VARIATION IN THE CAPACITY OF BLACK ALDER TO NODULATE IN CENTRAL ILLINOIS SOILS[1/]

Jeffrey O. Dawson and Mary T. Klemp[2/]

Abstract.--Although alders can nodulate and fix nitrogen naturally in a wide variety of soils within and outside their native ranges, nodulation does not always occur and little is known about how or why soils vary in their ability to facilitate nodulation of actinorhizal (*Frankia*-nodulated) hosts. In order to determine how they vary in infective capacity, soils collected from four locations in central Illinois were assayed for their ability to promote nodulation of European black alder (*Alnus glutinosa*) seedlings. Seeds of black alder were sown in field soil in a replicated greenhouse trial. Total numbers of seedlings, the number of nodulated seedlings, the number of nodules per seedling, the ability of seedlings to fix nitrogen and other plant and soil properties were determined. All soils produced effective nodules, however soils from a native prairie containing actinorhizal *Ceanothus americanus* had the highest infective capacity, followed by adjacent cultivated soil and, lastly, soil from hardwood forests.

Key words: actinorhizal plants, *Alnus glutinosa*, *Frankia*, microbial ecology, nitrogen fixation, soil bacteria.

INTRODUCTION

European black alder, *Alnus glutinosa*, is planted as a nurse crop and for minespoil reclamation in the eastern United States because it tolerates a wide range of soil acidity (pH) and fixes atmospheric nitrogen with nodule-forming actinomycetes of the genus *Frankia* (Funk 1965). Associations between *Frankia* and host plants occur in at least 23 genera of woody plants and one genus of herbaceous plants in 8 diverse families and are termed actinorhizal symbioses (Dawson 1983, 1986). Nitrogen fixed from the atmosphere is metabolized by the host plant and released primarily as a component of plant litter and sloughed root tissue, similarly to leguminous plant symbioses with *Rhizobium*. Thus soils low in nitrogen can be improved through the accumulation of nitrogen-rich organic material. Nitrogen deficiency is a major factor limiting tree growth throughout the world.

Although actinorhizal plants are able to become nodulated and fix nitrogen when planted in soils outside their native ranges (Benecke 1969), precise information on the infective capacity of soils is almost totally lacking. Schramm (1966) investigated the natural colonization by plants of waste dumps resulting from anthracite mining in Pennsylvania. Nitrogen deficiency was shown to be an important factor limiting plant growth on these waste dumps. One of the most successful native plant colonizers was actinorhizal *Comptonia peregrina*, which was regularly nodulated on these sites. *Alnus glutinosa* was also successful.

Alnus glutinosa seeds germinated in minespoil from strip mines in central Illinois produced less than 3% nodulated seedlings in spoil material from steep upper slopes, while 26% of the seedlings were nodulated in spoil material from a level terrace towards the bottom of the spoil heap (Dawson *et al*. 1983). The level terrace afforded conditions suitable for infiltration and retention of water, thereby

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2/Jeffrey O. Dawson is an Associate Professor, Department of Forestry, University of Illinois at Urbana-Champaign, Urbana, IL 61801. Mary T. Klemp is a former undergraduate student at the University of Illinois and is currently a Graduate Research Assistant, Department of Forestry, Virginia Polytechnic and State University, Blacksburg, VA 24061.

favoring microbial and plant growth.

In Oregon, McNabb and Cromack (1983) found a patchy pattern of nodulation of actinorhizal Ceanothus velutinus root systems on a Douglas-fir clearcut area 17 years after harvesting. Douglas-fir excludes intolerant Ceanothus and lessens the infective capacity of affected soils (Youngberg and Wollum 1976). This is one of many cases where actinorhizal pioneers nodulate where they have not been growing for many years (Rodriguez-Barrueco 1968, Wollum et al. 1968, Benecke 1969, Bermudez de Castro et al. 1976, Akkermans and Houwers 1979, van Dijk 1984). The occurence of Frankia in such areas has been explained by long-term survival in the soil and migration via water, wind, rainfall or animal vectors (van Dijk 1984, Weber 1986). We have found that 100% of black alder seedlings grown in silt deposits from a small, intermittent stream in central Illinois consistently becomes nodulated. Coarse sands from the same stream and adjacent soils promote infection of only 20-80% of planted seedlings (unpublished data available from J. Dawson). This suggests that Frankia is transported and deposited with fine particulate matter in small streams.

