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Michael Patrick Powers

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of the requirements for

Masters degree in Forestry

Douglas O. Lantagne
Major professor

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INTERACTION EFFECTS OF SOIL NITROGEN LEVELS AND INTERCROPPED MAIZE (*ZEA* MAYS) ON NITROGEN FIXATION IN AND TRANSFER FROM BLACK LOCUST (*ROBINIA PSEUDOACACIA*)

By

Michael Patrick Powers

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Committee Chair

ABSTRACT

INTERACTION EFFECTS OF SOIL NITROGEN LEVELS AND INTERCROPPED MAIZE (*ZEA* MAYS) ON NITROGEN FIXATION IN AND TRANSFER FROM BLACK LOCUST (*ROBINIA PSEUDOACACIA*)

BY

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Inclusion of black locust (*Robinia pseudoacacia*) in a temperate agroforestry system could benefit the soil-plant system through nitrogen-fixation and transfer of this fixed nitrogen to the intercrop. A greenhouse study was designed to gather initial data on these processes A randomized complete block design was used with four repetitions. Over three levels of applied liquid nitrogen, 50, 125 and 200 kg ha^{-1} equivalent, black locust alone was compared to black locust intercropped with maize, and maize alone was compared to maize intercropped with black locust. Nitrogen-fixation showed a dramatic downward trend with increasing levels of added nitrogen, however fixation was not completely suppressed. Intercropping had no effect on nitrogen fixation. Estimates of nitrogen transfer were variable and sometimes negative. Intercropped maize grew very poorly, presumably due to competition for phosphorus and potassium or allelopathic effects of black locust. Results indicated that black locust may well continue to fix atmospheric nitrogen despite high levels of added fertilizer nitrogen. However, strong competition for nutrients or allelopathic properties of black locust may be factors to consider in temperate agroforestry systems.

This research is dedicated to those who believe in the potential of agroforestry to take advantage of complex ecosystem interrelationships and produce more products from the same land, and do it in a socially and environmentally harmonious manner.

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PREFACE

This thesis is divided into six chapters. The first three include the introduction, literature review, and materials and methods for the entire study. The results of the study are then divided and discussed in the next two chapters, IV and V. Specifically, Chapter IV discusses results related to the impact of maize on the nitrogen-fixation of black locust. Chapter V reviews results on the potential of nitrogen transfer from the black locust to the maize. The final chapter presents the study's overall summary and conclusions with recommendations for future research. Although one of the original goals for this work was to study root growth, discussion is limited due to the poor growth of maize intecropped with black locust.

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I. INTRODUCTION

Agroforestry is the term given to systems where agricultural, horticultural, valuable wood crops, or animals are grown in the same temporal or spatial context as woody perennials. Agroforestry systems strive to achieve more production by optimizing the positive biological interactions and land-use efficiency in woody-woody and woody-nonwoody plant combinations. Incorporation of agroforestry systems into current land-use practice may: 1) make more productive use of marginal lands or lands in conservation reserves, including increased production of high value hardwoods; 2) contribute to reduced soil erosion losses due to increased ground cover; 3) diversify rural income sources; and 4) reduce nitrogen fertilizer inputs.

Two agroforestry systems appear feasible for Michigan:

- woody-woody intercropping of black locust (*Robinia pseudoacacia)* with high value timber species; and
- alley cropping or multi-row windbreak systems in which food crops are planted in alley-ways created by rows of woody legumes such as black locust.

This study attempted to provide a measure of black locust's ability to serve a positive biological role in temperate agroforestry systems. The nitrogen-fixing ability and fast growth of black locust make it a prime candidate as a temperate agroforestry tree. Research indicates that nitrogen-fixing woody species have proven ability for use in forest or agroforestry management systems in ways similar to that of legumes in agriculture. Legumes fix nitrogen from the atmosphere and thus reduce the need for nitrogen fertilizer. They improve soil nitrogen conditions for crops grown with the legume or planted later in the same field. Nitrogen-fixing trees and shrubs also may improve the nutrient cycling in a field. The deep roots of perennials access and take up nutrients leached beyond the root zones of annual crops. The nitrogen-fixing woody plants return these nutrients to the surface soil through leaf and stem loss as well as root turnover and rhizodepositions. It is a widespread practice in many parts of the world where low-input agriculture is common to plant food crops simultaneously with perennial woody legumes -- often called intercropping or agroforestry when perennials are used -- to take advantage of their nutrient cycling and nitrogen-fixation characteristics. The soil nitrogen balance is thereby improved using little to no chemical inputs.

As scientific and public pressures have demanded more sustainable land-use systems that also decrease inputs and costs, interest in agroforestry as a potential alternative to current systems has increased in the United States. There are many questions to be answered, including what species to use and what are their potential roles in highly mechanized, high-input agricultural systems.

In order to study the effect of leguminous plants on the soil-plant N budget, it is necessary to determine how much nitrogen is being added to the system. Several techniques have been developed, but the Isotope Dilution

Technique (IDT) is considered the most accurate in terms of its cost and ease of use. As almost all atmospheric nitrogen is ^{14}N and only 0.3663% of atmospheric N is ^{15}N, this method introduces a source of nitrogen with a higher than normal ^{15}N percentage. Through analysis of ^{15}N:^{14}N ratios, the IDT differentiates nitrogen fixed from the atmosphere from that already in the soil and that added as fertilizer N. In this way it is possible to measure the fixed nitrogen that a legume contributes to a soil-plant system.

Robinia pseudoacacia L. (black locust), a leguminous tree native to the United States and Michigan, was studied for its multiple uses and promise as a temperate agroforestry woody perennial. Maize was selected as the intercrop for its common use and importance in Michigan agriculture.

The study's hypotheses were:

1. A greater dilution of ^{15}N will occur in the black locust planted with maize than when black locust is planted alone.
2. The weight of nodules on black locust roots will increase when planted with maize.
3. A greater dilution of ^{15}N will also occur in maize planted with black locust.
4. Total maize root length is reduced when planted with black locust.

II. LITERATURE REVIEW

Introduction

Negative environmental effects and subsequent public outcry directed toward certain forestry and agricultural practices have increased interest in developing alternative sustainable production systems in the United States. Alternative systems would directly embrace the philosophy of resource stewardship over that of short-term production concerns. Some of the goals of these new production systems are to decrease farm chemical applications, sharply decrease erosion of our agricultural and forest soils, and increase agricultural biological diversity. By modifying typical monoculture environments, farmers may also be able to exploit beneficial plant and animal interactions, perhaps decreasing reliance on fertilizers and pesticides (Altieri and Leibman, 1986). Diversifying the farm ecology could mean the farmer is producing more than one product at a time, potentially diversifying farmer income as well (Arnold, 1987; Gruggenheim and Spears; 1991; Spears, 1987). Research is currently focused on developing appropriate land-use systems that increase diversity, build soil resources, and maintain agricultural production. Agroforestry is a multifaceted, adaptable land-use system that may be able to fill important roles in North American sustainable production systems. Black locust (*Robinia pseudoacacia* L.), a native nitrogen-fixing, fast growing tree, is being studied to determine its potential role in North American agroforestry.

Agroforestry

Agroforestry is the term given to systems where there are agricultural, horticultural, valuable woody crops, or animals grown in the same temporal or spatial context as woody perennials. Agroforestry systems attempt to achieve more production by: (1) optimizing the positive interactions between the two plant components of the system, and (2) diversifying output for the land in an ecologically and socially sustainable way. Incorporation of agroforestry systems into current land-use practices may also:

- make more productive use of marginal lands;
- diversify income sources; and
- add socioeconomic benefits from revitalization of rural areas through production of nontraditional agricultural products.

Some of the more specific benefits that agroforestry can bring to agricultural systems include soil erosion control, micro-climate stabilization, water infiltration enhancement, soil moisture retention, inputs to soil organic matter, better light-use efficiency, pest control through increased beneficial insect populations, and promotion of nutrient cycling (Altieri and Leibman, 1986; Dawson and Pasche, 1991; Jackson *et al.*, 1989; Kang and Wilson, 1987; Giller and Wilson, 1991; Risch et al., 1983; Sanchez, 1987; Young, 1985).

Agroforestry research indicates that nitrogen-fixing trees (NFTs) can be used in agroforestry management systems in ways similar to that of legumes in agriculture (Gordon and Dawson, 1979; Mishra, 1986; McIntyre and Jeffries, 1932; Young, 1989b). In addition, deeper rooted trees and shrubs access nutrients from soil depths unreachable by annual crop root systems. These nutrients are returned to the surface soil through leaf and stem abscission and resulting decomposition (Huxley, 1983). Decreased erosion and soil surface run-off helps maintain soil N, and improves fertilizer efficiency. NFTs and nitrogen-fixing shrubs are preferred because they biologically fix atmospheric N and add it to the soil-plant system (Dawson and Paschke, 1991; Khosla and Toly, 1986; Mulongoy, 1986). Many legumes fix N by forming a symbiotic relationship with soil bacteria such as *Rhizobium*. These microorganisms penetrate the legume roots, exploiting soluble plant carbohydrates to grow and form nodules. In return they fix N from the atmosphere and makes it available to the legume (Devlin and Witham, 1983).

Although agroforestry is a relatively new idea to North America, the inclusion of trees in agricultural systems is not new in many other parts of the world, and a large and growing body of literature exists covering tropical agroforestry (Steppler and Nair, 1987). Neither is agroforestry new in the temperate zones outside North America. In the temperate regions of the Peoples Republic of China, for example, extensive agroforestry systems exist that integrate tree (*Paulowina/Populus*) and wheat production as well as other agricultural commodities (Chirko, 1993). Farmers in the northern regions of India intercrop trees with wheat and other cereal crops (Dawson and Pasche, 1991; Sharma and Geyer, 1990), and a widely used agroforestry system in New

Zealand grazes cattle under fast-growing, managed pine (*Pinus radiata*) (Gold and Hanover, 1987).

Though agroforestry in North America is still new, it is becoming more established. Several national and international conferences on or related to these subjects have occurred recently (Garret, 1991; Hanover *et al.*, 1992; Schultz and Colletti, 1994; Williams, 1991). Research and farmer experience indicate that various agroforestry management systems appear feasible for North America, including Michigan, and include: (1) intercrops of NFTs such as black locust (*Robinia pseudoacacia* L.) or autumn olive (*Elaeagnus umbellata*) with crops, pasture, or high value timber trees; (2) live fences that provide forage as well as inexpensive fences or fence posts; (3) windbreaks and snowdrift fences that help prevent wind erosion and drifting snow; and (4) placement of food crops, ornamentals, or Christmas trees in alley-ways created by rows or hedgerows of woody legumes or high value timber species.

Below-ground Tree/Crop Interaction

As described earlier, agroforestry is a land-use system that attempts to achieve more production by optimizing the positive interactions between two plant components of the system. Below-ground interactions are less studied than those above-ground, yet they may have more influence on the system (Ong *et al.*, 1991). The use of deeper rooted NFTs may increase the total soil volume used by the agroforestry system and improve the potential for higher production.

Conversely, the benefits of NFTs may be negligible, and harmful conflicts may occur, if rooting densities increase in the intercrop's rooting zone (Huxley, 1983; Jonsson *et al.*, 1988; Ong *et al.*, 1991; Szott *et al.*, 1991; Young, 1989a), or if allelopathic incompatibility is found. Tree/tree or tree/crop competition for moisture and nutrients can result in yield and biomass reductions (Buck, 1986; Chirko, 1993; Dawson and Pasche, 1991; Huxley, 1983; Rachie, 1983).

One study serves as an interesting example of the competition problems and paradoxes that agroforestry systems can present. Willey and Reddy (1981) performed a millet and groundnut experiment that included a sole cropping of each species, an intercropping/control treatment, and an intercropping with a polythene below-ground barrier between the intercrops. Total intercropping yields were better than either sole crop, presumably due to an improvement in light-use efficiency. There was a decrease in millet production in the barrier intercropping treatment versus the control intercropping while groundnut performed better in the barrier intercropping compared to the control intercropping. This suggests a positive below-ground impact of the millet on the groundnut, but a negative below-ground impact of the groundnut on the millet. Below-ground impacts, then, are important in order to understand the ecology of agroforestry systems, and effects on production, yet little research has been aimed at them.

Soil moisture is an important below-ground factor in any agricultural system. Roots of intercropped trees can benefit plant/soil moisture relations through deep root systems that create macropores, thereby enhancing water infiltration and preventing soil compaction. This same root development can also reduce soil erosion and hence improve soil moisture retention (Gish and Jury, 1983). Tree roots may also reduce soil moisture to associated crops through competition from surface roots (Szott *et al.*, 1991; Young, 1989a). In an agroforestry soil management study on a tropical alfisol, both *Leucaena*- and *Gliricidia*-based systems showed greater available water capacity over no-till and plow-till no-tree systems. While moisture retention declined over time in all cropping treatments, the agroforestry systems showed the highest moisture retention (Lal, 1989a). Cumulative water infiltration was greatest for the plow-till treatment, but was followed closely by the *Leucaena* and then by the *Gliricidia* systems (Lal, 1989b). Alley cropping with perennial hedgerows of *Calliandra calothyrsus* (calliandra) and *Gliricidia sepium* (gliricidia) on a tropical Inceptisol in Western Samoa showed improved water holding capacity over the no-tree control (Rosecrance *et al.*, 1992). Conversely, many species such as *Leucaena* can fiercely compete for water with intercrops. When a soil partition was placed between *Leucaena* and cowpea and sorghum intercrops, yields of cowpea and sorghum increased versus the no-barrier treatment (Singh *et al.*, 1989). In a similar study, the soil barrier between a *Leucaena leucocephala* (leucaena) hedgerow and a millet intercrop virtually eliminated the loss in millet dry matter production due to root competition for soil moisture (Ong *et al.*, 1991). A note of caution is appropriate here: it can be difficult to obtain accurate measurements in field root competition work using soil barriers. Studies show that roots from tree-only and tree-intercrop plots have invaded no-tree control treatment plots, even when the soil barriers were present (Hauser and Gichuru, 1994).

