GROWTH AND FOLIAR NITROGEN CONCENTRATIONS OF INTERPLANTED NATIVE WOODY LEGUMES AND PECAN

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Abstract.—The interplanting and underplanting of nodulated nitrogen-fixing plants in tree plantings can increase early growth and foliage nitrogen content of hardwoods, especially black walnut and pecan. Recent studies have demonstrated that some non-nodulated woody legumes may be capable of fixing significant levels of atmospheric nitrogen. The following nine nurse crop treatments were established with and without interplanted northern pecan: the nodulated legumes black locust, false indigo, and smooth false indigo; non-nodulated thornless honeylocust, Kentucky coffeetree, and redbud; non-leguminous buttonbush; 16N-8P-8K tree food spikes; and a control without shrubs or fertilizer. Average foliage nitrogen content of the nurse trees ranged from 3.3 percent for black locust, false wild indigo, and smooth wild indigo, and 2.1 percent for honeylocust and Kentucky coffeetree, to 1.8 percent for redbud and buttonbush. In the fourth growing season, pecan foliage nitrogen was similar across all treatments (1.8 to 2.0 percent); however, black locust had increased pecan foliage nitrogen to 2.2 percent in the sixth growing season. Pecan growth is similar across all treatments except when interplanted with black locust that overtopped the pecan and is suppressing its growth. Interpretations include the possibilities that soil nitrogen was adequate to preclude any benefits from biologically fixed nitrogen, that nurse plants did not release sufficient fixed nitrogen to increase pecan growth, and foliage nitrogen in non-suppressed saplings, or that other soil factors are limiting pecan development.

INTRODUCTION

The interplanting of nodulated nitrogen-fixing woody shrubs and trees into hardwood plantings can substantially increase hardwood tree growth (Plass 1977, Hansen and Dawson 1982, Schlesinger and Williams 1984, Dawson and Van Sambeek 1993). Nodulated nitrogen-fixing woody plants can include species that form symbiotic associations with either actinomycetes (actinorhizal plants) or rhizobial bacteria (legumes). On low-nitrogen sites, nitrogen-fixing plants can obtain up to 70 percent of their nitrogen through fixation within root nodules (Tripp and others 1979). Some of this nitrogen can become available to adjacent non-nitrogen-fixing plants through root exudates or decomposition in soil of leaves and roots (Friedrich and Dawson 1984, Avery 1991, Dawson and others 1992).

Much of the early research on interplanting nitrogen-fixing woody plants in the Central Hardwood region has been done with non-native actinorhizal shrubs and trees. Species have included autumn olive (*Elaeagnus umbellata* Thunb.), Russian olive (*E. augustifolia* L.), and European black alder (*Alnus glutinosa* (L.) Gaertn.)(Schlesinger and Williams 1984, Van Sambeek and others 1985). Black locust (*Robinia pseudoacacia* L.) has been the most promising nodulated temperate woody legume (Haines 1978). Estimates of the annual nitrogen input within black locust stands range from 30 to 56 kg ha^{-1} (Ike and Stone 1958, Boring and Swank 1984).

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Most of the woody legume species found in the Central Hardwood region are included within the Caesalpinioideae subfamily of the Fabaceae. More than 70 percent of the legumes classified within this subfamily are not nodulated and were thought not to have the ability to fix atmospheric nitrogen (Allen and Allen 1981, De Faria and others 1989). Bryan and others (1996) examined 12 non-nodulated tree legumes to evaluate their nitrogen-fixing capacity. They found rhizobial bacterioids within roots of six species, including honeylocust (*Gleditsia triacanthos* L.), and reported all species had nitrogenase activity which indicated the capacity to fix nitrogen although fixation rates were 1- to 2-fold lower than for nodulated tree legumes. Navarrete-Tindall and others (1996) have also reported acetylene-reduction activity from honeylocust seedlings when inoculated with one of four rhizobial strains isolated from a tropical woody legume.

OBJECTIVES

The objectives of our study were 1) to determine if non-nodulated native woody legumes produce foliage high in nitrogen; and 2) to determine if native woody legumes established as nurse crops can improve foliage nitrogen content and growth of interplanted northern pecan.

