowa. The *A. fruticosa* cuttings that were field planted show normal growth and cannot be distinguished from seed derived plants after several growing seasons.

DISCUSSION

We found that both unhulled seed and seed left on pods harvested from *A. nitens* plants in natural stands had lower germination than hulled seed from more protected plants. The low germination rates from plants in natural stands supports the argument that the endangered status of *A. nitens* may be due to low reproductive rates. When hulling seed, we find insects have damaged many seed, thus the differences between unhulled and hulled seed are mostly likely due to damaged seed inside the pods rather than germination inhibitors.

Our results support earlier observations by Ure (1937) that an auxin treatment is not needed to root semihardwood cuttings of *A. fruticosa*. We found that semihardwood cuttings of the endangered legume, *A. nitens*, also do not require use of auxin treatments to produce roots. However, the number of roots was higher for *A. nitens* in the presence of Hormex 8, than in the rest of the treatments.

Our results show vegetative propagation of *A. nitens* plants is a feasible option for restoration of this endangered and threatened legume. It should be relatively easy to propagate a subset of plants from the few known populations to create a seed orchard for ex situ interbreeding and establish new seedlings on sites with declining populations or sites where *A. nitens* has been extirpated.

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Hardy Hemlocks and Salty Pines©

Brian K. Maynard

Department of Plant Sciences, University of Rhode Island, Kingston, Rhode Island 02881 U.S.A.

HARDY HEMLOCKS

Hemlock woolly adelgid (*Adelges tsugae*) is an aphid-like insect that feeds on several species of hemlock in Asia, its homeland, and in North America, where it was introduced early in the last century. Damage occurs from feeding on needles, and can result in death of the tree in as little as 4 years (McClure, 1996). Adelgids are readily distributed by mammals, birds, and wind; and with multiple generations each year, populations can increase dramatically. For the most part, populations of hemlock woolly adelgid cannot be managed effectively. Integrated pest management (IPM) of hemlock adelgid utilizes pest monitoring, cultural practices that enhance tree vigor, and mechanical, chemical, or biological control.

Within its native range, hemlock woolly adelgid feeds harmlessly on several hemlock species: *Tsuga chinensis* in Taiwan, and *T. diversifolia* and *T. sieboldii* in Japan. The adelgid also has been innocuous on Western hemlock (*T. heterophylla*) and mountain hemlock (*T. mertensiana*) during the 80 years it has had a foothold in the Pacific Northwest. However, in Eastern North America, where it was discovered about 50 years ago, hemlock wooly adelgid has caused extensive damage to forests and ornamental plantings of both Eastern (*T. canadensis*) and Carolina hemlock (*T. caroliniana*).