NODULATION BY *FRANKIA* OF *ALNUS GLUTINOSA* SEEDED IN SOIL FROM DIFFERENT TOPOGRAPHIC POSITIONS ON AN ILLINOIS SPOIL BANK

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Abstract.--*Alnus glutinosa* seeds were germinated in pots of minesoil collected at various topographic locations from the surficial 16 cm of an east-central Illinois spoil bank. After 10 weeks, spoil material from a level terrace at lower mid-slope had promoted nodulation of 26% of the *Alnus* seedlings while material from a slope below the terrace had promoted nodulation of 13% of the seedlings. Steep upslope spoil material had promoted nodulation of 3% or less of the seedlings. These results are consistent with speculation that the level terrace afforded conditions suitable for infiltration and retention of water, thus favoring microbial and plant growth. Results suggest that terracing or leveling of similar spoil banks would improve their capacity to support the actinorhizae-forming actinomycete *Frankia*.

Keywords: Alder, mine spoil, reclamation, nitrogen fixation.

INTRODUCTION

European black alder [*Alnus glutinosa* (L.) Gaertn.] is planted on mine spoils in the eastern United States because it tolerates a wide range of soil pH's and because it fixes atmospheric nitrogen symbiotically with nodule-forming actinomycetes of the genus *Frankia* (Funk 1965). Nitrogen deficiency is a major factor limiting plant growth on mine spoils.

Nitrogen-fixing plants can obtain the nitrogen necessary for their growth directly from the atmosphere. They do this with the help of procaryotic, nodular endophytes possessing the enzyme nitrogenase that can convert N_2 from air into NH_3. Nitrogen fixed in this way is metabolized by the host plant and released primarily as a component of plant litter and sloughed root tissue. As this organic material accumulates in soil, so does nitrogen. Thus soils low in nitrogen and organic matter can be improved by nitrogen-fixing plants.

The most-commonly cultivated nitrogen-fixing plants are legumes that are nodulated by bacteria of the genus *Rhizobium*. Some familiar examples are black locust trees, alfalfa, soybeans, and clovers. Another type of nitrogen-

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fixing symbiosis occurs between the actinomycete genus Frankia and species in 8 diverse families of hosts, most of which are woody plants (Akkermans and van Dijk 1981). These actinorhizal (Frankia-nodulated) plants include alder, autumn olive, Russian olive, and wax myrtle.

Although actinorhizal plants are able to become nodulated when planted in soils outside their native ranges (Benecke 1969, Dawson 1979), precise information on the infective capacity of mine spoils 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 plant colonizers was actinorhizal Comptonia peregrina, which was regularly nodulated on these sites. Alnus glutinosa was also successful.

The purpose of our work was to determine if there were differences in the capacity of minesoil from different topographic positions on a spoil bank to infect seeded European black alder. Since leveling and terracing mine spoils improve their ability to support plant growth, we wished to find out how a terrace differed from other slope positions in the capacity of its soil to promote nodulation of alders by Frankia.

MATERIALS AND METHODS

A spoil bank near the southeastern entrance to Kickapoo State Park in Vermilion County, Illinois was selected as the study site. The spoil bank faced due south (183° azimuth) with a 73% slope and a level terrace at mid-slope. The face of this slope was approximately 10 meters wide. The spoil material was a product of surface mining of coal in the early 1960's. Mining had been done with a dragline to a depth of up to 100 feet. The spoil material consisted primarily of shale and limestone.

Samples of minesoil were taken in 1980 at 6 topographic positions on the spoil bank (Figure 1). At each topographic position, 6 soil samples were taken at randomly selected points at the same elevation on the slope surface. The sample depth was 16 cm. Fragments larger than 1 cm were screened out and 300 cc samples of this material were added to pots that were 7 cm on a side. Each sample was put in a separate pot and approximately 175 (±25) Alnus glutinosa seeds were sown evenly in each. Pots were placed in a growth chamber at a 16-hour photoperiod provided by a combination of incandescent and white-fluorescent lights. The light and dark temperatures were 25°C and 18°C respectively. Plants were watered regularly with deionized, distilled water. No nutrients were added to the potted minesoils. After 10 weeks, all live seedlings were removed from their pots after gentle agitation in water to loosen the root systems. Counts of live seedlings and the number of nodules formed on each seedling were made. Sections of root nodules were examined by light microscopy for the presence and form of Frankia.

Chemical analyses were performed on additional samples taken at the same depth and level as the microbiological samples. The composite samples

were used to determine soil pH in a 1:1 mixture of dry soil with water, available phosphorus using the Olson test for calcareous soils, and calcium carbonate equivalent to estimate the amount of undissolved limestone present. Soil texture was determined using a soil hydrometer.