MATERIAL AND METHODS

The study was established on a 0.7-ha old-field site inside a 2-m-high, 6-strand electric fence at the Horticulture and Agroforestry Research Center (New Franklin, MO). The site is along a south-facing toe-slope with Memfro silt loams. The area was repeatedly tilled in spring 2001 before laying out twenty-four 42-m-long rows 4.5 m apart. Odd-numbered rows were planted to pecans (*Carya illinoensis* (Wang.) K. Koch) 3 m apart alternating between 1-0 bareroot stock from Forrest Keeling Nursery (Elsberry, MO) and 2-year-old grafts. Scionwood of Peruque, Kanza, or Posey were spring grafted in 1999 to 1-0 bareroot pecan rootstocks from Cascade Forestry Nursery (Cascade, IA) planted in 25-cm-tall 12-L plastic pots. Circling roots on the container-grown pecan grafts were cut before field planting. Because of the long taproots, both the bareroot stock and grafts were planted with tile spades in early May 2001. A 60-cm-long perforated semi-transparent tree shelter was placed over each grafted pecan after planting and removed in spring 2005.

Each row was divided into three 12-m-long treatment plots separated by 3-m-long border plots. The nine nurse crop treatments randomly assigned to the three odd-numbered rows within each block were interplanting with the nodulated legumes black locust (*Robinia pseudoacacia* L.), false indigo (*Amorpha fruticosa* L.), and smooth false indigo (*A. nitens* Boynton); the non-nodulated legumes thornless honeylocust (*Gleditsia triacanthos* var. inermis), redbud (*Cercis canadensis* L.), and Kentucky coffeetree (*Gymnocladus dioicus* (L.) K. Koch); the non-leguminous buttonbush (*Cephalanthus occidentalis* L.); slow-release 16N-8P-8K fertilizer spikes; or control without nurse plants or fertilizer. Within the odd-numbered rows, two nurse crop seedlings were planted within the tree row at a distance of 1 m on either side of each pecan seedling or grafted sapling. The same nine treatments were randomly assigned to the three even-numbered rows within each block. In these treatment plots, a plant of the same species as the nurse crop treatment was planted as the crop tree instead of a pecan.

Pecan and all nurse crop treatments except buttonbush were established in spring 2001. Bareroot 1-0 nursery of black locust and redbud seedlings were obtained from Forrest Keeling Nursery and planted with KBC planting bars. Kentucky coffeetree seed collected in southern Illinois were direct-seeded with two seeds in each planting spot in spring 2001. If seed failed to germinate, 1-0 nursery stock was added to planting spot in spring 2002. False indigo and smooth false indigo seedlings were grown from seed collected in southern Illinois (Taft 1994). Seed was sown in flats in the campus greenhouse and germinates transplanted to 0.5-L pots. Seedlings were inoculated and than transplanted as in-leaf stock. Honeylocust seedlings were planted as 3-0 in-leaf container-grown stock grown from seed collected from two thornless honeylocust trees. Because many of the seedlings developed thorns, only one seedling was available for planting within 1 m of a crop tree. Annually from 2001 through 2005, a 16N-8P-8K Jobe's tree food spike (Easy Gardener, Ltd., Waco, TX) was hammered 5 cm deep below soil line within the tree row at a distance of 1 m on either side of each pecan.

Each 12-m long treatment plot was divided in half and one of two weed control treatments randomly assigned to each subplot. One subplot was chemically treated each spring with a combination of glyphosate (6.4 L ha^{-1}) and simizine (4 kg ha^{-1}) for 2 years. For the other subplot, two 6-m-long strips of 1.2-m-wide woven polypropylene fabric were placed along the tree row, cut to make 20-cm long slits by each tree, and then overlapped within tree row before pinning down the center was pinned down and the edges buried. Quizalofop (77 g ha^{-1}) was sprayed in July 2001 and fluazifop (1.4 kg ha^{-1}) was sprayed over the top in May 2002 to control invading grasses within all tree rows. Alleys between tree rows were mowed periodically with a side discharge mower, allowing plant residues to cover the black weed barrier. In spring 2003, alleys between rows were disked and allowed to revegetate. All nurse crop trees were coppiced in late spring 2005, stems shredded, and residue placed back within the same tree row. The crop trees for both the pecan and nurse crop species have been annually pruned since 2004 to develop a single dominant stem with approximately 40 percent clear bole.

Crop and nurse trees were measured for height and basal diameter of largest stem and total number of vertical stems in the lower 30 cm in fall 2001, 2002, 2003, 2004, and 2006. In addition, if fruits were present, the approximate number of fruits or racemes was recorded in fall 2003, 2004, and 2006. Leaves, including the petioles, were collected from the center of the new growth from each crop tree in late July 2004 and late July 2006. Leaves of all crop trees within a treatment plot were combined, oven-dried at 60 °C, and ground to pass a 1-mm screen. Foliage samples were analyzed for total nitrogen by infrared spectrometry.