The literature appears to support the potential for agroforestry systems to promote nutrient cycling, another important feature of below-ground interactions. Tree roots are capable of absorbing nutrients from soil too deep for annual crops to access, and then returning these nutrients to the soil surface through leaf and stem abscission as well as through fine root turnover in the rooting zone of the crop (Young, 1989a). NFTs are also important for their contribution of atmospheric N to the soil/plant system. Transfer of nutrients via direct root contact and the hyphae of root mycorrhizae holds potential (Giller and Wilson, 1991; Robinson, 1991; van Noordwijk and Dommergues, 1990). Ewel *et al.* (1982) compared root to leaf biomass ratios in three agriculture systems, two forest systems, and four agroforestry systems in Costa Rica and Mexico. Three of the four agroforestry systems had greater root:leaf ratios than the other five systems, and all had greater absolute root biomass, representing potentially large nutrient and organic matter sources as roots turnover.

While the nutrient cycling potential of agroforestry may be great, tree root distribution and competition for soil nutrients may be costly in terms of intercrop biomass and yield losses. Competition seems especially likely for more mobile nutrients such as nitrate N (Gillespie, 1989; Grime *et al.*, 1991). Though it is often speculated that trees have deep root systems, they can also have root distributions similar to annual crops, as found in one study of five potential agroforestry tree species (Jonsson, 1988). In a similar study, root distribution patterns of four tree species were measured. *Alchornea cordifolia*, *Cassia siamea*, and *Gmelina arborea* had 73%, 76%, and 74% of their fine roots, respectively, in the top 20 cm of an acid Ultisol (Ruhigwa *et al.*, 1992). These root systems, therefore, show potential for nutrient competition with food crops. In contrast, the fourth species, *Acacia barteri*, had only 49% of its fine roots in the surface soil. Furthermore, the *A. barteri* root system was concentrated around its trunk and had deeper roots than the other three species. Central to efficient nutrient use between the annual and perennial species is the selection of species with complementary root behavior patterns, but more research is needed (Huck, 1983).

Direct Nitrogen Transfer In Agroforestry Systems

A potentially important benefit of below-ground tree/crop interaction is N transfer to the nonlegume. There are three main pathways of N transfer considered to be present in agroforestry systems:

- decomposition of fallen above-ground biomass and dead root material (Agboola *et al.*, 1972; Mulongoy, 1986);
- mycorrhizal hyphal interconnections between legume and nonlegume root systems (Giller and Wilson, 1991; Robinson, 1991) and

- direct contact of legume and nonlegume roots (van Noordwijk and Dommergues, 1990; Young, 1989b).

In the absence of manure from grazing animals, decomposition of dead leaves and stem is considered the major pathway for N transfer from legumes to nonlegumes in agroforestry and intercropping systems, with little direct transfer between root systems (Catchpoole and Blair, 1990; Giller *et al.*, 1991; Giller and Wilson, 1991; Henzell and Vallis, 1977). Reliable data on root N contributions to soil and the intercrop are lacking (Giller *et* al., 1991; Giller and Wilson, 1991), in part due to the unreliable research methods (Chalk, 1985).

Nodulation and resulting N-fixation are strongly influenced by below-ground interactions. They are generally greater in low N soils and decrease with added fertilizer N. Nodule response can be affected on three levels:

- complete suppression;
- reduction in N-fixation as well as mass or number of nodules; or
- reduction or inhibition of nitrogenase activity of mature nodules (Giller and Wilson, 1991; Heckmann and Drevon, 1987).

In a greenhouse study with treatments of 220, 22, and 0 mg l^{-1} nitrate-N fertilizer applied to sainfoin and lucern, N-fixation and nodulation were both suppressed at the high N treatment. There were no differences between the control and the low N treatment (Sheehy and McNeill, 1987).

There is also little research showing the effects of intercropping and root competition on legume N-fixation and nodulation. Van Noordwijk and

Dommergues (1990) postulate that roots of N fixing trees have more nodules, where N-fixation takes place, when they are in close contact with roots of non-N fixing plants. This increased N-fixation and nodulation may lead to the direct transfer of N to the nonnodulating plant. General results point to an increase in N-fixation and nodulation with intercropping. Using the ^{15}N isotope dilution technique (IDT), N-fixation in ricebean (*Vigna unbellata*) intercropped with maize increased to 97 kg N ha^{-1} from 62 kg N ha^{-1} in the monocropped ricebean. Further, the proportion of N derived from N-fixation increased from 72% to 90%. The authors believe that the increase in %N from N-fixation in rice bean was due to competition and depletion of soil N by the maize, thus increasing rice bean N-fixation and nodulation (Rerkasem and Rerkasem, 1988). However, caution must be used when interpreting results from isotope dilution studies as the differences in the ^{15}N enrichment used to determine the amount of N-fixation can vary according to method and different ^{15}N uptake patterns between the legume and nonlegume crops (Giller and Wilson, 1991). In addition, above-ground effects such as shading can decrease nodulation and N-fixation (Nambiar *et al.*, 1983).

Of current N transfer studies, most have been done using legume-grass swards, and estimates of N transfer in these systems vary greatly. No transfer was measured from legumes to grass by Henzell and Vallis (1977), yet in other studies 20% to 50% of legume N was transferred (Burity *et al.*, 1989; Ta and Faris, 1987). Using the ^{15}N IDT, Broadbent *et al.* (1982) found no transfer from clover (*Trifolium replias* L.) to ryegrass (*Lolium rigidum* L.) in a greenhouse experiment, but measured a transfer up to 79% of legume N in a field trial. Perhaps there was a time delay: the greenhouse experiment ran for two or four cuttings (exact time duration not given) while the field experiment ran for two years. Similar contrasting field and greenhouse results were found by Ledgard *et al.* (1985) between clover (*Trifolium subtgerraneum* L.) and annual ryegrass (*Lolium rigidum* Groud).

In the case of cereals such as corn, there are few definitive studies showing transfer of legume N without additions of above-ground biomass. No such studies have been performed under field conditions (Giller and Wilson, 1991; Ta *et al.*, 1989). Searle *et al.* (1981) found greater maize grain yield and residual soil N when maize was intercropped with peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.), but apparently no contribution of fixed N from the legumes to the maize. One attempt to measure N transfer to a cereal resulted in a series of greenhouse experiments using perilite and montmorilonite plant mediums, and compared the ^{15}N-foliar feeding technique against the ^{15}N IDT. With foliar feeding, *Phaseolus* bean transferred less than 5% of its N to the maize, and no N transfer was measured using the IDT (Giller *et al.*, 1991). There was also no proof of N transfer between maize and *Phaseolus* in either greenhouse or field trials utilizing the ^{15}N IDT by Reeves (1991). Thus, there is currently little evidence that direct transfer of N from legumes is generally important for cereal intercrops.

After a field trial found no significant N transfer to a grass (*Panicum maximum* c.v. Riversdale) from the leguminous trees *Calliandra calothyrsus*, *Gliricidia sepium, Leucaena leucocephala* cv. Cunningham, or *Sesbania grandiflora*, researchers measured N transfer in a greenhouse using the split-root technique. Leucaena and Gliricidia trees transferred 4.1% of labeled N after six weeks and up to 7.6% of labeled N to the intercropped *Panicum maximum* after 12 weeks in a 1:1:1 sand, silt, and clay medium (Catchpoole and Blair, 1990). There are few other reports investigating N transfer from tree roots to associated crops (van Noordwijk and Dommergues, 1990) as researchers have only recently begun testing NFTs.

The nutrient competed for the most is soil N, so the potential of direct transfer of N from NFTs to associated crops deserves more research with more species over a wider range of environments and plant combinations. Researchers must begin to systematically study the role played by NFT roots in agroforestry systems to control the positive and negative aspects of tree/crop root interaction (Buck, 1986; Gillespie, 1989; Huck, 1983; Szott *et al.*, 1991; van Noordwijk and Dommergues, 1990; Young 1989b), including allelopathy (Putnam and Duke, 1978).

Black Locust (*Robinia pseudoacacia* L.)

The introduction of NFTs into agricultural systems means we need to understand the below-ground interactions and the role of roots in N transfer. Primary to this understanding is selecting potential NFTs with complementary root dynamics for use in North American agroforestry systems.

Black locust (*Robinia pseudoacacia* L.) is a prime candidate for consideration in temperate agroforestry systems. Dawson and Pasche (1991) call this NFT "the only important nitrogen-fixing tree adapted to cool-temperate climates." Economically, black locust is used for lumber, poles, wood fiber/pulp, fuel, beekeeping, railroad ties, mine timbers, and more (Barrett and Hanover, 1991). Physically, its root system is characterized by a tap root with a broad lateral root system close to the soil surface that is normally about 1.0 to 1.5 times tree height. It can also produce a deep tap root in arid regions that allows it to evade droughts (Barrett and Hanover, 1991; Huntley, 1990). Physiological characteristics include its intolerance of competition and shade in natural systems (Huntley, 1990), although it is used successfully in agroforestry systems (Sharma and Geyer, 1990). It is tolerant of both acid and calcareous soils, and is host to a wide variety of common rhizobial strains, making field inoculation usually unnecessary. In addition, black locust has a high rate of photosynthesis and is capable of supporting high rates of N-fixation (Mebrahtu and Hanover, 1991), although estimates vary on its ability to improve soil fertility. Reports of soil N accretion range from 30-40 kg ha^{-1} in natural North American stands

(Boring and Swank, 1984; Ike and Stone, 1958; Reinsvold and Pope, 1985) to 109 kg ha^{-1} in a dense planting of 2½ year-old black locust (Dawson *et al.*, 1982).

Black locust response to fertilizers is typical of legumes, with nodulation and N-fixation decreasing with increasing levels of N (Dawson *et al.*, 1992; Johnsen, 1992). Using black locust seedlings, Johnsen and Bongarten (1991) demonstrated that nodule biomass and N-fixation, as measured by acetylene reduction, were lower in the 5.0 mM nitrate treatment than in the 0.0 or 1.0 mM treatments. Black locust seedlings grown in sand for 50 days treated with a plus-N treatment (5 mM NH_4NO_3) and a minus-N treatment (no N) showed decreased nodulation and lower N-fixation rates in the plus-N treatment as measured by acetylene reduction (Roberts *et al.*, 1983). A greenhouse study using a soil, sand, and vermiculite planting medium was conducted to determine the combined influence of different levels of added nitrogen (0, 25, 50, and 100 mg N kg^{-1} soil as nitrate and ammonium) and phosphorous on nodulation and N-fixation (Reinsvold and Pope, 1987). Low nodule weight in the low N treatment was attributed to a lack of starter N and therefore delayed physiological development at the time of measurement. Nodule dry weight and number of nodules were inhibited at the high and low N treatments, in contrast to the middle N treatments, though the results were not significant. N-fixation as measured by acetylene reduction assay was greater in the middle treatments than in the high N treatment, though again the differences were not significant.

Research on the effects of black locust in U.S. agroforestry is still new. In a greenhouse pot experiment, Ntayombya and Gordon (1993) found that intercropping black locust with barley decreased barley yield compared to the sole crop. However, total dry matter production increased and soil fertility improved with the black locust mulch treatment. In separate one year field experiments, two studies found that an alley cropping system with black locust hedgerows and corn alleys lowered total corn yield on a per hectare basis. However, these lowered yields were attributed to decreased corn plant populations in the agroforestry system (Seiter, 1994; Ssekabembe and Henderlong, 1991). Ssekambe and Henderlong (1991) used soil partitions to investigate root interaction between the corn and black locust. They found less corn total root length and better yields in the corn row closest to the black locust hedgerow in the soil partition treatment. Reasons for this were unclear as they could be due to higher soil moisture under the hedgerow, allelopathy, or a beneficial N effect of the black locust roots.

The multiple economic and biological uses of black locust may make it a viable option for farmers who are seeking to diversify their income and the ecology of their fields. However, the disadvantages of black locust must also be considered when designing research programs and when incorporating it into agricultural practices. Black locust has sharp thorns that can make management more difficult, though they will not puncture shoes and tires. Insect pests are common in pure stands, though not necessarily in less dense systems of intercropping. Its broad and shallow lateral root system indicates a strong ability to compete for nutrients and moisture. Finally, black locust has rapid growth from root suckers and heavy seed production that may allow it to escape cultivation and become a "weed" (Barrett and Hanover, 1991; Dawson and Pasche, 1991).

Proper and attentive management of black locust will be key in any agroforestry system that uses it. Such systems will require trained individuals (farmers or professionals) not only in tree management, but also in physiology, pest identification and control, and species selection. For farmers and foresters in the United States, this requires exploration and acceptance of using trees in nonconventional ways (Gold and Hanover, 1987).

Much research remains to be completed before confirming the role of black locust in temperate agroforestry. To date, there are no N transfer studies involving black locust. No studies have been found on the effect of root competition and intercropping on nodulation and N-fixation in black locust. This study attempts to address the potential of N transfer from black locust as well as effects of intercropping on nodulation and N-fixation.

Nitrogen-Fixation Measurement Techniques

Various methods exist to measure the symbiotically fixed atmospheric N_2 gas (fixed N) in legumes. The method used in this experiment is often called the ^{15}N Isotope Dilution Technique (IDT). The IDT differentiates the isotopic composition in the sources of N available for plant growth: soil N, fertilizer N, and atmospheric N (Chalk, 1985). Special terminology is required to explain this method (Table 1).

The amount of N in soil or biomass that contains the ^{15}N stable isotope is often expressed as the proportion of ^{15}N present -- $\%^{15}N$. Atmospheric N is almost all $^{14}N_2$, with $^{15}N_2$ making up only 0.3663%. Any material that has a $\%^{15}N$ greater than that in the atmosphere is said to be enriched with ^{15}N. A ^{15}N enrichment above the natural abundance of 0.3663% is the $\%^{15}N$ excess (Giller and Wilson, 1991) or ^{15}N ratio excess.

