SEASONAL CHANGES IN NITROGEN FIXATION ACTIVITY OF EUROPEAN BLACK ALDER AND RUSSIAN OLIVE[1]

Stephen F. Zitzer, Jeffrey O. Dawson
and George Z. Gertner[2]

Abstract.--Maximum rates of midday nitrogenase activity (acetylene-reduction) of 15 to 20 umoles C_2H_4 per g dry nodule per h were maintained for approximately 150 days for black alder. The seasonal length of maximum activity was similar for Russian olive, although specific nitrogenase rates were 25% lower. Nitrogenase activity increased exponentially between 10°C and 20 to 25°C for black alder and Russian olive with r^2 values of 27 to 73. No net hydrogen evolution by nodules was detected at any time during the assay period indicating efficient hydrogenase systems operating in nodules of alder and Russian olive under the conditions of this field assay. Alder trees were nodulated and had similar specific rates of nitrogen fixation in both upland and bottomland soils, though growth rate of alders was better on the bottomland site. In contrast, Russian olive grew best on the upland site, but nodulated roots were found only on the bottomland site.

INTRODUCTION

Actinorhizal plants form symbiotic root nodules with filamentous soil bacteria of the genus Frankia (Callaham et al. 1978). These procaryotic microsymbionts are capable of fixing atmospheric nitrogen by reducing dinitrogen from air to two ammonia molecules which can be assimilated by bacteria and plants. The reaction is catalyzed in nitrogen-fixing bacteria by the nitrogenase enzyme complex and can be quantified using acetylene reduction assays. Root nodules may provide up to 70% of the total nitrogen assimilated by actinorhizal plants (Tripp et al. 1979), and this nitrogen becomes available to other plants through the decomposition in soil of plant litter, roots and exudates from nitrogen-fixing plants.

Seasonal changes in nitrogenase activity frequently occur in temperate regions (Pizelle 1984); thus, the amount of nitrogen fixed will depend on these seasonal patterns. There may also be seasonal changes in the hydrogenase activities of the nitrogenase enzyme complex (Roelofson and Akkermans 1979), especially in hydrogenase efficiency which is defined as the relative amount of the total electron flow through the nitrogenase complex that is not lost via reduction of protons to H_2 (Winter and Burris 1976). Most Frankia species examined have uptake hydrogenases capable of oxidizing H_2 so that the energy is available for dinitrogen reduction and is not lost to proton reduction, measured as H_2 evolution. Actinorhizal nodules which evolve no hydrogen during nitrogen fixation and are 100% efficient at recycling hydrogen. Some recent research has shown that there are seasonal differences in patterns of nitrogenase activity and hydrogenase activity, suggesting that these activities are not controlled by the same regulatory system (Hafeez et al. 1984, Sellstedt et al. 1986).

Because of their ability to increase available nitrogen in soils (Paschke et al. in press), actinorhizal trees and shrubs are interplanted with other tree crops, such as black walnut, to increase their growth and yield (Dawson 1986).

The primary objectives of our research were to determine if seasonal patterns of nitrogenase and hydrogenase activity occur in alder and Russian olive root nodules, and to determine if their activities are related to soil moisture and temperature changes. A secondary objective was to describe nodulation of actinorhizal plants in this planting.

[1]Paper presented at the Seventh Central Hardwood Forest Conference, Carbondale, Illinois, March 5-8, 1989.

[2]The authors are Graduate Research Assistant and Associate Professors, respectively, Department of Forestry, University of Illinois, Urbana.

METHODS AND SITE DESCRIPTION

Two experimental interplantings of black walnut (Juglans nigra) with actinorhizal black alder (Alnus glutinosa) and Russian olive (Elaeagnus angustifolia) were established in 1978 by the U.S. Forest Service North Central Forest Experiment Station in cooperation with the Macon County Conservation District (Van Sambeek et al. 1985). Plantings were located on bottomland and upland sites in east central Illinois (89° 01' west, 39° 50' north). Their establishment, survival, growth and impact on interplanted black walnut through 1984 have been previously described (Van Sambeek et al. 1985). The experimental design was a randomized complete block. There were four replicate blocks of five plots each on each site. Pure black walnut plots were planted as controls with 42 plants per plot. Each plot was 18 x 21 m with 3 m between rows and trees within rows. In two plots of each block, the nitrogen-fixing plants were alternated with the black walnut to give 21 nitrogen fixing plants and 21 black walnut per plot.