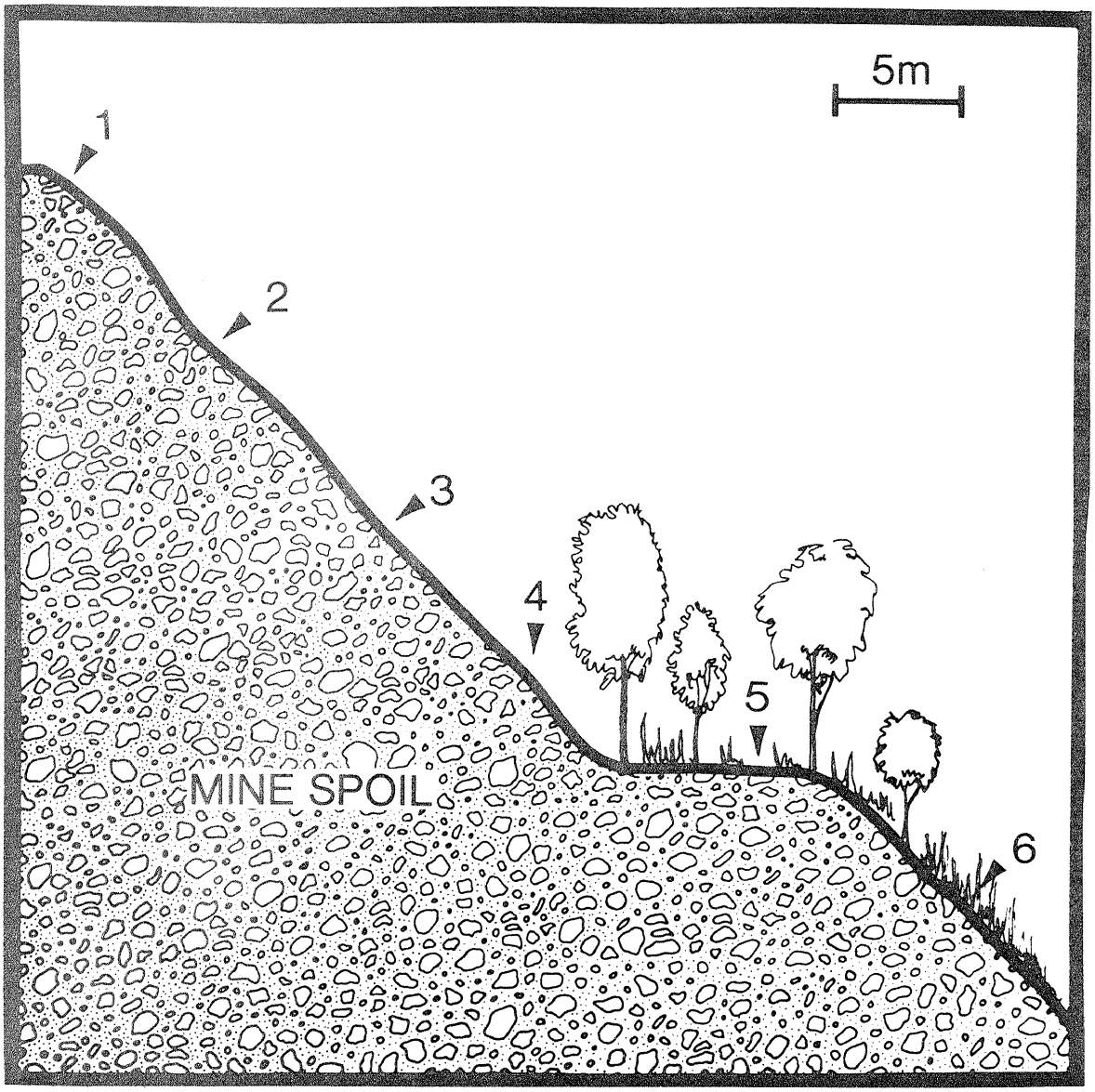

Figure 1. Topographic positions at which minesoil samples were collected on a spoil bank.