An enriched fertilizer can be added to the soil, thus labeling the soil with ^{15}N. IDT experiments performed in soil, versus a N-free media, must then attempt to discriminate between N derived from the soil, fertilizer, and atmosphere. A non-fixing system (nfs) reference plant is used to estimate the ^{15}N enrichment of the available soil N (Table 1). This requires that the fixing system (fs) and nfs both absorb N from soil with the same ^{15}N enrichment (Giller and Wilson, 1991). The IDT is considered by many researchers to be the most useful and comparatively accurate method for measuring N-fixation. Only techniques employing the principle of ^{15}N IDT yield a reproducible and precise estimate of N-fixation integrated over time under field conditions (Rennie, 1985; Ta *et al.*, 1989). The IDT requires that ^{15}N be uniformly applied to the soil (Rennie, 1986). This is vital so the fs and nfs roots grow in soil of very similar

Table 1: Terms for ^{15}N Isotope Dilution Technique

	Term		Definition
1.	atom % ^{15}N (or %^{15}N)	=	the relative amount of ^{15}N atoms versus ^{14}N atoms as a percentage of the total. (Giller & Wilson, 1991)
2.	natural abundance of the atmosphere	=	the atom %^{15}N of the atmosphere = 0.3663 %^{15}N (Giller & Wilson, 1991)
3.	atom %^{15}N excess (or %^{15}N excess)	=	the percentage of ^{15}N above 0.3663 = %^{15}N - 0.3663 (Giller & Wilson, 1991)
4.	^{15}N-enriched nitrogen (or ^{15}N-enriched)	=	nitrogen with a %^{15}N above 0.3663 (Giller & Wilson, 1991)
5.	Nitrogen-fixing system	=	fs
6.	Non-nitrogen-fixing system	=	nfs
7.	% of N derived from the atmosphere	=	$\%\text{Ndfa} = 1 - \left(\frac{\%^{15}\text{Nexcess (fs)}}{\%^{15}\text{N excess (nfs)}} \text{ x } 100 \right)$ (Rennie, 1986)
8.	% of N derived from the fertilizer	=	$\%\text{Ndff} = \frac{\%^{15}\text{N excess (plant)}}{\%^{15}\text{N excess (fertilizer)}} \text{x} 100$ (Rennie, 1986)
9.	% of N derived from the soil	=	%Ndfs of nfs = 100 - %Ndff of fs = 100 - (%Ndff + %Ndfa) (Rennie, 1986)

^{15}N enrichments (Danso, 1988; Giller and Wilson, 1991; Rennie, 1985; Vose *et al.*, 1982). Liquid fertilizer application of ^{15}N is believed to be the most precise method (Vose *et al.*, 1982). However, not all researchers agree and experiments have shown significant variation in IDT studies due to labeling technique (Chalk, 1985; Danso, 1988). Witty and Ritz (1984) showed conflicting results for reasons that are unclear. Rennie (1986) compared applications of ^{15}N enriched liquid fertilizer and applications of ^{15}N enriched legume and cereal organic residues that theoretically might provide a more consistent source of ^{15}N over time. No differences between these two techniques were found. Ta *et al.* (1988) found no differences in their work comparing direct $^{15}N_2$ exposure, foliar labeling of ^{15}N, and ^{15}N isotope dilution technique.

The discussion of labeling technique is centered around the best method to obtain an accurate estimate of maximum N-fixation and allow comparison between legume species and varieties. This experiment, however, is not designed to estimate maximum N-fixation in black locust but to observe its response to added N fertilizer and intercropping, conditions that might be present in an agroforestry system. Thus liquid labeled fertilizer was utilized in this study based on the existence of supporting literature and the desire to observe the interaction between added N fertilizer and intercropping on N-fixation and transfer.

The choice of the nfs reference plant is critical to the accuracy of the IDT (Danso, 1988; Giller and Wilson, 1991; Rennie and Rennie, 1983), regardless of the labeling method. It is difficult to ensure that ^{15}N uptake is not affected by variation in rooting depth, or in the timing of N uptake (Giller and Wilson, 1991). As the concentration of a fertilizer decreases with greater soil depth, so does the enrichment of ^{15}N. Deep and shallow rooted plants, then, would sample soil N of different ^{15}N enrichments, confounding any results. For example, if a legume (fs) has a deeper rooting pattern than its reference (nfs) plant, it would have access to a soil with a lower %^{15}N. Accessing soil of lower %^{15}N not available to the nfs leads to the overestimate of N-fixation (Giller and Wilson, 1991). In other words, the difference between the level of ^{14}N and ^{15}N can be due to availability in the soil, and not the legume's capacity to absorb and fix ^{14}N from the atmosphere.

Another potential source of error is that ^{15}N soil enrichment decreases as soil microbes immobilize added mineral N over time. Therefore, different N uptake patterns between the fs and nfs is of concern when using the IDT. For example, if in the early stages of growth the nfs plant absorbs soil N more rapidly, with the fs absorbing most of its N later, the nfs will accumulate a higher ^{15}N enrichment. This would lead to an overestimate of N-fixation. Conversely, if the fs samples soil N more rapidly in the early stages of growth than the nfs, N-fixation estimates are underestimated (Giller and Wilson, 1991).

Finally, N-fixation in trees is the most difficult to estimate of all legume types. Appropriate nfs reference plants are extremely difficult to find as sources of variation such as different N uptake profiles and unlike rooting patterns are magnified (Giller and Wilson, 1991).

Other techniques to measure N-fixation include indirect methods such as the Acetylene Reduction Assay (ARA) and the A-Value Method. They are indirect since they measure indicators of N-fixation, but do not measure fixation itself. The ARA is an attempt to measure nitrogenase activity. Nitrogenase is a complex of enzymes in legume nodules that reduce N_2 to NH_3 for plant use (Devlin and Witham, 1983). In the ARA, acetylene (C_2H_2) is reduced by nitrogenase to ethylene (C_2H_4) at the exclusion of N_2. The theoretical conversion ratio of acetylene reduced to N_2 fixed is 4 moles C_2H_4 reduced : 1 mole N_2 fixed. The ARA is generally performed by incubating the test material (roots and nodules or a nodule) in an atmosphere containing 10% acetylene in a closed vessel. The amount of ethylene produced after a period of incubation is then measured by gas chromatography. The nitrogenase activity, or ARA, is then expressed as uM C_2H_4 produced per plant (or nodule) per hour. The amount of N_2 fixed is calculated using the theoretical conversion ratio of 4:1. One can also calculate the actual conversion ratio using a direct measurement of the rate of N-fixation by incorporating $^{15}N_2$ analysis in parallel with an ARA (Giller and Wilson, 1991). The ARA is considered a sensitive, fast and inexpensive method for measuring N-fixation (LaRue and Patterson, 1981). However, the ARA requires many samples for a good field estimate, and it is a one-time estimate that must be done immediately after harvest. Furthermore, there is much plant to plant variation. Additional error is introduced through the uncertainty of obtaining the entire root and all the nodules (Giller and Wilson, 1991; LaRue and Patterson, 1981), as well as from the resulting plant disturbance that can cause a marked reduction in the rate of nitrogenase activity (Minchon *et al.*, 1986). Moreover, the 4:1 theoretical conversion factor of moles C_2H_4 to moles N_2 fixed can vary greatly, often ranging between 5:1 and 7.5:1 (Witty and Minchon, 1988). The best estimates come from 'flow-thru' systems made on undisturbed plants (Giller and Wilson, 1991), making this technique suitable only for smaller pot studies.

A second indirect method for measuring N-fixation is the A-Value Method. It refers to the amount of available N in the soil in terms of a standard (Fried and Dean, 1963). For an N-fixing plant there are three sources of N: A(soil), A(fertilizer), and A(fixed). A(fixed) is equal to the difference of A(soil + fixed) - A(soil). The actual amount of N fixed can be calculated as:

$$N_2 \text{ fixed (kg ha}^{-1}) = \text{A(fixed)} \quad \times \quad \frac{\text{total N yield in crop}}{\text{total N supply to crop}}$$

which then becomes:

$$N_2 \text{ fixed (kg ha}^{-1}) = [\text{A-value (fs) - A-value (nfs)}] \times \frac{\text{\%Ndff x total plant N (kg ha}^{-1})}{\text{fertilizer N applied (kg ha}^{-1}) \text{ to fs}}$$

(Vose *et al.*, 1982).

An advantage of this technique is the ability to provide different rates of N fertilizer to the fs and nfs (Vose *et al.*, 1982), allowing a lower fertilizer rate to be applied to the nfs in order to obtain an estimate close to maximum N-fixation. However, this method is yield dependent. In addition, A-values for reference plants may vary with increasing rates of applied N; some workers have shown decreasing A-values with increasing applied N (Chalk, 1985). This also assumes that the addition of a large amount of N fertilizer to the nfs has no effect on the availability of soil N, a questionable assumption (Witty, 1983). Finally, the equation term for the rate of N fertilizer means that any loss of fertilizer N by denitrification or volatilization will result in error (Vose *et al.*, 1982).

In addition to IDT, a direct technique for measuring N-fixation is the Nitrogen Balance Method (NBM). In the NBM, the N yield of a fs and a nfs are compared. The difference in the nfs, if greater, is attributed to N-fixation.

$$\%\mathrm{Ndfa} = \frac{\mathrm{N\ yield\ (fs)} - \mathrm{N\ yield\ (nfs)}}{\mathrm{N\ yield\ (fs)}} \times 100$$

(Rennie, 1985)

It provides a measure of the total amount of N fixed over the length of an experiment (Giller and Wilson, 1991). However, this method is yield dependent and is based on the assumption that the fs and nfs assimilate identical amounts of soil and fertilizer N (Rennie, 1985). Positive or negative values for N-fixation in the absence of nodulation are sometimes encountered when N-fixation is estimated using the NBM (Rennie, 1985), rendering its results rather questionable.

Of all methods used to measure N-fixation in legumes, measuring $^{15}N_2$ fixation is the most reliable and direct (Bremener, 1977; Giller and Wilson, 1991; Vose *et al.*, 1982). Plants are grown in an enriched atmosphere of $^{15}N_2$. After an appropriate period of time, the plant biomass can be harvested and later analyzed in a mass spectrometer to measure the $\%^{15}N$ excess. In this manner the amount of N-fixation can be accurately determined for that specific period of time. While using an enriched $^{15}N_2$ atmosphere is the most accurate method known, sophisticated incubation chambers are required, limiting this method to small greenhouse studies and nearly eliminating the possibility of field studies (Vose *et al.*, 1982).

Conclusion

Nitrogen-fixing trees and shrubs benefit agroforestry systems through soil erosion control, micro-climate stabilization, water infiltration, pest control, and promotion of nutrient cycling, especially of nitrogen. Major potential negative impacts include competition for light, nutrients, and moisture. More research is needed on below-ground root interactions, most notably on effects of intercropping on legume nodulation, N-fixation, and N transfer. Black locust currently holds high potential for use as a temperate agroforestry system component. However, we have only preliminary data on its potential impacts on intercrops. We do not have data showing how its high nodulation and N-fixation are affected by intercropping. In part through use of the IDT, this study attempts to provide some applicable data, and contribute towards the goal of determining the role of black locust in North American agroforestry systems.

III. METHODS

Study Site

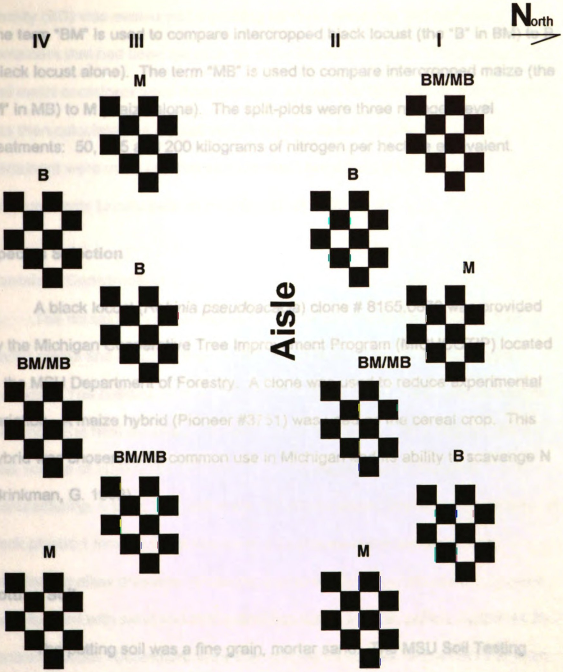
The study was located in the north greenhouse at the Michigan State University (MSU) Department of Forestry Tree Research Center, East Lansing, Michigan.

Experimental Design

A randomized complete block design (RCBD) with split-plots, four replications, and three subsamples per experimental unit was used. Figure 1 illustrates the arrangement of the experimental design. The main plots were plant treatments: one maize plant per container, one black locust plant per container, and an intercropping of one maize and one black locust. The following terminology is used to compare plant treatment results:

B → black locust alone;
BM → black locust in black locust + maize;
M → maize alone;
MB → maize in black locust + maize.

The BM and MB abbreviations represent the same plant treatment. The first letter in each term denotes the plant being referred to in that plant treatment.



Key: Roman numerals denote repetitions (blocks).
B → black locust main plot
M → maize main plot
BM/MB → black locust + maize main plot
N treatments randomly assigned within each main plot. Three subsamples per N treatment are used for a total of nine containers per main plot.

Figure 1: Experimental Design and Arrangement

The term "BM" is used to compare intercropped black locust (the "B" in BM) to B (black locust alone). The term "MB" is used to compare intercropped maize (the "M" in MB) to M (maize alone). The split-plots were three nitrogen level treatments: 50, 125 and 200 kilograms of nitrogen per hectare equivalent.

Species Selection

A black locust (*Robinia pseudoacacia*) clone # 8165.0879 was provided by the Michigan Cooperative Tree Improvement Program (MICHCOTIP) located in the MSU Department of Forestry. A clone was used to reduce experimental variation. A maize hybrid (Pioneer #3751) was used as the cereal crop. This hybrid was chosen for its common use in Michigan and its ability to scavenge N (Brinkman, G. 1993).

