NITROGEN-FIXING PLANTS FOR SILVICULTURE:
SOME GENECOLOGICAL CONSIDERATIONS

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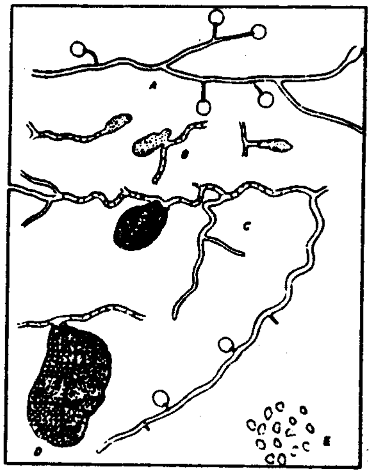

ABSTRACT

Nitrogen fixation as a silvicultural tool will require symbioses adapted to the relatively harsh temperate forest environment. Ability to survive and fix nitrogen in shade, cold soils, and under water stress are characteristics that must be selected. Research indicates a great deal of genetic diversity among legumes and Rhizobia strains in response to some environmental factors such as temperature, while other potentially important influences have been studied little. Virtually no studies have been done in genotype-environment interaction among nonleguminous symbioses. The small amount of work available suggests that legumes may fix N at higher rates in shade than nonlegumes, although no experiments have specifically tested this question. Legume N-fixation may also be greater in cold soils than that of nonlegumes. Many legumes and nonlegumes grow in droughty conditions, suggesting that searches for drought-adapted species and strains may be worthwhile.

INTRODUCTION

Natural nitrogen fertilization of forest stands can be tactically approached at least three ways: the N-fixers may be rotated with the crop species as in agronomic crop rotation; N-fixing plants can be interplanted in young tree plantations; or they may be underplanted in older stands (see Miller and Murray, these proceedings, for a more detailed analysis of these techniques). Crop rotation in forestry is less straightforward than in agriculture because clearing well established stands of N-fixers is either expensive or environmentally controversial. If commercially valuable N-fixers such as some Alnus species are used, this is, of course, not a problem. The second approach, interplanting, is feasible only if excessive competition between N-fixing plants and trees can be avoided by using herbaceous N-fixers, such as many legumes, or by heterogeneous planting designs that physically separate groups of N-fixers from trees (D. P. Lavender, Department of Forest Science, Oregon State University, Corvallis, personal communication). The third approach uses highly competitive N-fixing plants such as Alnus and Ceanothus sp. Evidence indicates that N-fixers of this type, despite their relative shade intolerance (Berg and Doerksen 1975; Silvester et al., these proceedings; Rehfuess, these proceedings), will survive and fix N under certain conditions--for example, in thinned stands.

In all these cases, especially in the third, N-fixing plants face relatively harsh conditions. In some cases, site scarfication and heavy fertilization are necessary to establish legumes in forest stands (Rehfuess, these proceedings;

Haines et al., these proceedings). An important characteristic of the "ideal" N-fixing plant for silviculture will be the ability to survive and fix nitrogen in cool temperatures, in low light, and in soils that are nutrient-poor, acidic, and droughty. Such an N-fixing ideotype likely can be developed. However, we must first increase our understanding of the nature and extent of genetic diversity among wild symbioses. This diversity is probably quite rich, but few studies have addressed this question, particularly in terms of adaptation to the temperate forest environment. In this paper, we briefly review genetic variation among nitrogen-fixing plants in response to three important environmental factors: irradiance, temperature, and water stress.