In the initial planting during the spring of 1978, the black alder seedlings planted were one-year-old rooted cuttings from three slow-growing clones. Significant mortality occurred by the end of the first year. Subsequent replacement plantings up to the spring of 1981 were ade with 1-0 nursery-grown seedlings from the Indiana State Tree Nursery. Russian olive plantings and replantings up to 1981 were also made with 1-0 nursery-grown seedlings from a North Dakota and a Tennessee tree nursery. Thus, by the spring of 1985 when we started sampling, plant ages, including walnut ages, on both sites varied from four to seven years.

The bottomland site had been in agricultural use prior to 1978, and no additional site preparation was done before planting the trees. It was a level site on the floodplain of the Sangamon river. The soil was in the Lawson series, a fine silty mixed mesic Cumulic Hapludoll (Carroll County Soil Survey 1975). The A horizon had a silt loam texture and was 1.4 m thick directly overlying the massive alkaline silt loam C horizon. We determined that the mean pH and total nitrogen concentration (Kjeldahl) of the top 15 cm were 6.80 ±0.12 and 0.136% ±0.004% N.

The Lawson series is somewhat poorly drained, i.e., being wet for significant periods but not all the time. Available water was very high, 0.20 to 0.24 inches of water per inch of soil. Permeability of this soil is typically slow, 0.6 to 2.0 inches per hour, but on this site mottling, which indicates poor drainage, did not occur above a depth of 1.1 m.

The upland site had been a red clover-brome grass pasture prior to 1978. It is located approximately 3 km southeast of the bottomland site. The soil was in the Birkbeck series, which is in the fine silty mixed mesic Typic Hapludalf family (Champaign County Soil Survey 1982). It formed in about 120 cm of wind deposited loess over alkaline loamy glacial till. The site sloped 2 to 4% towards the northeast. The A horizon was about 30 cm thick with a silt loam texture. The B horizon had a pedogenic accumulation of clay which resulted in a silty clay loam texture to a depth of 1.2 m. The mean pH and total nitrogen concentration (Kjeldahl) of the top 15 cm were 5.41 ±0.24 and 0.124 ±0.014% N respectively.

The Birkbeck soil series is moderately well drained having a slowly permeable layer within the lower B horizon, i.e., at a depth of about 95 cm. The A, B and C horizons were all slowly permeable, though decreasing from 2.0 to 0.6 inches of water per hour from the A to the C horizon. Available water was very high in the A horizon, 0.22 to 0.24 inches of water per inch of soil. The B and C horizons had slightly less available water, 0.22 to 0.14, because of their higher clay contents and greater bulk densities compared to the A horizon. Mottling occurred at a depth of 65 cm, about in the middle of the B horizon.

Crown volumes of actinorhizal trees and shrubs were estimated using geometric approximations of crown shape for each species in order to assess relative growth success. Nitrogenase activity and net H_2 evolution of actinorhizal plants were measured at two to three week intervals starting before bud break on March 24, and ending 246 days later on November 23 after severe frost had killed all remaining leaves.

Each sampling day three actinorhizal trees of one species at one location were randomly chosen, and five randomly-selected samples of nodules plus control pieces of non-nodulated roots were assayed for each tree. Sampling was without replacement, so no trees were sampled more than once during the course of the study. Nodules were collected from the surficial 15 cm of the soil. The number of infection sites (nodules) per assay varied from one to 16 and averaged about four. Nodule dry weights per assay varied from 0.1 to 2.0 g and averaged about 0.75 g. The nodules, attached to 3 cm of root, were excised and were placed in 50-ml syringes, sealed, and buried in the soil at a depth of 10-20 cm. The incubation period was between 1200 h and 1300 h on any given sample day. Soil, incubation vessel, and air temperatures were recorded. Initially, the nodules and 3-cm control root samples in were incubated in air inside the buried syringes, and after an hour 10-ml gas samples were collected and stored in 12 ml-vacutainer tubes. These samples were later analyzed on an Aerograph Master A100 gas chromatograph for net H_2 production. The lower limit for H_2 detection on this machine was 0.01 umol per ml sample injection volume.