Potting Soil

The potting soil was a fine grain, mortar sand. The MSU Soil Testing Service measured the pH to be 8.1, with calcium making up 97% of the soil bases, or 1760 kg Ca ha^{-1} equivalent. The Cation Exchange Capacity (CEC) ranged from 4.5 to 4.7 cmol kg^{-1}. NO_3^- - N was measured at 0.90 mg kg^{-1}, while NH_4^+ - N was 3.52 mg kg^{-1}, for a total N of 4.42 mg kg^{-1}. The average bulk density (BD) was measured by packing air dried sand into 140 cm^3 metal containers that had been set in an oven for 48 hrs at 105° C. The potting soil and metal containers were then placed in an oven for 24 hours at 105° C. BD was then calculated as dry soil weight (g) / container volume (cm^3). Two containers were used and this was repeated twice for a total of four measurements to calculate an average BD of 1.58 mg m^{-3}.

Container Construction

The containers for this experiment were constructed by stacking four plastic crates and securing them with 5 cm wide duct tape to form a single container. The bottoms from each of the top three crates were removed before stacking. The final dimension of each container was 30 x 30 x 100 cm., for a total volume of 90,000 cm^3 (Figure 2). Heavy paper inserts (Monarch Manufacturing, Salida, CO) within the containers added strength and stability. A black plastic 1 mm x 0.5 mm knit mesh was placed at the bottom of each container to allow drainage. Once the containers were constructed, they were partially filled with sand and then placed on top of wooden pallets supported by concrete blocks. Containers were then topped off with sand to within 3 to 4 cm of the top of the container. Foil catchments were placed under the containers to catch leachate, although no leachate was ever collected. The containers were arranged in a checkerboard pattern to reduce shading (Figure 2).

Fertilization Minus Nitrogen

To ensure an even distribution of macro- and micro- nutrients, the top 25 cm of sand was removed and mixed with three liters (L) of fertilizer solution. The solution was deionized water containing 7.81 g $MgSO_4 \cdot 7H_2O$, 3.56 g K_2HPO_4, 2.25 g K_2SO_4, and 1.89 g STEM micronutrient mix (Grace Sierra Horticultural Products Company, Richardson, Texas) to provide an equivalent of 75 kg ha^{-1} Mg, 140 kg ha^{-1} P_2O_5 and 300 kg ha^{-1} K_2O on an area basis as well as micronutrients S, B, Su, Fe, Mn, Mo, and Zn. Before replacement of the surface soil, the remaining container soil was excavated an additional 15-20 cm and compacted by hand along the sides of the container. This was done to favor percolation through the center of the container when six liters of deionized water were added to saturate the remaining soil. The goals were to bring each container to field capacity, maintain uniformity among containers, and further reduce available N through leaching. After saturation, the fertilized surface soil was placed back into the container. The compacted soil along the sides was not altered before returning the fertilized top soil. This was an effort to discourage root growth along the sides of the containers. Black locust seedlings were transplanted to the containers two days later.

Growth And Transplantation Of Black Locust

The black locust clone was grown in styroblocks, each holding 30 seedlings in a basic greenhouse potting mix. These seedlings were pruned back three times before transplanting to the containers. At transplanting, seedlings were taken from the styroblocks and their length measured from the fork below the terminal leaf down to the root collar. Seedlings were then root-pruned to 12.5 cm and the root system washed in deionized water to remove the potting mix and to minimize the amount of introduced N. Seedlings were then randomly assigned to containers by blocks according to height. They were transplanted on a diagonal 12.5 cm from the southeast corner of each container. The combination of high greenhouse temperatures, pruned root systems, and washed root systems resulted in the loss of all existing leaves. Seedlings were then top-pruned to a uniform height of 10 cm. Six seedlings died and were replaced, two having to be replaced a third time: one of those died and was not replaced, leaving only two subsamples in that treatment combination.

Method And Frequency Of Watering

All plants were watered as needed with deionized water. Deionized water was routinely sampled for nitrate-N and ammonium-N levels. All samples showed less than 0.1 mg kg^{-1} total N as measured for NH_4^+ - N and NO_3^- - N on a Technicon Autoanalyzer II System (Technicon, 1977 & 1978). Watering was initially done by hand using a 2 L Dram plastic watering can with a very fine water nozzle. Later a trickle irrigation system was installed.

^{15}N Application

Ninety-six days after transplanting, random samples consisting of five leaflets were collected from each tree to provide a baseline for N and ^{15}N levels in the leaves. The containers were then fertilized with a solution containing $(^{15}NH_4)_2SO_4$. Procedures used to determine the amount of N to apply and preparation of the labeled fertilizer came from Cabrera and Kissel (1989). The fertilizer enrichment was calculated to produce a uniform soil enrichment of 1%^{15}N. This would produce accurate ^{15}N tissue and soil results from the mass spectrometer (Harris, 1993) . The ^{15}N source in the fertilizer solution consisted of 10 g 99% ^{15}N and 19.22 g 10% ^{15}N for a total of 29.22 g of 38.09% ^{15}N enriched source of N as $(^{15}NH_4)_2SO_4$. The enriched source was mixed with 1257.27 g unenriched $(NH_4)_2SO_4$ and deionized water to make a volume of 3,240 ml of ^{15}N enriched fertilizer concentrate. Ten ml of this concentrate was removed for storage and later analysis. Although the target enrichment was 1.20% ^{15}N, analysis indicated an enrichment of 1.272%. The concentrate, 12, 30, or 48 ml, was mixed with deionized water to a volume of 2L and then applied to the appropriate randomly selected containers using a 2L Dram watering container with a fine stream nozzle. This resulted in an N application of 1.0, 2.5, and 4.0 g of N to approximate 50, 125, and 200 kg N ha^{-1} equivalent, respectively. The entire soil surface was saturated with the solution to provide an even N distribution. The watering container was rinsed with deionized water between applications to avoid cross contamination.

Maize Planting

After fertilization with ^{15}N, maize was planted on a diagonal 13 cm from the northwest corner of each container. In the intercropped treatment, this was 15 cm from the black locust seedling. Centered at each planting spot was placed a 5 x 5 x 10 cm planting band 5 cm into the sand. Sand was removed from the square to a depth of 5 cm and four to five kernels of maize were then placed inside and covered with dry sand and a paper bag. When above-ground maize growth was observed, the bags were removed. At seven to eight days of growth, the planting bands were carefully removed and the seedlings were thinned leaving only the tallest.

Measurements and Sampling

Final maize height was measured from the top of the whirl to the soil surface. Black locust height was taken from the root collar to the fork below the terminal leaf (of the tallest stem if the seedling had two or more stems). Diameter measurements were taken of the root collar using digital calipers.

Harvesting: above- and below-ground biomass, soil

Above-ground biomass was collected from the entire experiment. Each plant was cut at the root collar. In addition, maize plants were cut at the midpoint between the top of the whirl and the root collar and the two halves placed in separate paper bags. Black locust leaves (including the leaf stem) and woody stem were harvested separately and placed in paper bags. All plant biomass was dried for 24 hours at 65° C.

One of the three subsamples of each treatment in each repetition was randomly selected for soil sampling and root collection. After the above-ground biomass was harvested, a 5 cm x 5 cm inside diameter soil core was driven into the center of selected containers to collect root samples for root length and diameter measurements. Two soil depths were sampled: 0-25 cm and below 25 cm. These samples were placed in separate plastic zipper-type bags and frozen. Next the containers were tipped onto their sides and the long top panel of each container was removed. The panels were cut with little disruption to the soil and the root system. The opened container allowed easy sampling of soil and roots.

As part of the experiment, "test" containers were employed. Plant and N treatments were applied to test containers so they could be destructively sampled during the experiment to view root development. Harvesting of test containers showed a 25-30 cm plow-like layer resulting from the fertilization (minus N) procedures. There also appeared to be a wetting front at the 55-60 cm depth. Two soil samples were therefore collected with a standard 2 cm inside diameter soil sampler from each of the following ranges: 0-25 cm, 25-55 cm, and below 55 cm. Samples were placed in labeled paper bags and allowed to air dry. After soil sampling, root sub-samples were extracted by hand from the entire container and bulked. Maize and black locust roots were carefully separated by hand to avoid cross contamination. All root samples were placed in paper bags and dried for 24 hours at 65°C and later analyzed for total N and $\%^{15}N$.

After soil and root sub-samples were taken, the entire root system was separated from the soil of each container. Using fine stream nozzles and tap water, the sand was carefully washed from the root systems onto a 3 mm x 3 mm inside diameter mesh screen. Individual root systems, as in the maize or black locust plant treatments, were easily separated from the soil. Separating the two root systems in the black locust + maize plant treatment was difficult. Main roots were followed to the root collar for proper identification, and fine roots were traced to the appropriate root collar when feasible. Softer, whiter roots were attributed to maize, especially below the top 25 cm. Dark brown, coarse roots were determined to be black locust. Maize root growth in the black locust + maize treatment was poor, therefore roots in the bottom half of these containers were confidently identified as black locust. The one major potential source of error was in separating the fine roots between black locust and maize in the top 25 cm.

Nodule Measurements

Black locust root nodules were hand picked from each harvested root system, total N and $\%^{15}N$ root sub-samples, and length and diameter root subsamples. To determine the presence of nodule N-fixation activity, nodules were placed into one of two categories: dead or alive. Dead nodules were defined as having a dark brown or green exterior and/or interior. They were sometimes flaccid or contained a green liquid. Live nodules were light brown on the outside and red on the inside, due to the presence of oxygen-carrying leghemoglobin. Nodules without leghemoglobin cannot fix N (Devlin and Witham, 1983), so a red nodule is considered a general indicator of N-fixation. Large nodules with multiple red tips that were green or brown at the nodule base were considered dead. For root systems in block I, almost all nodules were opened to inspect the inside color. With experience from block I, nodule activity could be accurately determined without opening every nodule. Nodules were randomly opened to check for accuracy in succeeding blocks if any question of viability was indicated. After evaluation, collected nodules were stored in paper bags and refrigerated at 4°C.

Tissue And Soil Processing

All plant tissue (black locust leaves, black locust stem, upper and lower maize stalks, and roots) were stored in ambient temperatures in paper bags after drying. All plant tissue except for the entire root systems were dried again at 65° C for 24 hours before weighing. The biomass samples were then ground in a Thomas Willey Mill model 4 to pass a mesh screen and placed into zipper-type plastic bags. These ground samples were ground again in a Cyclone Sample

Mill (Udy Corporation, Fort Collins, CO) to pass a 1 mm screen and stored in glass vials. Plant N and %^{15}N were then determined from subsamples using a continuos-flow stable isotope ratio mass spectrometer (ANCA-MS, Europe Scientific). Subsamples were 4 mg to 10 mg in weight, with black locust leaf subsamples weighing approximately 4 mg due to the high N content. Though this method is accurate and comparable to conventional Kjeldahl procedures (Harris and Paul, 1989; Jensen, 1991), a random sub-sample of 30% of the above-ground biomass plant N readings were checked for accuracy by a Carlo Erba Nitrogen Analyzer 1500 Series 2 (Fisions Instruments, Danvers, MA) using the same weight of subsamples. To calculate N-fixation and N transfer, formulas for the Isotope Dilution Technique (IDT) were applied using maize as the nonfixing reference plant. The biomass percentage of N derived from the atmosphere (%Ndfa) was calculated as:

$$\%\text{Ndfa} = 1-\left(\frac{\%^{15}\text{Nexcess (fs)}}{\%^{15}\text{N excess (nfs)}}\text{x}100\right)$$

where:

fs = fixing system -- black locust
nfs = non-fixing system -- maize

(Rennie, 1986)

To calculate root length and diameter, the soil cores were thawed in a cooler and the roots separated from the sand using a hydropnuematic root elutriator. Recovered roots were immediately placed in whirly pac bags containing a 100 ml solution of deionized water and 20% alcohol. They were then stored at 4°C. Black locust and maize roots from the intercropped treatment were separated by hand. This proved difficult. Observations of roots from black locust alone and maize alone treatments indicated that black locust fine roots are not as transparent as maize fine roots. Maize fine roots have an almost clear translucence. Light clearly goes through the cells to the steele. Black locust fine roots can have a visible steele as well, but light does not travel through as well since the cells around the steele appear cloudier and more fibrous. In addition, the roots of black locust alone in the top 25 cm of the container were primarily brown and fibrous. Conversely, below 25 cm few maize roots were located due to the poor growth of the maize intercropped with black locust. After the root systems were separated, all roots were stained with malachite green. They were then video taped and computer analyzed to determine total sample root length and diameter (Smucker *et al.*, 1987).

To determine root dry weight, root samples were first manipulated to remove soil particles. Roots were shaken over a #10 mesh screen and a #250 mesh screen. Fine roots that broke off from the root systems were then collected from the screens. The roots were then dried for 24 hours at 65°C. Three 1 g dry weight random samples (a mix of fine and large roots) of each root system were placed in preweighed crucibles. The samples were placed in ovens at 550°F for four hours and allowed to cool overnight in the ovens. Crucibles were reweighed and a correction factor determined to account for soil particles attached to the roots in order to determine the true root dry weight.

To determine soil N content and %^{15}N, 50 ml of 2M KCl was added to 20 g of soil in a 100 ml flask. It was then mechanically shaken for one hour. The resulting solution was poured through Whatman #1 filter paper that had been rinsed with 0.1M HCl and placed in an oven at 50° C until dry. The resulting extracts were analyzed colormetrically for NO_3^- - N and NH_4^+ - N on a Alpkem RFA 300 (Alpkem Corporation, Clackamas, OR, USA). Diffusions for total N were performed according to the method of Brooks *et al.* (1992), with the following exceptions:

- filter papers were leached with 300 ml of deionized water as previous trials showed this to be adequate for leaching filter papers of excess N;
- a 5 ppm N spike as ammonium sulfate was used in the low N soil extract samples to boost extract N to a range above 40 ug per sample necessary for accurate measurements of total N and % ^{15}N;
- all samples were not shaken with glass beads at the beginning of the six day incubation period but were swirled for 15 seconds each day;
- reagent and spike N and ^{15}N were accounted for in the final N and ^{15}N estimates.