Treatment means for crop tree responses were subjected to analysis of variance for a split-plot design with four replications of nurse crop treatments as the main plot and methods of weed control and/or stock types as subplots. Separate analyses were done for the odd-numbered rows containing pecans as crop trees and for the even-numbered rows with nurse crop trees as the crop trees.

RESULTS

Foliage nitrogen content of the nurse crop trees ranged from 1.7 to 3.4 percent and separated into three distinct groups in both the 2004 and 2006 growing seasons (Table 1). Black locust, false indigo, and smooth false indigo are in the high foliage-nitrogen group, with foliage nitrogen ranging from 3.1 to 3.4 percent. Foliage nitrogen concentrations for black locust are within the range reported by Hacskaylo and others (1969) for seedlings grown with a complete nutrient solution or in solutions lacking a single essential mineral except nitrogen. Kentucky coffee tree and honeylocust were in an intermediate group with foliage nitrogen concentrations ranging from 2.1 to 2.6 percent. For these two species, foliage nitrogen was higher in the 2004 than in the 2006 growing season. Redbud and buttonbush were in the lowest group, with foliage nitrogen concentrations ranging from 1.7 to 1.9 percent.

Table 1.—Foliage nitrogen concentration of pecan and nurse trees in late July of fourth (2004) and sixth (2006) growing seasons

	Pecan foliage nitrogen (%)		Nurse crop foliage nitrogen (%)	
Treatment	July 2004	July 2006	July 2004	July 2006
Black locust	1.97	2.20	3.17	3.35
False indigo	1.85	1.77	3.16	3.25
Smooth false indigo	1.87	1.88	3.23	3.19
Honeylocust	1.93	1.74	2.35	2.02
Kentucky coffeetree	1.86	1.77	2.57	2.27
Redbud	1.97	1.92	1.76	1.84
Fertilizer spikes	1.92	1.78	----	----
Buttonbush	1.88	1.90	1.91	1.88
Control	1.86	1.78	----	----
LSD ($p<0.05$)	0.18	0.20	0.37	0.26

Table 2.—Survival percentages 2, 4, and 6 years after planting for pecan and nurse crop trees established with either weed barrier strips or chemical weed control

			Survival (%)					
		Number	Weed barrier			Chemical control		
Tree species	Planting stock type	planted	Year 2	Year 4	Year 6	Year 2	Year 4	Year 6
Pecan	1-0 seedlings	72	81	81	81	72	72	72
Pecan	3-0 grafts	72	100	100	100	100	100	97
Black locust	1-0 seedlings	80	68	78	90	78	85	92
False indigo	Germinant	80	98	98	95	90	90	90
Smooth indigo	Germinant	80	90	88	85	65	62	68
Honeylocust	3-0 container	44	100	100	100	100	100	100
Coffeetree	Seed	80	60	78	85	55	85	90
Redbud	1-0 seedlings	80	78	70	68	100	95	95

Nitrogen content of the pecan foliage ranged from 1.8 to 2.0 percent in the fourth growing season with no detected differences among treatments (Table 1). In the sixth growing season, pecan growing with black locust had higher foliage nitrogen than did pecan in any of the other nurse treatments. Foliage nitrogen concentrations of pecan in all plots are below those recommended for optimal growth of pecan (Sparks 1979, Mills and Jones 1996). Analyses of the other foliage macronutrients and micronutrients did not reveal any deficiencies that might explain why pecan foliage nitrogen was not raised by several treatments, especially by the slow-release fertilizer spikes.

Survival across species through the first 6 years was quite variable (Table 2). Survival of the pecan grafts, in-leaf honeylocust, and in-leaf container-grown false indigo has been excellent. Seedlings of pecan and black locust exhibited relatively high initial mortality regardless of type of weed control; however, many of the dead black locust seedlings were replaced by sprouts by year six. The increased survival percentages for Kentucky coffeetree may have resulted from a combination of delayed seed germination and poor survival of replacement nursery stock (Sander 1974). Mortality of smooth false indigo germinants was higher with chemical weed control than with the weed barrier fabric. In contrast, mortality of redbud seedlings was