IRRADIANCE

Shading

Light influences photosynthate supply to nodules and, hence, influences nitrogen fixation. Gordon and Wheeler (1978) found that both nodule number and acetylene reduction positively correlated with net photosynthetic rate in 12 clones of European black alder (*Alnus glutinosa*). Nitrogen fixation in experimental plants often shows a diurnal cycle that corresponds closely to photosynthetic activity and, presumably, export of carbohydrate to nodules (Wheeler 1969, 1971; Bond and Mackintosh 1975; Hardy and Havelka 1976). However, this is not always true. Nitrogen fixation seems to become less sensitive to diurnal changes in light intensity as alder seedlings age (Wheeler and Lawrie 1976) and may be absent in field-grown alder (Akkermans 1971). Wheeler and Lawrie (1976) found quite different diurnal behavior in two sets of 3-week-old pea plants tested 16 days apart; in neither case did changes in carbohydrate content of nodules absolutely correlate with nitrogenase activity. As they pointed out, diurnal fluctuations in nitrogenase activity likely reflect a complex of environmental factors.

The nitrogen-fixing ability of plant genotypes under low light will depend on photosynthetic efficiency, the ability of nodules to compete with other plant parts for available carbohydrates, and the efficiency of the nitrogen-fixing system. Nonleguminous nitrogen-fixers often have large leaf areas and very rapid growth, suggesting a high rate of energy capture; however, they tend to be classical pioneer species, poorly adapted to low light. Legumes, because they are herbaceous, may have evolved a greater ability to cope with shade.

Yet nonleguminous species have a possible advantage beyond light-gathering power. In many legumes, only 40% to 60% of electron flow to nitrogenase is transferred to nitrogen; the rest evolves as hydrogen (Schubert and Evans 1976). In at least some nonlegumes (e.g., *Alnus rubra* and *Purshia tridentata*), this is not true, and one might hypothesize that, other factors being equal, these non-H_2 evolvers are better able to maintain some N_2 fixation at low light levels.

Research on these speculations is limited. Only six studies (Table 1) have considered nodulation and nitrogen fixation of single species grown under continuous shade. Although differing experimental conditions make comparison awkward at best, these studies suggest that at least three legumes fix nitrogen more efficiently in low light than does European black alder (*Alnus glutinosa*). For comparison, we normalized light conditions by expressing all as a percent of full sunlight, assumed equivalent to 100,000 lux in photometric measure and 0.6 $cal \cdot cm^{-2} \cdot min$ in radiometric measure (Nobel 1974, p. 171-172). Dry nodule weights were converted to fresh weight using Sprent's (1973) ratio of 1:6.5, and nitrogen reduction was converted to acetylene reduction with the standard theoretical ratio of 1:3. We used a fixation period of 24 hours per day to convert Pate's (1962) values for total nitrogen fixed during 5 weeks to hourly rates and, therefore, they are likely underestimated. This is balanced some because Pate (1962) integrated fixation over the entire shaded period, whereas the other five reports measured acetylene reduction once at the end of the shading period. We stress that these results cannot be rigorously compared; however, the magnitude of difference between the legumes and the alder justifies properly controlled research along these lines.

TABLE 1. NITROGENASE ACTIVITY UNDER SHADED CONDITIONS

Species	% full sun	days in shade	C_2H_2 reduced per gram fresh nodule weight[a] ($\mu m \cdot hr^{-1}$)	Source
Lupinus arboreus	10	28	17	Sprent (1973)
Vicia atropurpurea	6	35	28-43	Pate (1962)
Phaseolus vulgaris	7	36	3.6	Sprent (1976)
Trifolium subterraneum	9	38	10	Gibson (1965)
Alnus glutinosa	10	10	5-9	Wheeler & Bowes (1974)
Alnus glutinosa	10	70	0.2	Gordon & Wheeler (1978)

[a] Explained in text.

Diversity exists within species as well as between broad plant groups, and it will form an important basis for future selection programs. For example, clones of two *Alnus* species, *rubra* (Gordon and Wheeler 1978) and *incana* (Huss-Danell and Lundmark, these proceedings) vary significantly in their photosynthetic efficiency and consequent reduction of atmospheric nitrogen, and cultivars of *Phaseolus vulgaris* differ in the effectiveness with which nodules compete with other plant parts for available carbohydrate (Graham and Rosas 1978).