Percentage ^{15}N of the diffusions was determined using a continuos-flow stable isotope ratio mass spectrometer (ANCA-MS, Europe Scientific).

Statistical Analysis

All statistical analysis was performed using analysis of variance (ANOVA) with linear, quadratic, and logical orthogonal contrasts. A generalized ANOVA table used in the analysis is presented in Table 2. Assumptions of ANOVA were tested using formulas provided by Little and Hills (1978). The assumptions tested were:

- Error terms are randomly, independently, and normally distributed;
- The variances of different samples are homogenous; and
- Variances and means of different samples are not correlated.

The fourth assumption, additivity, was not tested as the effects in a design using split-plots are not additive.

Table 2: Generalized ANOVA Table

Source
Main Plots
Block (B)
Plant Treatment (PT)
Error$_{(a)}$
Sub Plots
Main Plots
Nitrogen Treatment (N)
N linear
N quadratic
N x PT
N lin x PT
N qua x PT
Error$_{(b)}$

To test the assumptions, a table of error terms was constructed for each data set and analyzed for randomness, independence, and normal distribution. Homogeneity of variances was tested using the Bartlett's Test for Homogeneity of Variances (Little and Hills, 1978). The correlation of variances and means was determined by graphing each treatment variance and mean over the replications. A visual inspection determined whether the means and variances were correlated. If any of these assumptions were not satisfied, the data were transformed using log(10). Some data sets still did not satisfy one or more of the assumptions after transformation. Violations of the assumptions of ANOVA are identified and potential impacts are discussed as results are presented.

IV. ^{15}N-LABELED FERTILIZER AND MAIZE INTERCROPPING EFFECTS ON NITROGEN-FIXATION IN BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.)

Abstract

Black locust (*Robinia pseudoacacia* L.) is a temperate fast-growing, leguminous tree. As part of the effort to investigate its potential role in North American agroforestry systems, a two factor, split-plot greenhouse study was conducted to determine the effect of nitrogen (N) fertilizer and maize intercropping on the N-fixation ability of black locust. Cloned black locust seedlings from root cuttings were transplanted to containers of N deficient sand and grown for 98 days. N and plant treatments were then assigned. N as $(^{15}NH_4)_2SO_4$ was applied at 50, 125, and 200 kg ha^{-1} equivalent. Maize was planted by seed to form the following plant treatments: black locust alone, black locust and maize intercropping, and maize alone. These treatments were applied for 45 days and the experiment was harvested.

N-fixation was greatly reduced as N application increased ($p < 0.001$). There was a 60% reduction between the low and medium N application levels, and an additional 20% reduction from the medium to high levels. Intercropped and sole black locust derived similar portions of biomass N from the atmosphere ($p = 0.108$). There were no interaction effects ($p = 0.854$), indicating that the plant treatments had similar response patterns to added N. Intercropped maize grew poorly, showing nutrient deficiencies in phosphorus and potassium,

therefore results of intercropping on black locust N-fixation are inconclusive. Black locust may be able to continue to fix atmospheric N despite high applications of fertilizer N. However, competition for these nutrients as well as allelopathy may be important limiting factors in black locust agroforestry systems.

Introduction

N-fixation by NFTs is important in tropical agroforestry systems. It contributes to nutrient cycling by adding atmospheric N to the soil-plant system (Young, 1989a), perhaps reducing requirements for added nitrogen fertilizer. Research on the potential of temperate agroforestry, and what types of systems will define it, is still in its infancy. Thus, little research exists about the potential role of N-fixation in temperate systems. N-fixation is generally reduced with increasing levels of soil N, but little is known about the effects of intercropping on N-fixation. It is expected that N-fixation will increase with intercropping, in part due to depletion of soil N by the nonlegume intercrop (Rerkasem and Rerkasem, 1988). Conversely, above-ground effects such as shading can decrease nodulation and N-fixation (Nambiar *et al.*, 1983).

Current evidence points to black locust as a potential leguminous agroforestry tree because it has multiple uses and fast growth. Though data suggests that black locust can support a high rate of N-fixation (Dawson and Johnsen, 1992), it is reduced as available soil N increases (Johnsen and

Bongarten, 1991; Reinsvold and Pope, 1987; Roberts *et al.*, 1983). However, there are no data describing the effects of intercropping on black locust N-fixation. It is possible that intercropping will increase black locust N-fixation in response to the companion crop's competition for soil nitrogen. Therefore this study was designed to address the following hypothesis:

- A greater dilution of ^{15}N will occur in the black locust when planted with maize than in black locust planted alone.

The objective was to determine the extent to which black locust increases N-fixation in response to competition for soil N.

Methods

Cloned root cuttings of black locust were introduced to 30 x 30 x 100 cm containers of N-deficient sand with fertilizer (minus N) incorporated into the top 25 cm of sand. An error resulted in the application of 75 kg ha^{-1} Mg, 140 kg ha^{-1} P_2O_5, and 130 kg ha^{-1} K_2O equivalent, one-half of target levels. The seedlings were grown for 98 days to allow the internal N demands to equilibrate. This also provided a root distribution that would approximate that of an already existing tree in an agroforestry system when maize is planted. On the 99th day, N and plant treatments were applied. N treatments consisted of liquid $(^{15}NH_4)_2SO_4$ enriched to 1.27% ^{15}N applied at 50, 125, and 200 kg N ha^{-1} equivalent. Plant treatments consisted of one black locust, one maize, and one black locust + one maize. The following terminology is used to compare plant treatment results:

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B → black locust alone;
BM → black locust in black locust + maize;
M → maize alone;
MB → maize in black locust + maize.

The BM and MB terms represent the same intercrop plant treatment. The first letter in each term denotes the plant being referred to in that plant treatment. The term "BM" is used to compare intercropped black locust (the "B" in BM) to B (black locust alone). The term "MB" is used to compare intercropped maize (the "M" in MB) to M (maize alone). These treatments were applied for 45 days and then the experiment was harvested. Leaf and stem biomass were collected separately, dried for 24 hours at 65° C, and then stored. All biomass was ground in a Thomas Wiley Mill #4 and then pulverized in a Cyclone Sample Mill (Udy Corporation, Fort Collins, CO) to pass through a 1 mm screen. The biomass was then measured for total N and ^{15}N in a continuos-flow stable isotope ratio mass spectrometer (ANCA-MS, Europe Scientific). Total N readings were confirmed using a Carlo Erba Nitrogen Analyzer 1500 Series 2 (Fisions Instruments, Danvers, MA). N-fixation was estimated by the Isotope Dilution Technique (IDT. The biomass percentage of N derived from the atmosphere (%Ndfa) was calculated as:

$$\%\text{Ndfa} = 1-\left(\frac{\%^{15}\text{N excess (fs)}}{\%^{15}\text{N excess (nfs)}}\text{x}100\right)$$

where:

fs = fixing system -- black locust
nfs = non-fixing system -- maize

(Rennie, 1986)

Experimental design consisted of randomized complete blocks utilizing split-plots. Statistical analysis was performed using analysis of variance (ANOVA) with linear and quadratic orthogonal contrasts. Investigation to determine if the assumptions of ANOVA were satisfied found that the means and variances were not independent, variances were not homogeneous, and the error terms were not normally distributed. Transformation of the data to log(10) corrected most of these assumption violations. Validity of the ANOVA is discussed where necessary.

Results

Intercropping reduced both black locust and maize growth (Table 3). Maize growth as measured by above-ground dry weight (DW) and number of leaves per plant decreased dramatically. The reduction in black locust DW is significant at $p = 0.026$. However since black locust DW variation is not homogeneous and standard deviation is high, this statistical difference may not be real (Figure 2). Indeed, the reduction of black locust DW is not reflected in

Table 3: Average Maize Dry Weight and Leaves per Plant and Average Black Locust Dry Weight

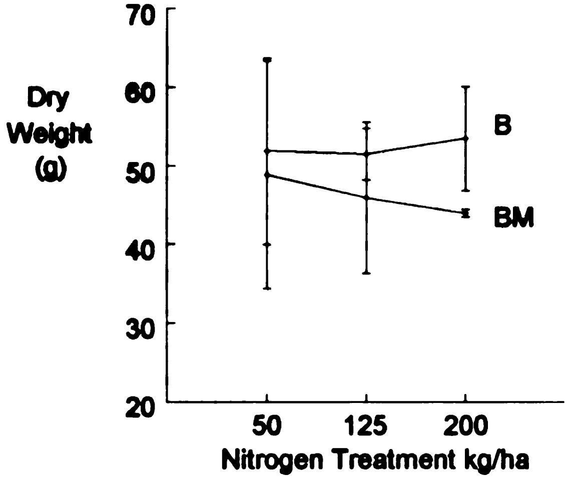
Key: M = maize alone; MB = maize in the maize + black locust intercropping
B = black locust alone; BM = black locust in the maize + black locust intercropping
Numbers in parentheses represent one standard deviation.

Maize

	Dry Weight (g)		# Leaves per Plant	
	Plant Treatment	Plant Treatment	Plant Treatment	Plant Treatment
N Level	M	MB	M	MB
N_{50}	34.50 (3.81)	4.79 (3.67)	12.50 (0.58)	7.5 (1.00)
N_{125}	30.62 (2.86)	12.88 (6.43)	12.25 (0.50)	10.5 (1.29)
N_{250}	25.63 (4.58)	9.52 (4.02)	12.25 (0.50)	9.0 (1.83)

Black Locust

	Dry Weight	
	Plant Treatment	Plant Treatment
N Level	B	BM
N_{50}	51.81 (11.91)	48.83 (14.56)
N_{125}	51.41 (3.26)	45.91 (9.64)
N_{250}	53.44 (6.98)	43.93 (0.51)



Key: B = black locust alone; BM = black locust in the maize + black locust intercropping
Error bars denote one standard deviation.

Figure 2: Average Black Locust Dry Weight

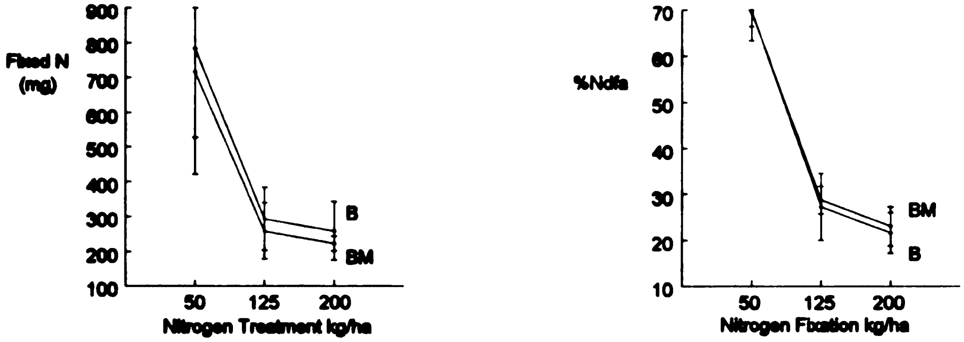
estimates of N-fixation as measured by % biomass N derived from the atmosphere nor milligrams of biomass N fixed from the atmosphere (Table 4; Figure 3). Therefore, no intercrop effect on N-fixation was observed and it was not affected by the potential DW difference between black locust plant treatments.

However, N application levels had strong influences on N-fixation, decreasing as N fertilizer levels increased ($p = 0.001$). There was a 60% decrease between the low and medium N treatments, and a further 20% drop between the medium and high N treatments (Figure 3). No treatment

Table 4: Average Black Locust N-Fixation as Measured by % Biomass N Derived From The Atmosphere (%Ndfa) and mg Biomass N Derived From The Atmosphere

Key: B = black locust alone; BM = black locust in the maize + black locust intercropping
Numbers in parentheses represent one standard deviation.

	Fixed Biomass N (mg)		% Biomass Ndfa	
	Plant Treatment	Plant Treatment	Plant Treatment	Plant Treatment
N Level	B	BM	B	BM
N_{50}	783 (256)	717 (296)	69.7 (3.3)	69.5 (6.2)
N_{125}	294 (90)	259 (81)	27.3 (7.2)	28.7 (2.9)
N_{200}	259 (84)	223 (21)	21.6 (4.4)	23.1 (4.2)



Key: B = black locust alone; BM = black locust in the maize + black locust intercropping
Error bars denote one standard deviation.

Figure 3: Average Black Locust N-Fixation as Measured by % Biomass N Derived From The Atmosphere (%Ndfa) and mg Biomass N Derived From The Atmosphere

interactions were observed, therefore the change in N-fixation was similar for each plant treatment as N fertilizer levels changed.

Due to an error, phosphorous and potassium application levels were lower than expected, perhaps contributing to the severe intercropped maize (MB) growth reduction. Black locust competition and or allelopathy may also have restricted MB development.