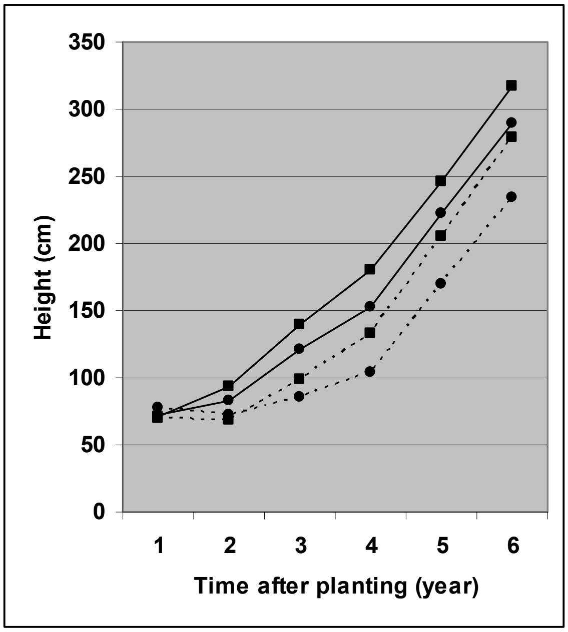

Figure 1.—Height of pecan seedlings (dotted line) and grafts (solid line) with 2 years of chemical weed control (circles) or weed barrier fabric (squares).

Table 3.—Mean sixth-year stem height and basal diameter of surviving pecans interplanted with woody legumes or fertilized with tree spikes

Nurse Crop Treatment	Height --cm--	Diameter --mm--
Fertilizer spikes	323 a	58 a
Kentucky coffeetree	308 a	55 ab
Control	295 a	54 ab
Buttonbush	295 a	52 ab
False indigo	292 a	51 ab
Smooth false indigo	281 a	49 ab
Honeylocust	291 a	48 ab
Redbud	281 a	42 bc
Black locust	183 b	31 c

lower with chemical weed control than with the weed barrier fabrics, suggesting redbud may be sensitive to the high soil temperatures associated with use of black weed barrier fabrics (Van Sambeek and others 1995). No additional mortality occurred between the fourth and sixth year, which suggests all the nurse crop species trees were tolerant of being coppiced in the spring of the fifth year.

Pecans interplanted with the fast-growing black locust were shorter and smaller than pecans in any other nurse crop treatment except for redbud after the sixth growing season (Table 3). By the fourth growing season, black locust had overtopped and slightly reduced stem height but not basal diameter of the pecans, which prompted us to coppice the nurse trees in all treatments the following spring. The grafted pecans planted as container stock with a 3-year-old rootstock grew faster after the second growing season than the pecans planted as bareroot nursery stock (Fig. 1). Both stock types exhibited the initial slow above-ground growth expected with transplanted pecans as they develop deep, large taproots. After the third growing season, pecans established with a 2-m-wide strip of weed barrier fabric grew faster than pecan established with a 2-m-wide herbicide strip (Fig. 1).

Differences in average height of the nurse tree species grown as crop trees were detected after the first growing season (Fig. 2). Unlike with pecan, no statistical differences were found between the two types of weed control in height or diameter of any nurse crop species. The height of honeylocust and redbud saplings most closely matched the height of the pecans. Black locust had very rapid height growth and overtopped the pecan seedlings or grafts even when coppiced. In contrast, initial height growth of Kentucky coffeetree, a common associate of pecan on bottomland sites, was quite slow but shows signs of accelerating now that the saplings are established.

Germinants of false indigo initially grew faster than smooth false indigo the first growing season; however, growth rates declined for both species when they started flowering in the second growing season. Similar

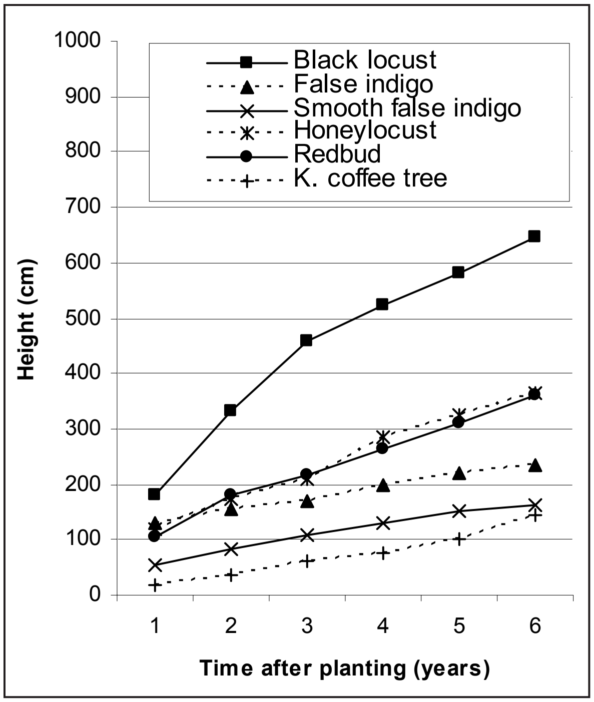

Figure 2.—Height of nurse crop trees and shrubs grown as noncoppiced crop trees within the nurse crop treatments.