Light Quality

Lie (1969, 1971a, 1971b) has shown that light quality strongly influences nodulation in legumes; nodulation is excellent in red, poor in blue, and strongly inhibited by far-red wave lengths. This suggests that phytochrome plays an important role in nodulation, perhaps by altering membrane permeability (Lie 1974) or by correspondingly changing ionic balance. Light below a plant canopy shifts toward the far red (Robertson 1966, Vezina and Boulter 1966, Atzet and Waring 1970), implying that shifts in light quality may be important in the silvicultural use of N-fixing plants. No research that we are aware of has explored phytochrome-dependent genetic variability in nodulation; however, other plant characteristics controlled by the phytochrome system (e.g., budset) do exhibit considerable genetic variability.

TEMPERATURE

Both nodulation and nitrogenase activity depend on temperature, but specific response shows considerable genetic variation. Pate (1976) lists 17 publications showing temperature adaptation in legumes or *Rhizobium*; not surprisingly, symbioses are usually adapted to the temperature regimes of their native locales. Mes (1959) reported that temperature dependence of nitrogen fixation differs considerably in tropical and nontropical legumes. *Casuarina cumminghamiana*, native to an area of Australia with high summer temperatures, must have at least 36°C for optimum N-fixation (Bond and Mackintosh 1975). In contrast, optimum temperatures for N-fixation range from 20° to 25°C in temperate nonleguminous plants such as *Alnus viridis*, *A. glutinosa*, and *Hippophae rhamnoides* (Benecke 1970, Akkermans 1971, Wheeler 1971). This is not always true. *Alnus rubra*, although a temperate species, has an optimum temperature of 30°C (Wheeler et al. 1979).

Rhizobia exhibit considerable temperature adaptation. Some strains are effective over a wider temperature range than others (Roughley and Dart 1969). Among several *Rhizobia* strains, only the one isolated from a cold environment could form bacteroids at 7°C (Roughley 1970). In Scandinavia, northern strains of *Rhizobia* nodulate more effectively at low temperatures than those from the south (Ek-Jandér and Fåhraeus 1971).

The nitrogenase activity of legumes appears less temperature sensitive than that of nonlegumes. *Alnus glutinosa*, *Myrica gale*, *Hippophae rhamnoides*, and *Casuarina equistefolia* all respond exponentially to temperature between 5° and 20°C; very little or no fixation occurs below 5°C (Wheeler 1971, Waughman 1977). In contrast, all temperate leguminous species studied by Waughman (1971) were active at 5°C, and two (*Pisum sativum* and *Lupinus polyphyllus*) fixed nitrogen at 2°C. Nitrogenase activity occurred at low temperatures (5°C or less) in six of eight legume species Dart and Day (1971) studied. During the winter in the Oregon Coast Range, *Cytisus scoparius* maintains 50% of optimum acetylene reduction with soil temperatures as low as 5°C (Wheeler et al. 1979) (Fig. 1). Legumes also vary considerably in response to high temperature (Dart et al. 1976), although in temperate forestry, this probably is less important than low temperature behavior.

WATER STRESS

In many temperate forests, soil dries as it warms to a temperature range conducive to nitrogenase activity. Sprent (1971) suggested that water supply has a major effect on nitrogen fixation in the field, especially in those plants with nodules close to the surface.