Discussion and Summary

The reduction in black locust N-fixation with increasing N levels was expected, and agrees with other work (Johnsen and Bongarten, 1992; Reinsvold and Pope, 1987; Roberts *et al.*, 1983). In this study, black locust continued to fix atmospheric N despite the highest N application rate, a rate near the upper range of the amount applied to field maize (Christenson *et al.*, 1992). It is well known that N-fixation decreases with increasing soil N levels. Still, black locust may be able to maintain N-fixation despite high soil N concentrations present in this study. In earlier work, black locust seedlings that were grown in a greenhouse sand and fed with 5 mM NH_4NO_3 applications for 50 days continued to fix atmospheric N (Roberts *et al.*, 1983). N-fixation in black locust seedlings supplied with an initial application of 0, 25, 50, or 100 mg N kg^{-1} and sufficient phosphorus was greater in the middle treatments than in the high treatment after 60 days. Then after 105 days black locust supplied with 100 mg N kg^{-1} fixed as much N as those supplied with 50 and 25 mg N kg^{-1} (Reinsvold and Pope, 1987). Similar increases in N-fixation at the high N treatment over time was reported by Johnsen and Bongarten (1992). Greenhouse grown black locust seedlings in sand that received 0.25 mM and 2.00 mM NO_3^- in daily applications initially showed decreased N-fixation at the high N level. However, as growth and N-demand became greater after 93 days, N-fixation at the high N treatment nearly equaled that of the low N treatment. It would appear that after a high initial fertilizer application or with stable soil N levels, black locust N-fixation eventually increases. In addition, black locust shows high initial growth when supplied with adequate amounts of soil N (Dawson *et al.*, 1992; Johnsen and Bongarten, 1992; Reinsvold and Pope, 1987; Roberts *et al.*, 1983). Implications of the high initial growth and continued N-fixation over time despite high soil N levels may have the following implications: fixed atmospheric N could serve as a source of additional N in North American agricultural systems; and the regular addition of fixed atmospheric N through incorporation of senesced or coppiced black locust leaves, stems, and roots in managed settings may decrease needed levels of fertilizer N required to maintain intercrop yields.

It was expected that N-fixation would have been greater in the intercropped plant treatment due to competition for available soil N. It was also expected that this difference would be especially apparent at the low N treatments, resulting in a significant plant treatment by N treatment interaction. However, intercropping had no effect on N-fixation in black locust. It is assumed that black locust competed effectively for soil N, despite the presence of maize.

In studying the rooting habits of both B and M, both were found to have a substantial portion of their roots near the soil surface. Both species are also known to be very competitive in N uptake.

However, the shoot and root growth of intercropped maize (MB) were poor. If intercropping has an effect on N-fixation, any intercrop difference may not have been allowed to develop due to the poor growth of intercropped maize. It is assumed that the poor growth of intercropped maize did not provide the anticipated level of N competition required to stimulate additional black locust N-fixation.

Intercropped maize germinated normally, but later showed serious symptoms of nutrient stress: burned leaf tips, purple lower leaves, and weak lower stalks. The symptoms reflected phosphorus (P) and potassium (K) deficiencies. Three additional applications of a complete nutrient mix (minus N) were applied to the surface soil with no improvement. MB did not suffer from N competition; MB biomass %N increased with increasing N levels. Although the intercropped maize absorbed more N as it was provided, growth did not improve. This indicates that other factors limited growth. In this case they appear to be P and K deficiencies, but allelopathic effects of black locust on the intercropped maize may have played a role as well. The increasing severity of the nutrient deficiency symptoms necessitated harvest at 45 days, 15 days earlier than originally planned.

There is little information available on black locust competition with intercrops. In a study with similar results, black locust in a greenhouse reduced the yield of intercropped barley compared to the sole crop, although N concentrations in the intercropped barley increased (Ntayombya and Gordon, 1993). The authors determined that competition for soil resources, especially moisture, resulted in the lower barley yields. Conversely, two field studies intercropping black locust hedgerows with maize alleyways showed that maize yields increased at the hedgerow edges (Seiter, 1994; Ssekabembe and Henderlong, 1991). However, in both field studies one year old seedlings were used and interspecies root interactions most likely did not approach that found in the barley study or my study.

There is also little data available on allelopathic effects of black locust. A cursory review of the literature did not uncover any North American studies. However, an abstract of a study performed in India indicates that leaf leachates of black locust decreased maize (*Zea luxurians*) germination and growth after five days (Bhardwaj, 1993). Five day growth was reduced, though not germination, in *Tricicum aestivum*, *Glycine max*, *Brassica campestris*, and *Vigna mungo*. Abstracts of studies conducted in the former Soviet Union indicate that black locust leaf exudates adversely affected germination and growth of numerous herbaceous plants (Matveev, 1975), and crown and root excretions (phytolines) from black locust adversely affected Oak (species not provided)

(Kolesnicenko, 1964). Therefore black locust may have had an allelopathic affect on intercropped maize in this study.

Evidence of satisfactory maize growing conditions were clearly seen when maize was grown alone. The sole maize showed no symptoms of P and K deficiencies and its root system explored the entire soil volume. Therefore, poor intercropped maize growth appeared to be related to black locust competition or allelopathy. Black locust grew well in both plant treatments, but better alone. The competitiveness or allelopathic effects of black locust may be an early warning regarding the compatibility of this tree species with maize production. In addition, nutrient deficiencies could have been intensified due to the low P and K application rates and from black locust absorbing a proportion of the P and K during the 98 days before maize planting. Therefore, a shortage may have been created before the maize was planted.

Overall, black locust fixed N regardless of the added N level of the study. Maize, however, may have difficulty competing with established black locust, not necessarily for N but for P and K. Black locust allelopathy may also retard maize growth. These results may be indicative of future compatibility problems associated with using black locust as a close intercrop with maize. This may be especially true for the first row of maize in a black locust and maize alley cropping.

V. ^{15}N-LABELED NITROGEN FERTILIZER AND INTERCROPPING EFFECTS ON NITROGEN TRANSFER FROM BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.) TO MAIZE (*ZEA MAYS*)

Abstract

Black locust (*Robinia pseudoacacia* L.) is a temperate fast-growing, leguminous tree. As part of the effort to investigate its potential role in North American agroforestry systems, a two factor, split-plot greenhouse study was conducted to determine the effect of nitrogen (N) fertilizer on transfer of nitrogen from black locust to intercropped maize. Cloned black locust seedlings from root cuttings were transplanted to containers of N deficient sand and grown for 98 days. N and plant treatments were then assigned. N as $(^{15}NH_4)_2SO_4$ was applied at 50, 125, and 200 kg ha^{-1} equivalent. A heavy isotope of nitrogen, ^{15}N, was used to measure N-fixation and N transfer. Maize was planted by seed to form the following plant treatments: black locust alone, black locust and maize intercropping, and maize alone. These treatments were applied for 45 days and then the experiment was harvested.

Intercropped maize biomass had a significantly lower %^{15}N excess at the low N treatment than did maize alone ($p < 0.001$), and similar ^{15}N levels at the two higher N treatments. Average intercropped maize soil ^{15}N and total available soil N levels at low N were also lower than for maize alone, however these plant treatment differences were not significant at $p = 0.680$ and 0.200, respectively. Black locust planted with maize showed greater live nodule weight and root length than black locust alone at low N, but these plant treatment differences were also not significant at $p = 0.458$ and 0.143, respectively.

In comparing plant treatment means it would appear that the lower %^{15}N excess in intercropped maize biomass was due to N transfer. Close examination of the data reveals that either differential maize root access to soil ^{15}N pools or black locust root interaction with maize roots might explain the ^{15}N dilution in intercropped maize biomass. A clear interpretation of the data, however, is difficult due to the poor growth of intercropped maize in this study.

Introduction

Improved nutrient cycling is an important benefit of agroforestry systems over traditional agriculture. Deeper perennial root systems access nutrients at soil depths too deep for agronomic plants and deposit them at the soil surface as fine root turnover and foliage from leaf and stem abscission (Young, 1985). The use of nitrogen-fixing trees (NFTs) and shrubs is especially beneficial: they fix N and add it to the soil-plant system through the nutrient cycling process (Mulongoy, 1986; Young, 1985). In this way N can be transferred from the NFT to the intercrop in agroforestry systems. Another potential path for the transfer of N is by way of direct root contact, direct root mycorrhizae contact, or uptake of NFT rhizodeposition before being immobilized by soil microorganisms (Agboola *et al.*, 1972; Giller and Wilson, 1991; Mulongoy, 1986; Robinson, 1991; van Noordwijk and Dommergues, 1990; Young, 1989b). Direct transfer is not considered a major source of N to companion crops in tropical grass swards, and even less in legume-cereal intercroppings (Catchpoole and Blair, 1990;

Henzell and Vallis, 1977; Giller *et al.*, 1991; Giller and Wilson, 1991). However, there has been little investigation of direct N transfer from NFTs to crops in agroforestry systems (van Noordwijk and Dommergues, 1990). No literature or studies have been found that have investigated the potential of N transfer from black locust to intercrops in temperate systems. With its broad, shallow root system and high N-fixation, there is potential for direct transfer. Research in this area is needed to better understand the beneficial roles black locust may have in Michigan and U.S. agricultural systems, and in particular, to determine its ability to transfer N directly to companion crops.

In order to gain greater knowledge about the potential of black locust to directly aid the nitrogen status of a companion crop, the following hypotheses were developed:

- A greater dilution of ^{15}N will occur in maize when planted with black locust than in maize planted alone.
- Total maize root length is reduced when planted with black locust.

The objectives were to measure N-transfer from the black locust to the intercropped maize as well as to determine if maize root length is reduced in the presence of black locust. In an effort to understand the interactions between black locust and maize, improve experimental validity, and aid interpretation of results, supporting data were collected. These data included soil ^{15}N, total available soil N (ppm), black locust root length, black locust nitrogen-fixation, and black locust live nodule weight.

Methods

Cloned root cuttings of black locust were introduced to 30 x 30 x 100 cm containers of N-deficient sand with fertilizer (minus N) incorporated into the top 25 cm of sand. An error resulted in the application of 75 kg ha^{-1} Mg, 140 kg ha^{-1} P_2O_5, and 130 kg ha^{-1} K_2O equivalent, one-half of target levels. The seedlings were grown for 98 days to allow the internal N demands to equilibrate and to provide a root distribution that would approximate that of an already existing tree in an agroforestry system when maize is planted. On the 99th day, N and plant treatments were applied. N treatments consisted of liquid $(^{15}NH_4)_2SO_4$ (1.27% ^{15}N) applied at 50, 125, and 200 kg N ha^{-1} equivalent. Plant treatments consisted of one black locust, one maize, and one black locust + one maize. The following terminology is used to compare plant treatment results:

B	→	black locust alone;
BM	→	black locust in black locust + maize;
M	→	maize alone;
MB	→	maize in black locust + maize.

The BM and MB terms represent the same intercrop plant treatment. The first letter in each term denotes the plant being referred to within that treatment. The term "BM" is used to compare intercropped black locust (the "B" in BM) to B (black locust alone). The term "MB" is used to compare intercropped maize (the "M" in MB) to M (maize alone). These treatments were applied for 45 days and then the experiment was harvested. Leaf and stem biomass were collected separately and dried for 24 hours at 65° C. These were ground in a Thomas Wiley Mill # 4 and then pulverized in a Cyclone Sample Mill (Udy Corporation, Fort Collins, CO) and passed through a 1 mm screen. The biomass was then measured for total N and ^{15}N in a continuos-flow stable isotope ratio mass spectrometer (ANCA-MS, Europe Scientific). Total N readings were confirmed using a Carlo Erba Nitrogen Analyzer 1500 Series 2 (Fisions Instruments, Danvers, MA). The biomass percentage of N derived from the atmosphere (%Ndfa) and the amount of N transfer were calculated as :

$$\%\text{Ndfa} = 1-\left(\frac{\%^{15}\text{Nexcess (fs)}}{\%^{15}\text{N excess (nfs)}}\text{x100}\right)$$

where:

$\%^{15}$N excess = the percentage of ^{15}N above background levels, or that above 0.3663%.
fs = N-fixing system, i.e. black locust
nfs = non N-fixing system, i.e. maize

(Rennie, 1986).

$$\%\text{N in maize derived from black locust} = \frac{\text{R sole maize - R intercrop maize}}{\text{R sole maize - R intercrop black locust}}\text{ x 100}$$

where R = $\%^{15}$N excess.

(Ta and Faris, 1987).

Root and soil harvesting, sampling, and measurement are discussed in detail in chapter III. The experimental design consisted of randomized complete blocks utilizing split-plots. Statistical analysis was performed by analysis of variance

(ANOVA) with linear, quadratic, and logical orthogonal contrasts. Assumptions of ANOVA were tested using formulas provided by Little and Hills (1978).

After log(10) transformation, some data sets still did not satisfy the assumptions of ANOVA. Table 5 presents all the data sets reported in this thesis and whether they meet the assumptions of ANOVA as normal or transformed data. It is believed that the ANOVA is robust enough that the problems of normality did not compromise statistical analysis given the data sets to be evaluated (Ries, 1995). Other violations must be analyzed and described. For example, the black locust live nodule weight variance was not independent of the means. A greater variance at the low N treatment (high nodulation/low nodule death) than at high N (low nodulation/high nodule death) presents a danger that true mean differences at the high N treatments were masked. In this

Table 5: Data Sets and Assumptions of ANOVA.

Data Set	Assumptions Not Satisfied
Black locust Root Length	Error Term Normality
Live Nodule Weight	Independence of Means and Variances Error Term Normality
Maize Biomass ^{15}N Ratio Excess	Homogeneity of Variances
Maize Biomass %N	None
Soil ^{15}N Ratio Excess	None
Soil N in ppm	Independence of Means and Variances Homogeneity of Variances

particular case the possibility that true mean differences were masked appears remote.

Another example is in the maize biomass %^{15}N ratio excess data. The intercropped maize (MB) had greater variance than M, especially at the high N treatment. This is not surprising in light of its poor growth. When pooled with other variance terms to calculate mean square error terms and f-statistics, the inclusion of the high MB could affect the final f-statistic and mask real differences in other treatments. This again does not appear likely: the low N mean of the MB was significantly less than the other treatments, even with its high variance. The remaining means were so close that real differences likely do not exist. Even the highest variance was only 0.4% of its mean, so some differences among variances seem large in part because the variances themselves were so small.

Finally, in the total soil N data set, there was a tremendous amount of variation. Plant treatment means varied greatly, as do mean rankings over the three N treatments. Soil N response to N applications was significant and this was consistent across the measured N variables, therefore, N treatment differences should be accurate. It is interesting that in M and MB plant treatments, soil N variation in the top soil section was low compared to the middle and bottom sections, whereas in B it was comparatively high. The means were independent of this pattern. Overall, trends were hard to identify due to the large number of treatments and variance, so simple caution is suggested while interpreting total soil N data set.