Immediately after sample collection for H_2 analysis, 5 ml air and 5 ml C_2H_2 were added to the syringes, and they were reburied and incubated for another hour to determine nitrogenase activity. In some cases, assays were allowed to run an additional hour to determine the length of linearity of the nitrogenase activity. After collection and storage of 10-ml acetylene-incubation samples in 12-ml vacutainer tubes, C_2H_4 production was determined with a Packard 433 gas chromatograph. The lower limit for C_2H_4 detection was 0.04 nmol per ml sample injection volume.

The nodules were washed, oven dried at 70° for 24 hours, and weighed. Soil from the upper 15 cm was also collected, and soil moisture determined from three replicate 1 kg samples. Soil pH was determined with a glass electrode in 1:1 soil to water ratio totaling 100 g with soil subsampled from that used to determine moisture content.

Analysis of variance and least significant differences for seasonal measurements of nitrogenase activity (umol C_2H_4 per g dry nodule per hr) were calculated for both sites and species. Nitrogenase activity was plotted and fit to regression curves as a function of soil temperature and soil moisture percentage.

RESULTS AND DISCUSSION

Black alder crown volumes averaged about 140 m^3 for black alder on the bottomland site, which was about 140 and 190 percent greater than the crown volumes for Russian olive and pure walnut, respectively. Russian olive on the upland site had the largest mean crown volume of about 110 m^3. Upland alder and walnut crown volumes were much smaller than the upland Russian olive as well as their counterparts on the bottomland site. Crown competition was not yet occuring among interplanted trees and shrubs in the study plots.

All plants were abundantly nodulated except for the Russian olive plants in the upland Hapludalf soil. Extensive excavation indicated that nodulation was extremely sparse on roots of upland Russian olive, precluding measurements of nodular activity.

There is a major difference between the two soils in surficial pH, with the upland soil being more acidic than the bottomland soil. The pH of the top 15 cm of the upland A horizon was significantly more acidic than the bottomland surface soil in agreement with soil descriptions. In contrast, the Lawson series is near neutral throughout the solum and C horizon. Frankia isolates are generally more infective at neutral pH values (Knowlton and Dawson 1983). This may account for differences in nodulation, but pot studies that we have performed show little difference in the infective capacity of upland and bottomland soils for Russian olive.

Midday acetylene-reduction rates of 15 to 20 umoles C_2H_4 per g dry nodule per h were maintained for approximately 150 days for black alder (Figures 2 and 3). The length of maximum activity was similar for Russian olive nodules, though specific rates were less than 75% those of black alder (Figure 1). Nitrogenase activity of black alder nodules was detected two to three weeks after the March 19 bud break when soil temperatures exceeded 7°C. Initial detection of nitrogenase activity in Russian olive nodules coincided with an April 6 budbreak at soil temperatures exceeding 12°C. Nitrogenase activity for both species declined sharply in late September, but low levels of activity continued into November. Nitrogenase activity increased exponentially between 10°C and 20 to 25°C for black alder and Russian olive with r^2 values of 27 to 73 (Figures 4, 5 and 6).

Lower seasonal rates of acetylene reduction by Russian olive nodules were probably due to the greater percentage of necrotic nodule tissue of Russian olive plants. A higher percentage of necrotic tissue was consistently found in Russian olive nodules compared with alder nodules. For correlation measurements we adjusted specific rates of acetylene-reduction activity so that only functional nodule tissue was included in the nodule dry weight determination. The necrosis of nodule tissue from the bottomland site and apparent lack of nodulation of plants in upland soils suggest that Russian olive may be able to reduce its own nodulation with increased plant size, because the larger, more mature plants on the upland site lacked nodules even though the upland soil was capable of nodulating Russian olive seedlings in a pot study. Alternatively, it is possible that the symbiotic relationship is not optimal between Russian olive and Frankia in this locale, or that annual turnover of nodular tissue occurs to a greater extent in Russian olive than in black alder. This last idea is consistent with the increase in specific nitrogen-fixation activity during the course of the growing season that occurred for Russian olive (Figure 1).

Nitrogenase activity declined linearly with values of soil water percentage between 10 and 30% (Figures 7, 8 and 9), though the correlation was not as strong (r^2 values of 6 to 17), and moister soil periods in spring and early fall also corresponded with lower soil temperatures. Neither the amount of nitrogen fixed by this symbiotic system, nor the amount that becomes available to other plants in soil can be reliably estimated using the enzyme assays employed in this study. Methods described by Paschke and coworkers (in press) and by Van Sambeek and others (1985) can be applied to estimate increases in available nitrogen in soil and tissue nitrogen attributable to interplanted actinorhizal plants.