There is a growing body of evidence from Europe that spore-negative Frankia, which do not sporulate in the nodular tissue of infected plants, are more abundant than spore positive Frankia in soil from areas lacking actinorhizal plants (van Dijk 1984, Weber 1986). This suggests that the spore-negative Frankia type is able to grow saprophytically in soil.

In central Illinois the only native actinorhizal plant is Ceanothus americanus which occurs scattered in distribution in tall-grass prairie and open oak woodlands. Alnus incana ssp rugosa occurs from northern Illinois northward into Canada, and probably occurrerd in central Illinois following the retreat of continental glacial ice 12,000 years ago. No historical record of this Alnus species exists for central Illinois.

Root excavation of exotic actinorhizal Elaeagnus angustifolia in mixed plantings with black walnut in Macon County, Illinois revealed differences in their nodulation on two different soils (personal communication from Stephen Zitzer, Department of Forestry, University of Illinois at Urbana-Champaign from dissertation research in progress). Zitzer found no nodules on Elaeagnus plants on Birkbeck silty clay loam, a Typic Hapludalf developed under upland hardwood forests with an acidity of pH 5.4. Whereas nodules on roots of Elaeagnus growing on nearby Lawson silty clay loam, a Cumulic Hapludoll with an acidity of pH 7.0 formed under prairie on the floodplain of the Sangamon River, were abundant. Soil acidity was thought to be the factor limiting nodulation.

Since we are interested in using Alnus glutinosa and other actinorhizal plants in experimental plantings as nurse crops with N-demanding tree species in central Illinois, these findings led us to look more carefully at the occurence of infective Frankia in soil. The objectives of this study were to determine the variation in the capacity of Alnus glutinosa seedlings to nodulate in some central Illinois soils and to relate this variation to soil and vegetation characteristics.

METHODS

Four study sites were selected in east central Illinois (Table 1). Two adjacent sites in McLean County had similar soil types, but differing treatments. The prairie site consisted of native tallgrass prairie on the old Weston Cemetery. The dominant grass on this prairie was big bluestem (Andropogon geradii) and 2% of the area had Ceanothus americanus cover. The adjacent site was former prairie that had been under cultivation for more than 100 years, most recently in row crops of corn and soybeans. The soils were black clays or silt loams developed on level, loess-capped glacial till.

Table 1.--Study locations in east central Illinois

Site Type	Soil Type	County	Survey Location
Tall-grass Prairie	Ashkum, Elliot	McLean	T26N, R4E
Cultivated Former Prairie	Ashkum, Elliot	McLean	T26N, R4E
Oak-Hickory Forest	Russell, Strawn, Miami	Piatt	T18N, R5E
Beech-Oak Forest	Blount	Vermillion	T19N, R12W

The oak-hickory site in Piatt County was in the University of Illinois' Allerton Park, a large complex of upland and bottomland forests along the Sangamon River. Quercus alba, Quercus rubra, Quercus imbricaria, Carya cordiformis, Carya ovata, Acer saccharum and Juglans nigra characterized the upland forests along the river. Soils were loams and silt loams on moderate to steep slopes near the river. Soils had developed on deep, dissected glacial till with loessal caps on ridges.

The beech-oak site in Vermillion County was in a designated natural area on the west bank of the Middle Fork River in Kickapoo State Park. Fagus grandifolia, Quercus alba, Quercus rubra, Populus grandidentata, Acer saccharum, Aesculus glabra and Juglans nigra characterized the forest in a cove ajacent to the river. Soils were loams on steep slopes formed in glacial till.