Results

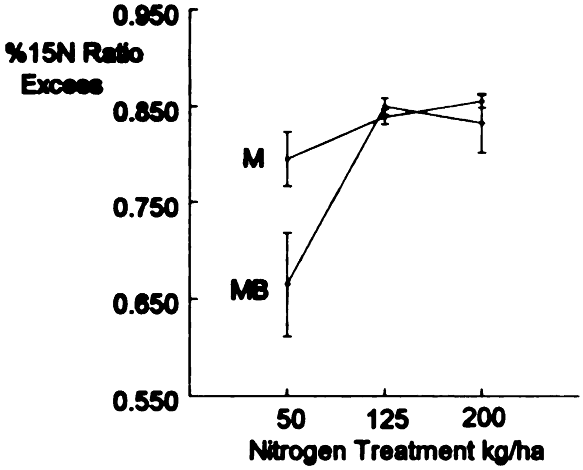
Black locust and maize dry weight (DW) are reported in chapter IV. MB growth and development was severely reduced while BM growth showed some decline (Table 3). In maize above-ground biomass %^{15}N excess, both plant treatment (PT) and nitrogen (N) level responses were highly significant ($p < 0.001$). However, the PT x N interaction was also significant at $p = 0.001$, indicating that M and MB biomass ^{15}N were affected differently by N (Table 6). Logical orthogonal contrasts indicate that at low N, MB above-ground biomass had a significantly lower %^{15}N excess than did M (Table 6, Figure 4). Conversely, M and MB were not different at the greater N treatments. In the analysis of N-fixation by black locust, N fertilization affected both plant treatments equally. Specific comparisons found both B and BM nitrogen responses to be linear ($p = 0.001$) and quadratic ($p = 0.002$). These results indicate that a definable negative relationship between available soil N and N-fixation was found in this study, but that no interaction was shown between intercropping and N-fixation at $p = 0.854$. Similar responses to added N were observed in analysis of black locust live nodule weight. However, B and BM root length actually increased at the highest N treatment level while B root weight

Table 6: ANOVA for %^{15}N Excess in Maize Above-Ground Biomass

$R^2 = 0.913361$ C.V. = 3.631855

Source	DF	Sums of Squares	Mean Square	F Value	p
Main Plots	7	0.01746			
Block (B)	3	0.00055	0.00018	0.21	0.8843
Plant Treatment (PT)	1	0.01349	0.01349	15.73	0.0019
Error$_{(a)}$	3	0.00342	0.00114		
Sub Plots	23	0.11873			
Main Plots	7	0.01746			
Nitrogen Treatment (N)	2	0.06928	0.03464	40.41	0.0001
N linear	1	0.05156	0.05156	60.15	0.0001
N quadratic	1	0.01772	0.01772	20.67	0.0007
or					
N_{125} v N_{200}	1	< 0.00001	< 0.00001	0.00	0.9532
N_{50} v (N_{125} x N_{200})	1	0.06928	0.06928	80.82	0.0001
N x PT	2	0.02169	0.01085	12.65	0.0011
N lin x PT	1	0. 01147	0. 01147	13.38	0.0033
N qua x PT	1	0.01022	0.01022	11.93	0.0048
or					
(N_{125} v N_{200}) x PT	1	0.00116	0.00116	1.35	0.2678
[N_{50} v (N_{125} x N_{200})] x PT	1	0.02053	0.02053	23.95	0.0004
Error$_{(b)}$	12	0.01029	0.00086		

N_{50} = 50 kg N ha^{-1} equivalent
N_{125} = 125 kg N ha^{-1} equivalent
N_{200} = 200 kg N ha^{-1} equivalent



Key: M = maize alone; MB = maize intercropped with black locust
Error bars denote one standard deviation.

Figure 4: Maize %^{15}N Ratio Excess in Above-Ground Biomass

decreased and BM root weight remained stable (Table 7; Figure 5). Trend analysis and contrasts determined that intercropping maize with black locust had no statistical effect on root weight, root length, or live nodule weight, and that there were no differential plant treatment responses to applied N in these data sets (Table 8). The black locust root weight data set had high standard deviations and a low correlation of variation at 0.470. Root weight variation may simply be high and more samples would be needed to measure any treatment differences if they exist.

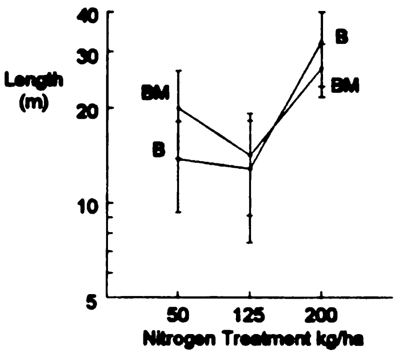
There were significant increases in available soil ^{15}N and total available soil N as N increased ($p < 0.001$). Maize PT x N interaction terms in both data

Table 7: **Black Locust Average Root Length Distribution for Two Soil Depths as Measured in Soil Cores and Average Total Root Weight and Average Live Nodule Weight**

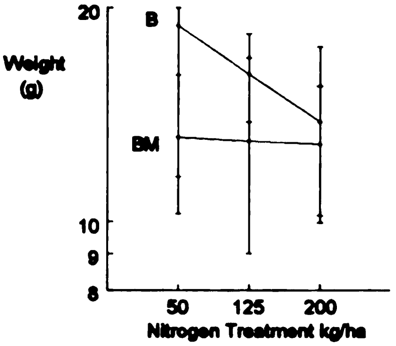
Key: N_{50} = 50 kg N ha^{-1}; N_{125} = 125 kg N ha^{-1}; N_{200} = 200 kg N ha^{-1}
B = black locust alone; BM = the black locust in the black locust + maize intercropping
The numbers in parentheses are standard deviations.

		Root Length (m)		Root Weight (g)		Live Nodule Weight (g)	
		Plant Treatment		Plant Treatment		Plant Treatment	
N Level	Soil Depth	B	BM	B	BM	B	BM
N_{50}	0 - 25 cm	9.13 (4.28)	13.50 (5.38)	18.87 (7.32)	13.14 (2.90)	3.10 (2.78)	4.56 (2.80)
	25 cm & below	4.58 (1.34)	6.42 (1.69)				
N_{125}	0 - 25 cm	6.70 (3.67)	9.37 (2.78)	16.08 (2.28)	12.97 (3.67)	0.53 (0.26)	0.86 (0.64)
	25 cm & below	6.10 (1.76)	4.75 (2.36)				
N_{200}	0 - 25 cm	22.55 (6.51)	13.34 (7.01)	13.76 (3.81)	12.81 (2.64)	0.34 (0.09)	0.33 (0.16)
	25 cm & below	9.76 (2.63)	13.31 (10.22)				

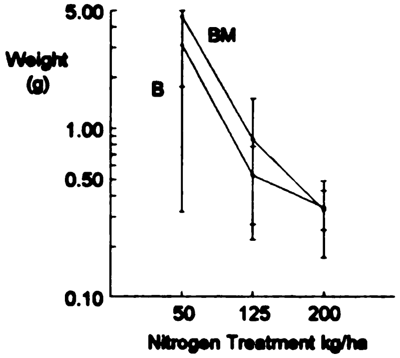
Total Black Locust Soil Core Root Length



Average Black Locust Root Weight



Average Black Locust Live Nodule Weight



Key: B = black locust alone; BM = black locust in the maize + black locust intercropping
Error bars denote one standard deviation
Y-axes use log)10) scale.

Figure 5: **Black Locust Total Soil Core Root Length, Average Root Weight, and Average Live Nodule Weight**

Table 8: Selected p-values for Black Locust Root Length, Root Weight, and Live Nodule Weight Responses to Plant and Nitrogen Treatments

Key:
PT = plant treatment
N linear = linear trend contrast for nitrogen treatment
N quadratic = quadratic trend contrast for nitrogen treatment
PT x N = plant treatment by nitrogen treatment interaction

	p-values		
Source	**Root Length**	**Root Weight**	**Live Nodule Wt.**
PT	0.441	0.093	0.280
N linear	0.010	0.236	< 0.001
N quadratic	0.013	0.949	0.071
PT x N	0.242	0.562	0.530

sets were not significant, and contrasts confirmed the lack of statistically different plant treatment responses to added N. Logical contrasts indicated that both soil ^{15}N and total soil N were significantly greater in the middle section of the containers (p = 0.014 and 0.003, respectively) and statistically equal at the top and bottom depths (Table 9). MB total root length and root weight were greatly reduced compared to M (Table 10). However, poor top growth of intercropped maize confounded the treatment effects so statistical analysis was inappropriate. The differential root growth likely resulted in differential sampling of soil ^{15}N pools.

Intercropped maize above-ground biomass $\%^{15}N$ excess levels, when analyzed by block, were higher than M at the medium N application and sometimes at the high N application level. Positive N transfer estimates depend

Table 9: Average Pools of Soil %^{15}N Excess and Available Soil N by Soil Depth 45 Days After Nitrogen Treatments Were Applied

Averages were calculated using over all N treatments and all plant treatments. Soil ^{15}N used weighted means to account for different soil weights by depth.

Soil Depth	Soil-^{15}N Ratio Excess	Available Soil N (ppm)
0 - 25 cm	0.242	0.960
25 cm - 55 cm	0.419**	2.538**
55 cm and below	0.253	1.177
	**greater than the top and bottom depths at $p = 0.014$	**greater than the top and bottom depths at $p = 0.025$

Table 10: Maize Average Root Length Distribution for Two Soil Depths as Measured in Soil Cores and Average Total Root Weight

Key: N_{50} = 50 kg N ha^{-1}; N_{125} = 125 kg N ha^{-1}; N_{200} = 200 kg N ha^{-1}
M = maize alone; MB = the maize in the maize + black locust intercropping
The numbers in parentheses are standard deviations.

		Root Length (m)		Root Weight (g)	
		Plant Treatment		Plant Treatment	
N Level	Soil Depth	M	MB	M	MB
N_{50}	0 - 25 cm	5.33 (1.85)	0.65 (0.52)	18.45 (3.81)	1.53 (0.73)
	25 cm & below	12.34 (2.77)	0.37 (0.18)		
N_{125}	0 - 25 cm	3.19 (1.24)	2.00 (1.97)	13.26 (2.34)	3.57 (1.80)
	25 cm & below	8.60 (5.26)	1.66 (1.48)		
N_{200}	0 - 25 cm	3.12 (1.64)	0.57 (0.37)	12.44 (4.02)	2.05 (2.12)
	25 cm & below	5.06 (1.79)	0.55 (0.78)		

Table 11: Nitrogen Transfer from Black Locust to Maize

Key: N_{50} = l50 kg N ha^{-1} equivalent
N_{125} = 125 kg N ha^{-1} equivalent
N_{200} = 200 kg N ha^{-1} equivalent
B = black locust

Replication	N Level	% B N in Maize	% Maize N from B	Total mg N Transferred
I	N_{50}	1.02	13.30	10.08
II	N_{50}	0.39	25.64	5.64
III	N_{50}	0.14	7.45	1.94
IV	N_{50}	1.22	42.41	8.73
I	N_{125}	-4.53	-11.22	-54.31
II	N_{125}	-0.99	-5.25	-10.08
III	N_{125}	-1.16	-7.09	-13.72
IV	N_{125}	-1.07	-6.16	-9.82
I	N_{200}	5.17	20.12	66.36
II	N_{200}	-1.19	-4.75	-16.05
III	N_{200}	13.21	41.04	141.97
IV	N_{200}	0.30	3.13	3.03

on the intercropped nonlegume biomass having a lower $\%^{15}N$ excess than the nonlegume planted alone. Because the opposite occurred, N transfer estimates were often negative. Transfer estimates at low N were positive, though variable (Table 11).

Discussion and Summary

With a cursory view of the data, the first hypothesis, that a greater dilution of ^{15}N will occur in MB versus M, would be accepted at the low N application. MB had a significantly lower biomass $\%^{15}N$ excess than M, but only at the low N treatment (Figure 4). However, a critical review of the data does not support acceptance of this hypothesis. Hypothesis number two, that MB root length would be reduced versus M due to the presence of black locust, was not addressed due to the confounding effects of the poor MB growth.

There are at least two potential explanations for the dilution of MB biomass ^{15}N. First, the differential root growth patterns between M and MB resulted in differential access to soil ^{15}N pools. Soil pools of ^{15}N do not vary by plant treatment, but they do vary by soil depth, with the middle (25-55 cm) being significantly greater than the top (0-25 cm) and bottom (55 cm and below) (Table 9). M explored the entire soil volume and MB did not. Soil core sampling revealed that well over one-half of the MB root length was located in the top 25 cm of soil whereas M incorporated over one-half of its root length 25 cm and below. Over all three N treatments, M had much more root length than MB below 25 cm, and greater total root weight throughout the container (Table 10), therefore M and MB did not equally explore the soil pools of ^{15}N. This was confirmed through visual inspection of opened containers.

Another potential explanation for the significantly lower MB above-ground biomass %^{15}N excess at low N is the presence of greater BM root length and live nodule weight than B (Figure 5). High root and nodule concentrations in the root zone of nonlegumes may lead to direct N transfer (van Noordwijk and Dommergues, 1990). In this case, black locust always maintained at least one-half of its root length in the top 25 cm of soil, more than half at the low and middle N treatments (Table 7). Competition for soil N may have increased BM root length and live nodule weight, while BM root weight decreased. The greater

BM versus B root length and live nodule weight may explain the lower MB soil ^{15}N than M over all N treatment levels, especially at low N, although this soil ^{15}N difference is not significant at $p = 0.344$ (Figure 6).