differences in growth rate have also been found with stem cuttings (Navarrete-Tindall and others 2002). Although they each have unique morphological characteristics, smooth false indigo, a threatened and endangered species, is sometimes classified as a subspecies of false indigo (Wilbur 1975). False indigo has cuspidate leaflets with a sharp tip at the apical end, and the new shoots originating from buds basal to the flowering spikes tend to elongate past and hide the developing fruit. In contrast, smooth false indigo has emarginated leaflets with a slight indentation at the tip, and new shoots with fewer and smaller leaves leave developing fruit visible.

DISCUSSION AND CONCLUSIONS

The foliage nitrogen concentrations of the nurse crop species tended to follow the pattern suggested by McKey (1994) and Bryan and others (1996). The nodulated legumes had the highest percent nitrogen, non-nodulating legumes had intermediate levels, and the nonleguminous plants the lowest levels. Black locust, false indigo, and smooth false indigo seedlings had high foliage nitrogen concentrations and have been shown to quickly produce effective root nodules in association with native rhizobial populations (Dawson and others 1992, Navarrete-Tindall and others 2003).

Honeylocust and Kentucky coffeetree, but not redbud, had intermediate foliage nitrogen concentrations. These three species are included in the Caesalpiniodeae subfamily, where most taxa do not exhibit nodulation. The intermediate foliar nitrogen concentration and reports of nitrogenase activity and presence of rhizobial-like bacteroids inside infections of cortical cells (Bryan and others 1996), strongly suggest honeylocust and Kentucky coffeetree can fix nitrogen, but at much lower rates than nodulated legumes. The low-foliage nitrogen concentrations for redbud were unexpected. Bryan and others (1996) measured low rates of nitrogenase activity in Judas tree (*Cercis siliquastrum* L.) when exposed to a highly diverse population of rhizobia to enhance the likelihood of finding a compatible symbiont. Perhaps efficient compatible rhizobial bacteria were not present within our old-field soils, or redbud is incapable of non-nodular nitrogen fixation.

Several reasons can be given for the lack of response by pecan to nitrogen apparently being fixed by several of the woody legumes and fertilizer treatment. Schlesinger and Williams (1984) reported that non-native nitrogen-fixing shrubs and trees increased black walnut growth on the poorest sites, but not the best sites. This observation could apply to pecan as well as soil fertility is naturally high in a deep Memfro silt loam; however, foliage nitrogen in the range of 1.8 to 2.0 indicates a nitrogen-deficiency that may not be correctable by the addition of just nitrogen. This hypothesis is partially supported by the observation that pecan foliage nitrogen was not improved in two of the four blocks that had been invaded by hairy vetch, an excellent nitrogen-fixing cover crop (White and others 1981). An alternative explanation is that fixed nitrogen is not being released into the ecosystem. It is possible the non-nodulated nurse crop trees were fixing nitrogen, but in the fall were translocating the fixed nitrogen to bark tissues rather than to the leaf litter, as found with some actinorhizal species (Dawson and others 1980). Trends in seasonal foliage nitrogen remain to be determined for the nurse crop species used in our study.

The major problem with using nitrogen-fixing trees in hardwood plantings is matching the growth of the woody nurse crop species to the crop trees. We found pecan was rapidly overtopped by black locust and will need to be coppiced multiple times if the pecan trees are to survive. False indigo and smooth false indigo were expected to stimulate early pecan height growth by adding fixed nitrogen to the soil, reducing competition from herbaceous weeds, and providing up to 3 m of side-shade. Although both species are multi-stemmed, the wide-spreading crowns allow too much light to penetrate the canopy to reduce herbaceous competition, and height growth was inadequate to provide much side shade to the pecans. Height growth of honeylocust, Kentucky coffeetree, and redbud most closely match the height growth of pecan. Although there is evidence that honeylocust is fixing nitrogen, the tendency to develop thorns on seed-origin seedlings precludes its use as a nurse crop without vegetative propagation. Saplings of Kentucky coffeetree, a common associate of pecan on bottomland sites, were slow to establish, but eventually had height growth rates similar to pecan. Additional studies with interplanted seedlings are needed to confirm the growth rates. Although redbud and pecan growth rates are compatible, the low foliage nitrogen of redbud suggests it has not formed an association with native rhizobial species. Results indicate it may be too early in the study to determine which native woody legume, if any, should be interplanted with northern pecan to improve foliage nitrogen, growth, and pecan production.

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