RESULTS AND DISCUSSION

A summary of the nodulation results in Table 1 reveals that the terrace position yielded minesoils that promoted the greatest amount of seedling nodulation, with a total of 359 nodules produced on 692 seedlings. The minesoils downslope from the terrace were about 1/3 as infective as the

terrace minesoils. The steep slopes above the terrace had soils with almost no infective capacity. The numbers of seedlings per pot and nodulation were reasonably consistent within each topographic position (Table 2). Topographic positions with soils that promoted the greatest nodulation also supported the greatest amount of vegetative cover. Invading sycamore and cottonwood trees had achieved the greatest height and diameter growth on the level terrace, with less growth downslope. The slopes above the terrace were devoid of vegetation. Soil tests revealed that the surficial soil of the spoil bank had a considerable amount of undissolved limestone and a slightly alkaline pH (Table 3). Phosphate availability was in the low to medium range when compared with local soils, but was sufficient to support plant growth.

Nodular tissue contained hyphae and vesicles characteristic of Frankia. Nitrogen fixation was evidenced by greener color (more chlorophyll) and greater growth of nodulated plants compared with those lacking nodules.

Table 1. Nodulation of Alnus glutinosa seedlings in surficial soils from different topographic positions on a mine spoil bank.

Relative topographic position	Vegetative cover on spoil bank*	Number of living seedlings	Total number of nodules formed
1 steep upslope	-	547	13
2 steep upslope	-	422	2
3 steep upslope	-	622	20
4 steep upslope	-	546	6
5 level terrace	+	692	359
6 steep downslope	+	689	114

*The symbol (-) denotes absence, while the symbol (+) denotes presence of vegetative cover.

Table 2. Variation in nodulation among individual seedlings and soil samples from different topographic positions on a mine spoil bank.

Relative topographic position	Mean number of living seedlings per pot ± 1 s.d.*	Mean percentage of nodulated seedlings per pot ± 1 s.d.	Mean number of nodules per nodulated seedling ± 1 s.d.
1 steep upslope	91 ± 30	2.0 ± 2.0	1.0 ± 0.0
2 steep upslope	70 ± 12	0.4 ± 1.0	1.0 ± 0.0
3 steep upslope	104 ± 20	3.0 ± 5.9	1.2 ± 0.5
4 steep upslope	91 ± 23	1.1 ± 0.9	1.0 ± 0.0
5 level terrace	115 ± 44	25.7 ± 5.3	2.0 ± 1.4
6 steep downslope	115 ± 16	12.7 ± 3.9	1.3 ± 0.6

*s.d. = sample standard deviation.

Table 3. Properties of spoil-bank soils.

Relative topographic position	Soil pH	Olson P (lbs/acre)	%sand	%silt	%clay	Calcium carbonate equivalent
1 steep upslope	8.0	26	39	37	24	11.65
2 steep upslope	6.9	74	36	43	21	9.89
3 steep upslope	7.7	50	57	24	19	10.69
4 steep upslope	7.9	34	40	41	19	5.32
5 level terrace	8.1	26	70	22	8	31.22
6 steep downslope	8.0	36	27	49	24	9.78

The minesoil surface was stony, although soil particles less than 2 mm were of a loamy texture. The bare slope which faced the south, undoubtedly had high surface temperatures in summer, thus discouraging the establishment and growth of plants and microbes.

Surface runoff on the upper slope, evidenced by gully erosion, probably added to the input of water from precipitation on the level terrace. Runoff reaching the sandy clay loam soil of the terrace from the upper slope would decrease in velocity and infiltrate more readily than on the 73% slope. This extra water input, together with the increased infiltration capacity of the level terrace, may have created soil water conditions favorable for survival and growth of plants and soil microoogansims. Conversely, the steeper slopes above and, to a lesser extent, below the terrace would have had less favorable soil moisture conditions for the support of plants and microorganisms.

The sources and methods of dissemination of Frankia are not known, though European alder, autumn olive, and Russian olive planted in undisturbed soils nearby commonly form effective nodules. Ceanothus americanus L. is the only native actinorhizal plant found in the locale of these mine spoils. It is possible that propagules of Frankia, whatever they may be, reached and were washed from upper slope positions with surface runoff, resulting in greater infective capacity of terrace and lower-slope minesoils. It is also possible that better soil-moisture conditions for plant establishment on the terrace and lower slope resulted in higher soil organic matter, lower temperatures, and improved water-holding capacity, thus creating a more hospitable environment for Frankia. Because of the high calcium carbonate and shale content of these minesoils, no accurate determination of soil organic matter quantities was possible.

Leveling and terracing slopes to improve their capacity to support plant establishment is accepted practice in mine-spoil reclamation (Vogel 1981). Our observations indicate for the first time that these practices might also increase the ability of similar mine spoils to harbor infective

agents of Frankia, as well as to promote growth of other beneficial soil microorganisms. Thus, both establishment and early nitrogen fixation of alders and other actinorhizal plants on mine spoils might be enhanced by leveling and terracing.

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