In this study it is evident that there is a trend showing an interaction between plant treatments and N levels on black locust root length, nodulation, and soil ^{15}N levels. Though not statistically significant, the presence of more BM versus B root length and nodules coincide with the lower MB soil ^{15}N at low N. The lower MB soil ^{15}N presumably resulted from the lower N application in combination with competition for soil N between maize and black locust. It may be that the lower MB biomass %^{15}N excess at low N resulted from the ensuing lower soil ^{15}N as well as greater maize and black locust root contact that allowed maize to take up black locust rhizodeposition. Still, it is difficult to separate the potential effect of differential M and MB rooting patterns and resulting differential ^{15}N uptake from the potential effect of black locust roots and nodules in contact with maize roots. While it is plausible that MB received some its N directly from black locust, the difference in biomass ^{15}N ratios may simply have resulted from differential access to ^{15}N.

The difficult interpretation of the data is due to the poor growth of intercropped maize. The poor growth was presumably caused by soil nutrient competition, most likely for phosphorous (P) and potassium (K). Physical

K

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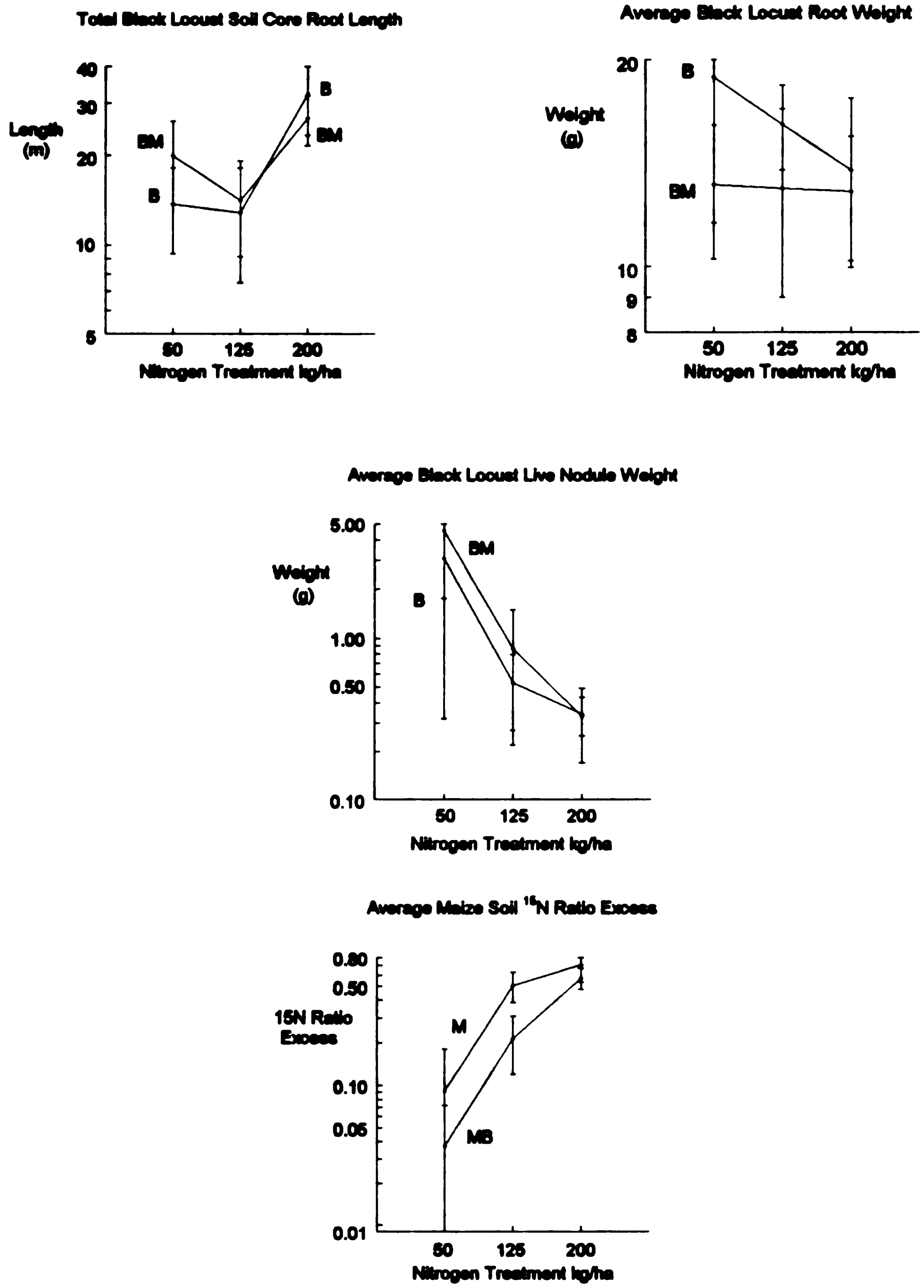

Key: M = maize alone; MB = the maize in maize + black locust intercropping;
B = black locust alone: BM = the black locust in maize + black locust intercropping
Error bars denote one standard variation.
Y-axes use log(10) scale.

Figure 6: Black Locust Root Length, Root Weight, and Live Nodule Weight Compared to Maize Soil ^{15}N Ratio Excess

manifestations included brown, burned-looking leaf tips, dark purple lower leaves, and weak and broken stalks. Nutrient deficiencies could have been intensified due to the low P and K application rates and from black locust absorbing a proportion of the P and K during the 98 days before maize planting. Since MB biomass %N increased with increasing N fertilizer with no improvement in MB health status, it is apparent that N was not limiting.

Related effects of black locust on companion crops vary. In a separate greenhouse experiment, black locust reduced the yield of intercropped barley compared to the sole crop (Ntayombya and Gordon, 1993). Conversely, two field studies intercropping black locust hedgerows with maize alleyways showed that maize yields increased at the hedgerow edges (Seiter, 1994; Ssekabembe and Henderlong, 1991). However, Ssekabembe and Henderlong (1991) reported results with one year old seedlings, so interspecies root interactions most likely did not approach that found in the barley study or in my study. It will be very interesting to see if the positive edge effects seen in the maize continue with time as interaction with black locust roots increases. In the Seiter study workers chisel plow between the black locust hedgerow and the first row of maize, greatly reducing the potential for root competition. After four years of intercropping these species no negative affects have been observed in the maize (Seiter, 1995)

Shading of the intercropped maize may have prevented adequate growth as well. As this was not a treatment and shading was not expected to be a problem, light readings at the maize level were not taken. However, black locust lacks a dense canopy, therefore shading should not have been a major factor in decreasing MB growth.

VI. CONCLUSIONS

The broad objective of this study was to aid in efforts to determine black locust's role in temperate agroforestry systems and contribute to the debate on agroforestry's role in safer and more sustainable Michigan and U.S. agroecosystems. Specifically, this work attempted to collect initial data that would elucidate this NFT's ability to contribute to the soil-plant N cycle through N-fixation and N transfer to an intercrop.

An important aspect of this study was validation of the assumptions of the IDT to assure that the estimates of N-fixation are accurate. It is important that both the nfs reference plant (maize) and fs (black locust) plants have similar N uptake patterns. However, this is not critical when soils are low in carbon sources, such as organic matter, as was the case in this study. Initially, maize has low demand for soil N, greatly increasing soon after initiation of the V6 growth stage (i.e., presence of six leaves) while black locust seedlings scavenge for N and increase growth throughout their development. Considering this and the fact that black locust had a well-developed root system when the $(^{15}NH_4)_2SO_4$ was applied, it is assumed that N-fixation was underestimated. However, the fact that the soil medium was a sand low in organic matter and had a low cation exchange capacity (CEC), the immobilization of applied ^{15}N over time would have been minimal. Furthermore, visual inspection of the B and M root systems showed them to be similarly distributed, indicating equal access to soil ^{15}N at the time of harvest. In addition, analysis of soil total N and ^{15}N at three levels of depth showed no significant differences by plant treatment. Considering the low soil N immobilization, equal root distributions at harvest, and similar soil %^{15}N excess levels across plant treatments, the discrepancy in final biomass %^{15}N excess at the time of harvest caused by low initial maize N uptake should result in only a slight underestimate of N-fixation, and at least allow a confident ranking of the treatments.

This study presented data on N-fixation, nodulation, above-ground biomass %^{15}N excess, root length, root weight, available soil N, and soil ^{15}N. Added fertilizer N significantly suppressed N-fixation and live nodule weight, while available soil N, soil ^{15}N, and above-ground %^{15}N excess increased. Intercropping showed one significant difference in these data: maize grown with black locust had lower biomass %^{15}N excess at low N than did sole maize. Similar trends due to intercropping at low N were also noted in black locust root length and live nodule weight, though these intercrop differences were not significant. Intercropping also greatly reduced maize growth which resulted in much less above- below-ground biomass. This repressed growth was caused by nutrient competition for P and K, allelopathy, or a combination of these. Competition may have been intensified due to an error in nutrient applications (minus N) where the experiment received one-half the target amounts of P and K. In addition, intercropped black locust absorbed P and K for 98 days before the introduction of maize. N was provided in amounts as called for in the experimental proposal and no N competition was observed.

It was expected that there would be lower biomass %^{15}N excess at low N in intercropped maize. The presumption was that the presence of black locust would absorb soil N in competition with the maize companion crop thereby reducing available soil N and soil ^{15}N. The reduced soil N would require black locust to increase N-fixation as well as root length and nodulation in the presence of maize roots. The high amount of root interaction and direct maize root contact with black locust roots and rhizospheres would aid N-transfer. This N-transfer would be observed as lower biomass %^{15}N in the intercropped maize. Indeed, there was high black locust/maize root interaction and contact at the low N application, and intercropped maize had a significantly lower %^{15}N excess. However, the poor growth of the intercropped maize introduced a second hypothesis explaining the lower biomass %^{15}N -- dissimilar access to pools to soil ^{15}N that resulted in the different biomass ^{15}N levels. Thus the poor intercropped maize growth confounded the results and no definitive conclusions can be made at this time regarding the potential of black locust to transfer N to a companion crop.

Still, the author finds it intriguing that at low N intercropped black locust root length and live nodule weight were greater than sole black locust. It is conceivable that if the intercropped maize had not grown poorly and the experiment had been longer in duration, these differences would have been greater. N-fixation did not follow this trend, but a time delay between the development of greater root length and increased live nodule weight and ensuing lower %^{15}N excess in the plant tissue used to estimate N-fixation may provide an explanation for this result. Only further studies can validate or reject this hypothesis.

Despite the poor growth of intercropped maize, and also in light of it, there are some interesting observations to be made from this work. The presence of one-half of black locust root length in the top 25 cm of soil, and presumably most of the nodule weight, confirms the potential of N-transfer, and the results of this study allude to this potential. The normal growth of maize alone, black locust alone, and intercropped black locust, despite less than optimum soil nutrient levels, indicate that black locust competes well for nutrients. While N competition does not appear to be important, P and K competition deserves study. Intercropped maize P and K deficiencies indicated that black locust will out compete maize for these nutrients, perhaps because they are key for maintaining high N-fixation rates (Reinsvold and Pope, 1987). This points to the importance of maintaining ideal P and K levels in systems incorporating black locust.

In this study, as in others (Dawson *et al.*, 1992; Johnsen and Bongarten, 1992; Reinsvold and Pope, 1987), black locust continued to fix N under the highest N application rate. This could mean that black locust will continue to fix N despite the high fertilizer N applications necessary for maize. In temperate agroecosystems this may result in the addition of atmospheric N, and in combination with the N cycling ability of black locust, the additional N introduced into the system may eventually reduce conventional rates of expensive N fertilizer.

Finally, to confirm the assumptions under which the IDT is used, it is vital to collect and analyze root and soil data, in addition to plant biomass, as done in this study. Without this data it would have been tempting to claim solid evidence of N-transfer as a result of the lower biomass $\%^{15}N$ at low N in intercropped maize. The additional data, though time consuming to collect, provided an important check on the validity of the results and allowed the findings to reflect a realistic interpretation. Readers should question similar studies that do not provide such important collaborative data.

If this work was to be repeated, a few changes are warranted. Though a simple error calculating the correct P and K applications necessary for this work may have played a role in the nutrient deficiency and poor growth observed in the intercropped maize, managing nutrients in a nutrient-less medium such as sand can be difficult. The researcher must have a good understanding of how to develop an appropriate balance and avoid problems such as high salt concentrations. An alternative to a nearly sterile sand is to use a soil a little higher in N but sufficient in the other nutrients, such as a Boyers Field Sand. This would provide a soil allowing for study of reactions to added fertilizer N, but would simplify nutrient management. Another change would be to place the experiment outside a greenhouse. Black locust seedlings could be started in a sand, and when weather permitted, transplanted into outside containers. Atmospheric deposition of N and ^{15}N from air and rain could be reduced by covering the containers at the root collar of the plants, allowing only the drip irrigation tube for the deionized water. With this done, environmental conditions would be a bit more realistic while still providing a closed and accessible system for biomass and soil analysis. Incorporating minirhizotrons into the containers to observe intercrop root interaction would enable another measurement of interaction as well as root and nodule turnover. Ultimately, repeated above- and below-ground measurements should be taken to provide a time series of results. This would provide a greater understanding of the development of interactions and treatment effects.

For an initial study such as this, the methods used were appropriate to validate and satisfy the assumptions of the IDT. However, this would be much more difficult in the field. The rooting behavior of maize and black locust was similar in this greenhouse study using sand, but in the field their root systems are not similar, making maize a less suitable reference plant for calculating N-fixation in, and N transfer from, black locust. A proper field study would have to give a great amount of consideration to a satisfactory reference plant. If greenhouse studies provide results that encourage further study, then the time and resources necessary needed for a field experiment might then be appropriate. Reeves (1991) provided an excellent example of the process of shifting from greenhouse to field studies to investigate N-fixation and N transfer.

The scientific and practitioner communities both recognize the need to develop agroecosystems that are safer and more sustainable. Rural communities look to diversify their products from agriculture to survive. Agroforestry is a land-use system gaining recognition as one alternative to help fulfill these parallel goals, and black locust has surfaced as a native temperate nitrogen-fixing woody perennial with great potential as an agroforestry tree. It is hoped that this study contributes to the ongoing investigation into the suitability of agroforestry and the usefulness of black locust.

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