Five-hectare areas representative of each type of site were chosen. Then ten surficial soil samples (top 30 cm) were taken at randomly selected points within each area. Soils were gathered in the fall and kept frozen until their use during the winter. Five additional samples were randomly taken at each site for soil testing and to be steam-sterilized for checks of stray contamination with Frankia in the subsequent greenhouse study. For each of the samples from each site, 500 cc of soil were placed in individual round pots with top diameters of 10 cm and seeded to yield approximately 40 seedlings per pot of European black alder. The reason for this dense planting was to allow the roots to thoroughly bait the soil for infective Frankia. Pots were set out on a greenhouse bench in a completely randomized fashion. All alder seeds were from the same lot gathered from a single parent tree in Urbana, Illinois and used immediately after collection in early winter. Seedlings were grown in a greenhouse at 24°C ± 2° day temperature and 18°C ± 3° night temperature. Photoperiod was extended to 16 h using a combination of incandescent and white fluorescent lamps.

Twelve weeks after germination, the roots were washed to remove all soil and the total number of seedlings and the total number of nodulated seedlings were counted in each pot. Up to ten randomly selected nodulated plants per pot were measured to determine the number of nodules per plant, nodule dry mass, shoot length, root length, shoot dry mass and root dry mass. Five nodulated plants from each of the four sites were randomly selected after washing and incubated individually for 1 h in 0.5 cc of acetylene and 4.5 cc of air in glass tubes sealed with serum stoppers. Then acetylene reduction (nitrogenase activity) was measured using a gas chromatograph with a flame ionization detector according to Dawson and Gordon (1979).

Duplicate determinations for at least 5 soil samples per site were made for total N (Gallagher et al. 1976); exchangeable N (Bremner 1965); K, Ca and Mg by atomic absorption spectrophotometry (Ediger 1973); total P according to Jackson (1958); soil pH in a 2.5:1 distilled H_2O:soil mixture; soil texture by the hydrometer method; and organic matter by combustion in a muffle furnace at 700°C for 12 h. Least significant differences (LSD) were used to test the statistical significance of differences between mean values for soil source. Standard deviations are given to indicate the amount of variation overall or within a treatment.

RESULTS AND DISCUSSION

The prairie soil had the greatest infective capacity, followed by the adjacent, cultivated prairie soil, the oak-hickory forest soils and, lastly, the beech-oak forest soil (Table 2).

Rankings are based on the total estimated number of nodules formed, which can be derived by multiplying the number of seedlings by the percentage of seedlings nodulated and the mean number of nodules per nodulated seedling in Table 2. The prairie soil was the only soil supporting an actinorhizal plant, Ceanothus americanus. None occurred on the cultivated, former-prairie site nor in the forest sites.

Shoot lengths averaged 6.2 cm with a standard deviation of 1.4 cm. Root lengths averaged 9.0 cm with a standard deviation of 2.6. Shoot mass averaged 44 mg ± 40 mg (SD) while root mass averaged 24 mg ± 22 mg. There were no significant differences in these values associated with soil type or nodulation.

Table 2.--Nodulation of black alder seedlings in soils from various sites in east-central Illinois. Each number is derived from 10 soil samples. SD = standard deviation.

	Total Number of Seedlings	Percentage of Seedlings Nodulated	Mean Number of Nodules per Nodulated Seedling ± SD	Number of Nodulated Seedlings Sampled for Nodule Tally
Tall-grass Prairie	403	99%	6.7 ± 6.2	97
Cultivated Former Prairie	402	35%	6.6 ± 9.9	59
Oak-Hickory Forest	371	48%	2.7 ± 2.2	81
Beech-Oak Forest	373	14%	1.3 ± 0.6	55

Variation was equally high within soil-type treatments and differences in nodulation were probably associated more with the amount of infective Frankia in a soil than with root length or mass.