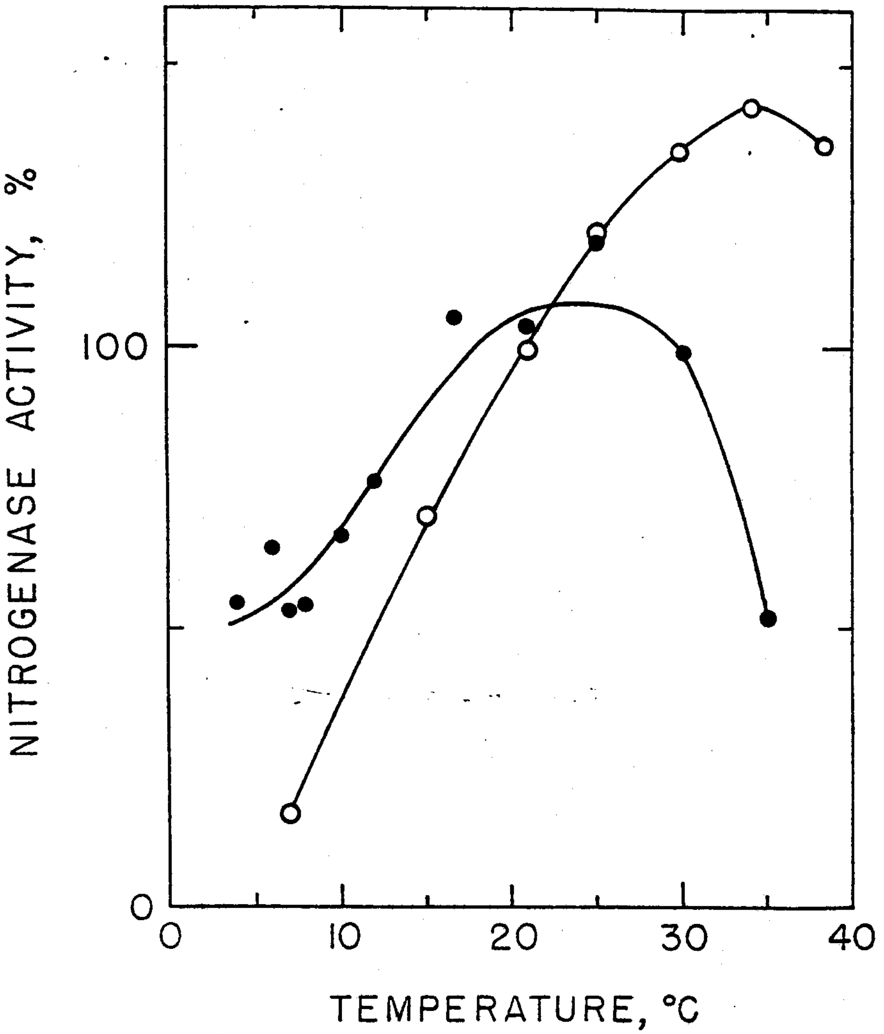

Figure 1. The effect of temperature on nitrogenase activity (acetylene reduction) in Scotch broom (•) and red alder (O). Nitrogenase activity is expressed as a percentage of that measured at 22°C (from Wheeler et al. 1979).

Water stress affects nitrogen fixation both directly through desiccation of nodules and indirectly by lowering host plant efficiency and, thus, carbohydrate supply to the nodules. Kummerow et al. (1978) speculated that the nodule biomass of Ceanothus in southern California is lower than in northern California because of lower soil moisture. Nodules will recover after desiccation if water content does not drop below approximately 60 percent of maximum fresh weight. Plants with meristematic nodules recover much more rapidly than those with spherical nodules (Engin and Sprent 1973, Sprent 1976), suggesting this may be an important trait for selection.

> established practices of legume selection for climate, soil type, or agricultural management...a second order of variation is manifest at the level of host cultivar and Rhizobium strain sufficient to justify selection for improved symbiotic yield...

For example, nodulation is determined by genetics of both the host and Rhizobium (Nutman 1969); temperature dependence of nodulation in peas is determined by one major gene in the host plant (Lie et al. 1976).

The diverse taxa present in the nonleguminous nitrogen-fixing plant group likely represent an extremely rich source of natural variation, adapted to a wide range of environments. Future research is needed to better define (1) the relationship between the operational environment and nitrogen fixation, and (2) the degree to which we can genetically manipulate symbioses to fix significant amounts of nitrogen within these environments. If natural nitrogen fertilization is to work, it will be through clever and knowledgable management of these two factors--environment and genetics--and the interaction between them.

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