

ADAPTATION OF FOUR AMORPHA SHRUBS TO FOUR LIGHT LEVELS

Nadia E. Navarrete-Tindall, J.W. Van Sambeek, Steven D. Kirk, and Robert L. McGraw¹

Native legumes can play an important role in natural ecosystems and in tree plantings as a source of nitrogen through their symbiosis with rhizobial bacteria. The genus *Amorpha* of the subfamily Papilionoideae within the Fabaceae contains 20 to 25 shrubby species native to North America (Wilbur 1975). Several species are documented as nodulated by rhizobial bacteria (Allen and Allen 1981, Navarrete-Tindall 1998).

The most common *Amorpha* species, false wild indigo (*Amorpha fruticosa* L.) is found throughout most of the continental United States. It forms multiple stems reaching heights of 5 to 7 meters and has been used in windbreaks and for wetland restoration. The closely related smooth false indigo (*Amorpha nitens* Boyton), is documented from Kentucky and Arkansas and listed as an endangered species in Illinois and Georgia. Both false and smooth wild indigo can be found in canopy gaps following disturbance and in the understory of riparian and mesic hardwood forests (Taft 1994).

The *Amorpha* genus also includes smaller shrub species found in prairies including leadplant (*A. canescens* Pursh) and dwarf false indigo (*A. nana* Nutt). The objective of our studies was to evaluate the effect of four light levels on growth, development, and rhizobial nodulation of four *Amorpha* shrubs grown in pots in the greenhouse and under natural conditions.

In the experiment using natural conditions, we established two to three seedlings of false wild indigo, smooth wild indigo, leadplant, or dwarf wild indigo in 2 gallon white plastic pots filled with Metro-mix®-380. Previously, we collected seed of smooth wild indigo from plants found in two populations in Southern Illinois. Seed of

other *Amorpha* species were purchased from a commercial nursery in Minnesota. We placed six pots of each species in each of nine shade structures located at the Horticulture and Agroforestry Research Center in New Franklin, MO.

Shade structures (5-m wide x 15-m long x 2.5-m high) in the University of Missouri Center for Agroforestry outdoor shade laboratory were covered with shade cloth to maintain light levels of 20, 45, and 100 percent full sunlight. The experiment was established using a randomized block design with three replicates of each light treatment. The experiment ran from March to November for three consecutive years. We harvested aboveground stems each fall to determine oven-dried stem biomass. Stems with leaves were also harvested in early fall and late spring and analyzed for total plant nitrogen. We used the Combustion Analysis Technique (LECO), AOAC Official Method 990.03, to determine percent plant nitrogen.

In the greenhouse experiment, we established seedlings of false wild indigo, smooth wild indigo, and leadplant in D40 Deepots® filled with sterile vermiculite. Two pots of each species were placed in each of 18 shade frames set on benches in the greenhouses at the Horticulture and Agroforestry Research Center in New Franklin, MO. Light inside the greenhouse was approximately 50 percent of full sunlight and was supplemented with high-pressure sodium lamps to maintain a 16 hr photoperiod. Six of the eighteen shade frames were covered with shade cloth to reduce light levels by an additional 0, 55, or 80 percent.

We established the greenhouse experiment using a randomized block design with three

¹ Plant Ecologist (NEN-T) and Research Plant Physiologist (JWVS), North Central Research Station, USDA Forest Service, 202 Natural Resources Building; Research Associate (SDK), Center for Agroforestry, 202 Natural Resources Building, University of Missouri; Associate Professor (RLM), Department of Agronomy, 200 Waters Hall, University of Missouri, Columbia, MO 65211. NEN-T and JWVS are corresponding authors: to contact, call (573) 875-5341 or e-mail at nnavarretetindall@fs.fed.us or jvansambeek@fs.fed.us.

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replications of three light treatments with and without rhizobial inoculation. We inoculated half of the plants 1 and 3 weeks after establishment with a rhizobial mixture that included bacteria isolated from smooth wild indigo. All plants were watered as needed with sterile deionized water in addition to 50 ml Broughton and Dilworth solution biweekly (Somasegaran and Hoben 1994). We also added a potassium nitrate solution (6 mg N per plant) during the first and second week of the experiment. The greenhouse experiment ran for 4 months in two consecutive years.

At the conclusion of each experiment, we removed each plant from the vermiculite under water, measured stem and root length, and determined number of root nodules. Plants were oven dried to determine plant biomass and then ground to determine percent nitrogen using the LECO procedure.

Table 1.—Stem biomass from sprouts on 2-year-old plants of four native *Amorpha* shrubs grown in outdoor shadehouses under three light levels

Amorpha species	Light (% of full sunlight)		
	100 %	45 %	20 %
	- g -	- g -	- g -
False wild indigo	45 b	64 a	68 a
Smooth wild indigo	15 a	28 a	34 a
Lead plant	1.5 a	3.4 a	2.3 a
Dwarf wild indigo	2.6 a	5.8 a	5.4 a
Means within rows followed by different letters are significantly different ($p \leq 0.05$).			