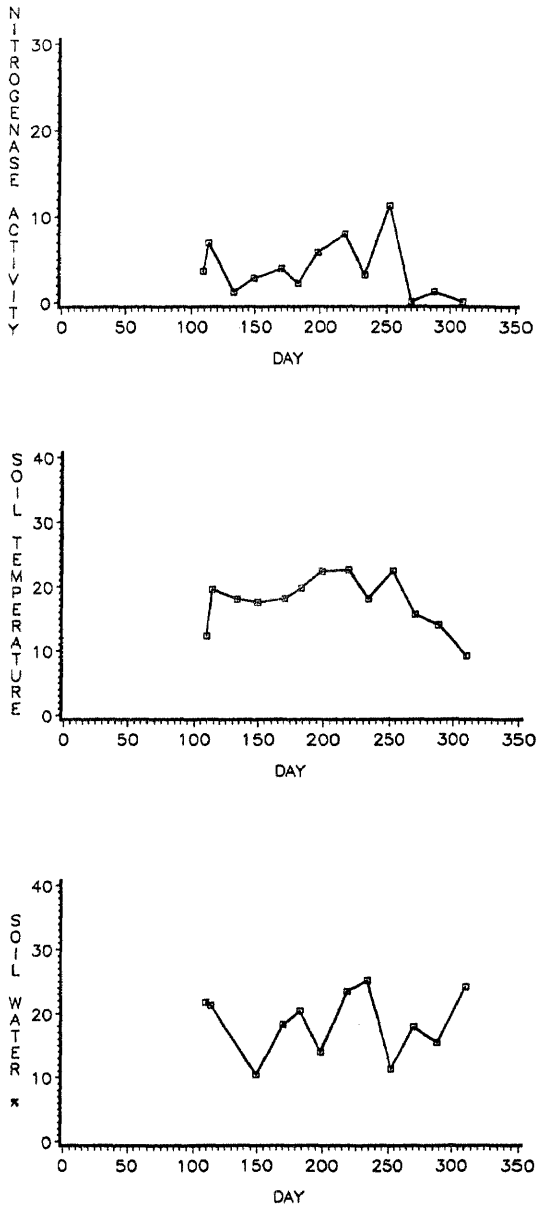

Figure 1. Seasonal pattern for nitrogenase activity (umoles C_2H_4 per g dry wt. per h), soil temperature (C) and soil moisture percent associated with root nodules of Russian olive growing on a bottomland site in central Illinois. Least Significant Difference (alpha= 0.05) is 4.31 umoles C_2H_4 per g per h for nitrogenase activity.