The number of nodules per nodulated plant ranged from 1 to 28 and nodule dry mass per plant ranged from 0.1 to 3.4 mg. Nodules that developed in each soil type were capable of acetylene reduction, indicating that actinorhizal symbioses between exotic Alnus glutinosa and Frankia present in central-Illinois soils are effective at fixing nitrogen from the atmosphere. Acetylene-reduction rates were variable and ranged from 2 to 35 nanomoles per plant per hour when freshly-washed seedlings were incubated in 10% acetylene in air at 25°C in a lighted growth chamber. All plants assayed exhibited some nitrogenase activity, though levels of activity did not exhibit any pattern related to soil type. No nodules developed on plants in steam-sterlized soil samples from the 4 study sites. This finding eliminates random contamination of soils by Frankia in the greenhouse as a possible source of variation in soil infective capacity.

The soil properties measured are outlined in Table 3. Forest soils had at least 3 times the amount of exchangeable NH_4^+ as prairie soils and about the same amount of exchangeable NO_3^-. This difference could explain, in part, lower nodulation of alder seedlings baited in forest soils, since higher levels of combined N inhibit nodulation of actinorhizal plants (Stewart and Bond 1961).

Soils from the beech-oak forest, which had the lowest nodulation capacity, were lowest in all nutrients except N, and had the lowest organic matter concentration of any of the soil sources analyzed. Smaller amounts of organic matter in this soil might have resulted in reduced saprophytic growth of Frankia in soil, assuming that such growth occurs. Lower nutrient levels, such as P levels, might also have affected alder-seedling growth and the nodulation process in beech-oak site soils. Although seedling-growth differences were highly variable and did not differ significantly among soil types, mean total plant mass varied from 80 mg in the prairie soil and 79 mg in the cultivated former-prairie soil to 50 mg in the oak-hickory soil and 42 mg in the beech-oak soil. These

Table 3.--Properties of experimental soil (standard deviation is in parentheses).

Soil Property	Prairie	Cultivated Former Prairie	Oak-History Forest	Beech-Oak Forest
Acidity, pH	6.0 (0.1)	6.6 (0.2)	6.6 (0.3)	6.0 (0.4)
P, ppm	68 (1)	44 (12)	59 (26)	33 (10)
Exchangeable NH_4^+, ppm	6 (2)	4 (3)	26 (10)	20 (7)
Exchangeable NO_3^-, ppm	11 (2)	10 (4)	6 (13)	11 (3)
Total N, ppm	3919 (256)	2066 (318)	3127 (965)	2200 (415)
Organic matter, %	3.9 (0.3)	2.0 (0.3)	2.3 (0.6)	1.8 (0.5)
K, ppm	11,357 (2913)	6464 (997)	5852 (1556)	3197 (1023)
Ca, ppm	87,586 (17,403)	114,172 (32,089)	72,086 (18,960)	48,439 (17,882)
Mg, ppm	9949 (2519)	11,811 (6466)	13,023 (2761)	4423 (1335)
Sand, %	21 (3)	21 (3)	36 (15)	38 (6)
Silt, %	59 (3)	42 (4)	50 (14)	51 (5)
Clay, %	20 (3)	37 (5)	14 (3)	11 (3)

differences in plant growth might account in part for reduced nodulation in forest soils compared with prairie soils. But plant growth differences do not explain the great reduction in percentage of seedlings nodulated in cultivated former-prairie soils compared with adjacent prairie soils (Table 2).

Our results indicate that the presence of actinorhizal Ceanothus americanus on a site is most strongly associated with an increased capacity of central Illinois soils to nodulate Alnus glutinosa seedlings. Other soil properties, such as higher exchangeable nitrogen levels, lower organic matter content and lower soil nutrient levels may also reduce nodulation by limiting Frankia growth in soil, limiting alder seedling growth and, thus, the chances of root contact with infective Frankia propagules in soil, or by inhibiting the infection process.