Table 2.—Seasonal aboveground plant nitrogen in stems of four native *Amorpha* shrubs grown in outdoor shadehouses under three light levels

Amorpha species	Harvest time	Light (% of full sunlight)		
		100 %	45 %	20 %
		- % -	- % -	- % -
False wild	Fall	2.3 a	2.2 a	2.0 a
Smooth wild	Fall	2.3 a	2.1 b	2.0 b
Leadplant	Fall	1.8 a	1.6 a	1.6 a
Dwarf	Fall	1.5 a	1.6 a	1.8 a
False wild	Spring	3.9 a	3.6 a	3.1 a
Smooth wild	Spring	3.5 a	3.1 b	2.8 c
Leadplant	Spring	2.8 a	2.4 a	2.6 a
Means within rows followed by different letters are significantly different ($p \leq 0.05$).				

In the outdoor shade laboratory, we found biomass on sprout growth of 2-year-old false wild indigo plants was less when grown under full sunlight than when grown at under 45 and 20 percent of full sunlight (table 1). Although not statistically significant, the same trend was found for smooth wild indigo, leadplant, and dwarf wild indigo.

Of the four species, only smooth wild indigo showed decreasing aboveground plant nitrogen in response to decreasing light both in the fall and in the spring (table 2). When averaged over season and light intensity, false wild indigo had the highest plant nitrogen content (2.9 percent) followed by smooth false indigo (2.6 percent), then leadplant (2.1 percent), and, finally, dwarf indigo (1.6 percent). Plant nitrogen in the spring was nearly double that of plant nitrogen in the fall. One reason for the large differences may be that a 5- to 6-month-old stem was included with leaves in the fall samples while spring samples only had a 3- to 4-month-old stem with younger leaves.

In the greenhouse studies, we found a highly significant interaction among the three *Amorpha* species, with and without rhizobial inoculation, and three light levels (table 3). Although non-inoculated seedlings showed small changes in response to light, inoculated seedlings of all three species showed a marked decrease in plant biomass with decreasing light. Except for plants grown at 10 percent of full sunlight, shoot and root biomass for plants inoculated with rhizobia was 3- to 6-fold greater than plant biomass for non-inoculated plants. Plants grown in the greenhouse under 10 percent of

Table 3.—Biomass of 4-month-old plants under three light levels for three *Amorpha* shrubs with and without rhizobial inoculation

Amorpha species	Rhizobia	Light (% full sunlight)		
		50 %	22 %	10 %
		- g -	- g -	- g -
False wild	With	2.3 a	2.5 a	0.5 b
Smooth	With	1.2 a	0.6 b	0.4 c
Lead plant	With	2.0 a	1.1 ab	0.2 b
False wild	W/out	0.4 a	0.4 a	0.2 b
Smooth	W/out	0.3 a	0.3 a	0.1 b
Lead plant	W/out	0.7 a	0.4 a	0.1 a
Means within rows followed by different letters are significantly different ($p \leq 0.05$).				

full sunlight were distinctly etiolated, seldom upright, and had the fewest nodules if inoculated.

In general, there was a trend for nitrogen content to decrease slightly with decreasing light intensity 4-month-old plants with and without rhizobial inoculation (table 4). We found that the number of nodules decreased with decreasing light levels and that non-inoculated plants could be nodulated from air-borne rhizobia (data not included).

The average plant nitrogen concentration across all light levels was slightly higher (2.4 percent) for smooth wild indigo than for wild false indigo (2.2 percent) and leadplant (2.0 percent). The lower values for plant nitrogen in the greenhouse plants (table 4) compared to those in the outdoor shade tolerance laboratory (table 2), may be a consequence of including the root and nodules along with the stem and leaves for the greenhouse-grown seedlings.

Our results suggest that the riparian species, false wild indigo and smooth wild indigo, and the prairie species, leadplant and dwarf wild indigo, are tolerant of moderate shade and were readily nodulated by native rhizobial bacteria. All four *Amorpha* species should be evaluated further for possible inclusion in various agroforestry practices as woody nurse crops or in savanna restoration projects. Field trials are needed to test how effectively nitrogen is lost from these species and made available to other plants in managed ecosystems.

Table 4.—Plant nitrogen concentration for three *Amorpha* shrubs grown for 4 months under three light levels with and without rhizobial inoculation

Amorpha species	Rhizobia	Light (% of full sunlight)		
		50 %	22 %	10 %
		- % -	- % -	- % -
False wild	With	2.0 b	2.4 a	2.1 b
Smooth	With	2.7 a	2.3 b	2.3 b
Leadplant	With	2.1 a	2.2 a	2.1 a
	MEAN:	2.3	2.3	2.1
False wild	W/out	2.4 a	1.9 b	2.4 a
Smooth	W/out	2.7 a	2.5 ab	2.1 b
Lead plant	W/out	2.0 ab	2.3 a	1.8 b
	MEAN:	2.4	2.3	2.1
Means within rows followed by different letters are significantly different ($p \leq 0.05$).				

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