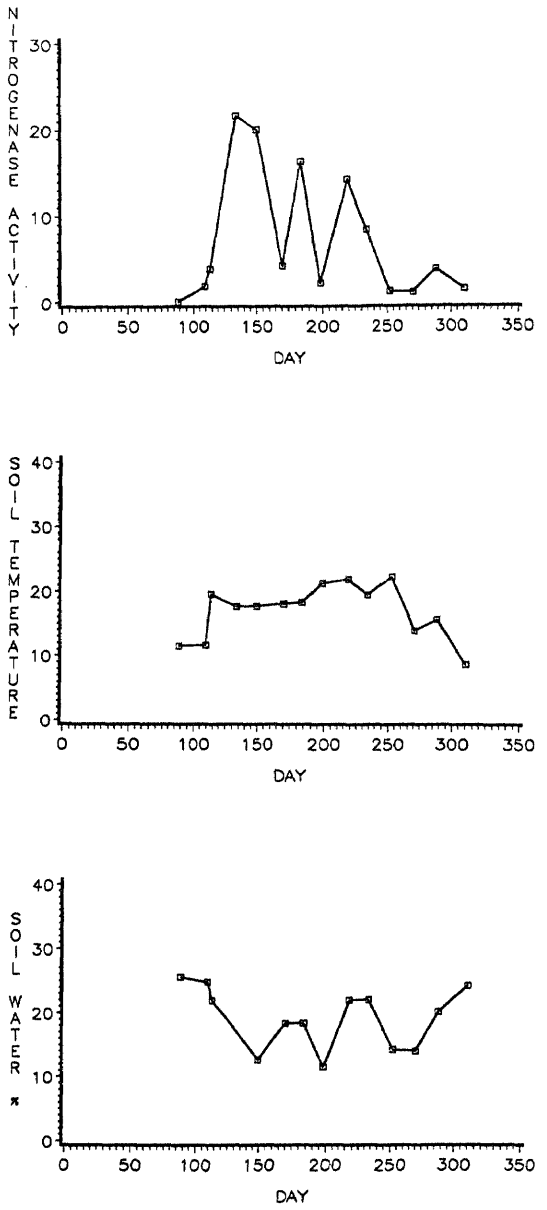

Figure 2. Seasonal pattern for nitrogenase activity (umoles C_2H_4 per g dry wt. per h), soil temperature (C) and soil moisture percent associated with root nodules of black alder growing on a bottomland site in central Illinois. Least Significant Difference (alpha= 0.05) is 4.86 umoles C_2H_4 per g per h for nitrogenase activity.

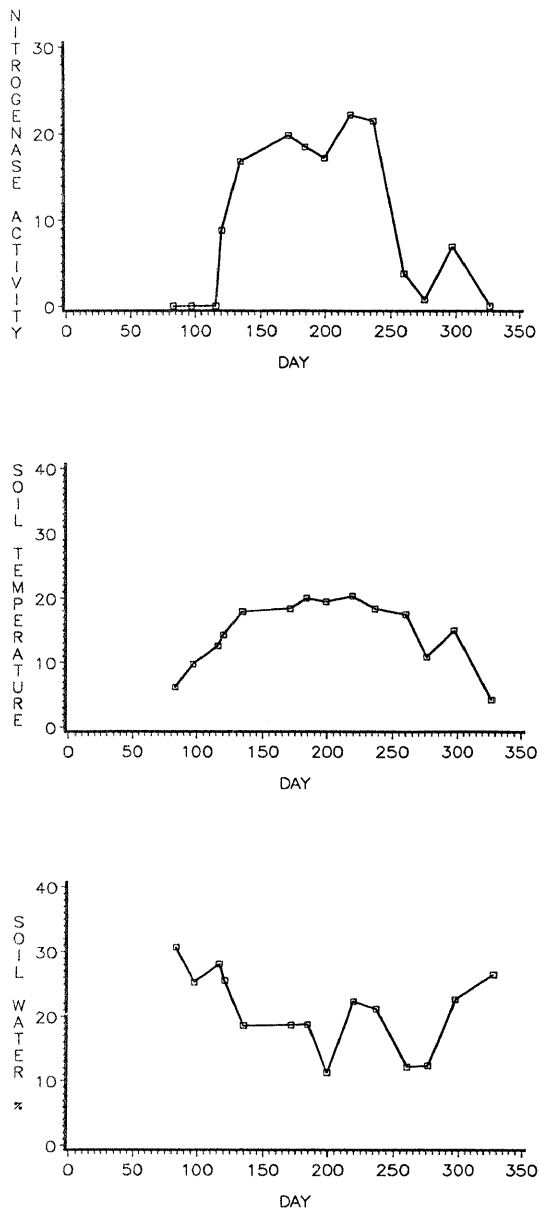

Figure 3. Seasonal pattern for nitrogenase activity (umoles C_2H_4 per g dry wt. per h), soil temperature (C) and soil moisture percent associated with root nodules of black alder growing on an upland site in central Illinois. Least Significant Difference (alpha= 0.05) is 5.12 umoles C_2H_4 per g per h for nitrogenase activity.

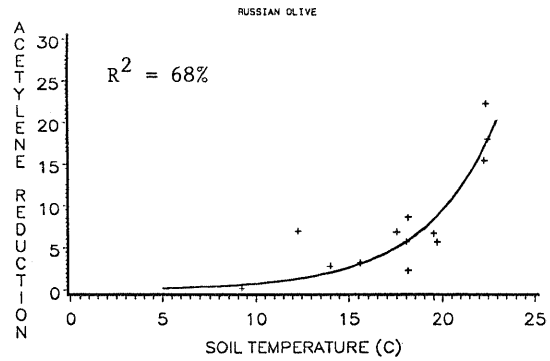

Figure 4. Relationship between soil temperature and nitrogenase activity (umoles C_2H_4 per g dry wt. per h) in root nodules of Russian olive growing on a bottomland site in central Illinois.