Although our data were too variable to assess the individual importance of various soil factors in influencing nodulation, this study indicates clearly that soils vary in their capacity to support infection of actinorhizal plants. This may be of concern only where soils have no capacity to support infection of an actinorhizal plant, or where nodules are not effective in fixing nitrogen. However, because such situations can occur in nature, it is important to observe whether nodulation has occurred, to assay nodules for nitrogen-fixation capacity and to monitor changes in soil and plant nitrogen over time in forest plantations with actinorhizal plants if we are to better understand and exploit the nitrogen fixation process silviculturally.

LITERATURE CITED

Akkermans, A. D. L. and A. Houwers.
1979. Symbiotic nitrogen fixers available for use in temperate forestry. In Symbiotic Nitrogen Fixation in the Management of Temperate Forests (J. C. Gordon, C. T. Wheeler and D. A. Perry, eds.), pp. 23-38. Forest Research Laboratory, Oregon State University, Corvallis.

Benecke, U.
1969. Symbionts of alder plants in New Zealand. Plant Soil 30:145-149.

Bermudez de Castro, F., C. Miguel and C. Rodriguez-Barrueco.
1976. A study of the capacity of soils to induce nodules in Alnus glutinosa (L.) Gaertn. and Myrica gale L. with special reference to the specificity of the endophytes. Ann. Microbiol. (Inst. Pasteur) 127a:307-315.

Bremner, J. M.
1965. Inorganic forms of nitrogen. In Methods of Soil Analysis (C. A. Black, ed.), pp. 1179-1273. American Society of Agronomy. Madison, Wisconsin.

Dawson, J. O.
1983. Dinitrogen fixation in forest ecosystems. Can. J. Microbiol. 29:979-992.

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

Dawson, J. O., T. W. Christensen and R. G. Timmons.
1983. Nodulation by Frankia of Alnus glutinosa seeded in soil from different topographic positions on an Illinois spoil bank. The Actinomycetes 17:50-60.

Dawson, J. O. and J. C. Gordon.
1979. Nitrogen fixation in relation to photosynthesis in Alnus glutinosa. Bot. Gazette 140:70-75.

Ediger, R. D.
1973. A review of water analysis by atomic absorption. Atomic Absorption Newsletter 12:151-157.

Funk, D. T.
1965. Silvics of European alder. USDA Forest Service, North Central Forest Experiment Station. 20pp.

Gallagher, R. N., C. O. Weldon and F. C. Boswell.
1976. A semiautomated procedure for total N in plant and soil samples. Soil Sci. Soc. Am. J. 40:887-889.

Jackson, M. L.
1958. Soil Chemical Analysis. Prentice-Hall. Englewood Cliffs, New Jersey.

McNabb, D. H. and K. Cromack, Jr.
1983. Dinitrogen fixation by a mature Ceanothus velutinus (Dougl.) stand in the Western Oregon Cascades. Can. J. Microbiol. 29:1014-1021.

Rodriguez-Barrueco, C.
1968. The occurrence of the root-nodule endophyte of Alnus glutinosa and Myrica gale in soils. J. Gen. Microbiol. 52:189-194.

Schramm, J. R.
1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans. Am. Phil. Soc. 56:1-194.

Stewart, W. D. P. and G. Bond.
1961. The effect of ammonium nitrogen on fixation of elemental nitrogen in Alnus and Myrica. Plant Soil 14:347-359.

van Dijk, C.
1984. Ecological aspects of spore formation in the Frankia-Alnus symbiosis. Ph.D. Thesis, University of Leiden, The Netherlands.

Weber, A.
1986. Distribution of spore-positive and spore-negative nodules in stands of Alnus glutinosa and Alnus incana in Finland. Plant Soil (in press).

Wollum, A. G., C. T. Youngberg and F. W. Chichester.
1968. Relation of previous timber stand age to nodulation of Ceanothus velutinus. For. Science 14:114-118.

Youngberg, C. T. and A. G. Wollum.
1976. Nitrogen accretion in developing Ceanothus velutinus stands. Soil Sci. Soc. Am. J. 40:109-112.