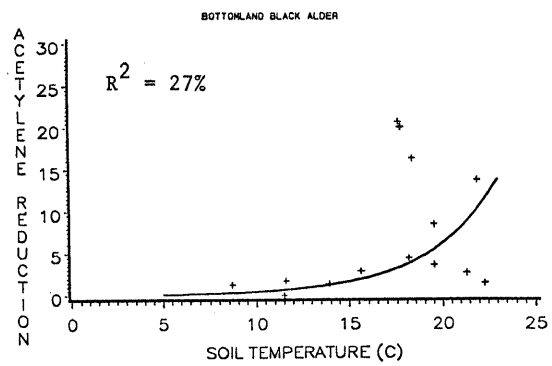

Figure 5. Relationship between soil temperature and nitrogenase activity (umoles C_2H_4 per g dry wt. per h) in root nodules of black alder growing on a bottomland site in central Illinois.

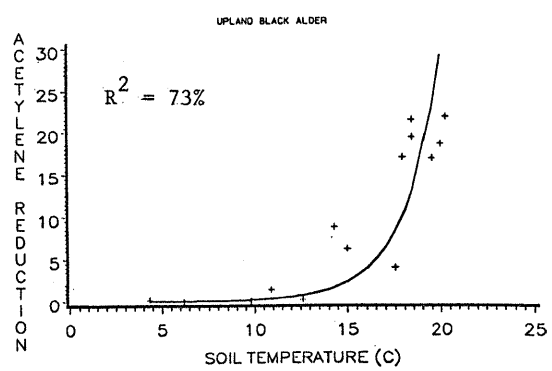

Figure 6. Relationship between soil temperature and nitrogenase activity (umoles C_2H_4 per g dry wt. per h) in root nodules of black alder growing on an upland site in central Illinois.

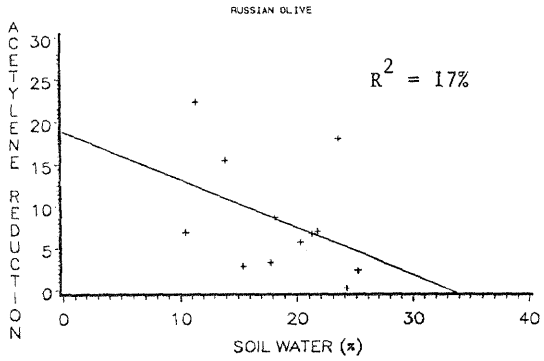

Figure 7. Relationship between soil water percentage and nitrogenase activity (umoles C_2H_4 per g dry wt. per h) in root nodules of Russian olive growing on a bottomland site in central Illinois.

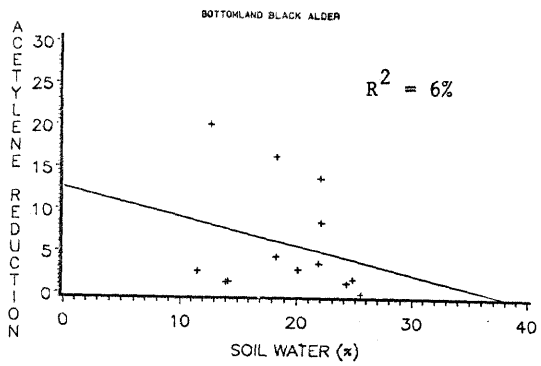

Figure 8. Relationship between soil water percentage and nitrogenase activity (umoles C_2H_4 per g dry wt. per h) in root nodules of black alder growing on a bottomland site in central Illinois.

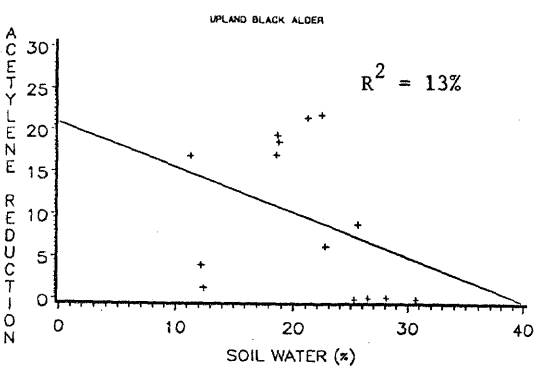

Figure 9. Relationship between soil water percentage and nitrogenase activity (umoles C_2H_4 per g dry wt. per h) in root nodules of black alder growing on an upland site in central Illinois.

No net hydrogen evolution by nodules was detected at any time during the assay period, and net hydrogen uptake was measured once by incubating black alder nodules in 10% H_2 (unpublished data). This indicates that efficient hydrogenase systems are operating in nodules of alder and Russian olive under the conditions of this field assay.

Our results indicate that symbiotic nitrogen fixation occurs in nodules of actinorhizal plants interplanted at this site with black walnut trees, and that the process is biochemically efficient. Nodular nitrogen fixation can occur during a 220-day period in actinorhizal plants in central Illinois, although the highest rates occur when soil temperatures approach 25°C during a 150-day period of peak activity during the growing season. Our finding that Russian olive plants lacked nodules on one site illustrates the importance of a systematic examination of the nodulation and nitrogen-fixation status of actinorhizal plants employed to improve N fertility in silvicultural systems.

ACKNOWLEDGEMENTS

The authors express their thanks to Jerry Van Sambeek, the U.S. Forest Service and the Macon County Conservation District for permission to use the experimental interplantings for this study.

REFERENCES

Callaham, D., P. Del Tredici and J. G. Torrey. 1978. Isolation and cultivation *in vitro* of the actinomycete causing root nodulation in *Comptonia*. Science 199: 899-902.

Carroll County Illinois Soil Survey. 1975. U.S.D.A. Soil Conservation Service with Ill. Agr. Exp. Sta.,University of Illinois, Urbana. 138 pp.

Champaign County Illinois Soil Survey. 1982. U.S.D.A. Soil Conservation Service with Ill. Agr. Exp. Sta., University of Illinois, Urbana. 178 pp.

Dawson, J. O. 1986. Actinorhizal plants: their use in forestry and agriculture. Outlook on Agriculture 15: 202-208.

Gleason, H. A. and A. Cronquist. 1963. Manual of vascular plants of northestern United States and Canada. D. Van Nostrand Co., New York. pp. 456-457.

Hafeez, F., A. H. Chaudhary and A. D. L. Akkermans. 1984. Physiological studies on N_2-fixing root nodules of *Datisca cannabina* L. and *Alnus nitida* Endl. from Himalaya region in Pakistan. Plant and Soil 78: 129-146.

Knowlton, S. and J. O. Dawson. 1983. Effects of *Pseudomonas cepacia* and cultural factors on the nodulation of *Alnus rubra* roots by *Frankia*. Can. J. Bot. 61: 2877-2882.

Paschke, M. W., J. O. Dawson and M. B. David. 1989. Soil nitrogen miner-alization in plantations of black walnut interplanted with actinorhizal autumn olive or black alder. This proceedings.

Pizelle, G. 1984. Seasonal variations of the sexual reproductive growth and nitrogenase activity (C_2H_2 reduction) in mature Alnus glutinosa. Plant and Soil 78: 181-188.

Roelofson, W. and A. D. L. Akkermans. 1979. Uptake and evolution of H_2 and reduction of C_2H_2 by root nodules and nodule homogenates of Alnus glutinosa. Plant and Soil 52: 571-578.

Sellstedt, A., K. Huss-Danell and A. Ahlquist. 1986. Nitrogen fixation and biomass production in symbioses between Alnus incana and Frankia strains with different hydrogen metabolism. Physiol. Plant. 66: 99-107.

Tripp, L. N., D. F. Bezdicek and P. E. Heilman. 1979. Seasonal and diurnal patterns and rates of nitrogen fixation by young red alder. Forest Sci. 25: 371-380.

Van Sambeek, J. W., R. C. Schlesinger, F. Ponder and W. J. Rietveld. 1985. Actinorhizal species as nurse cropsfor black walnut. In Proceedings of the Fifth Central Hardwood Forest Conference. J. O. Dawson and K. A. Majerus, eds. University of Illinois Dept. of Forestry Urbana. pp.257-263.

Winter, H. C. and R. H. Burris. 1976. Nitrogenase. Annual Review of Biochemistry 45: 409-426.