Nitrogen accumulation and acetylene reduction activity of native lupines on disturbed mountain sites in Colorado

S.T. KENNY AND R.L. CUANY

Abstract

Lupines are pioneering plants in many disturbed mountain habitats in Colorado. The purpose of this work was to determine if *Lupinus argenteus, L. caudatus,* and *L. alpestris* could be useful revegetation plants in a reclamation program. Paired soil samples from 33 disturbed sites supporting native lupines were used to determine if lupines increased the nitrogen content of the soil. Soil samples collected 10 cm from lupine tap roots averaged 13.8 mg kg^{-1} more exchangeable ammonium and 2.7 mg kg^{-1} more nitrate than soil samples collected 3 m from lupine plants. Field measured acetylene reduction rates of detached lupine nodules averaged 10.0 μmol ethylene g^{-1} nodule dry weight h^{-1} for *L. argenteus* and 17.3 μmol ethylene g^{-1} nodule dry weight h^{-1} for *L. alpestris*. Soil adjacent to lupines had higher levels of inorganic nitrogen than soils 3 m from lupine plants and lupines had the ability for biological nitrogen fixation as shown by the acetylene reduction assay, suggesting that native lupines are potentially useful revegetation plants in a reclamation program.

Key Words: *Lupinus alpestris, Lupinus argenteus, Lupinus caudatus,* dinitrogen fixation, soil nitrogen, nodule activity

Soil disturbances in mountain regions require revegetation to reduce environmental degradation. Disturbed soils often have low soil fertility with nitrogen and phosphorus deficiencies most common (Bauer et al. 1978). Bauer et al. (1978) report several studies which show that annual applications of nitrogen fertilizer result in more efficient utilization of nitrogen. Legumes are plants capable of symbiotic biological dinitrogen fixation associations which convert atmospheric nitrogen into a form of nitrogen used by the plant. Establishing legumes may be beneficial to a revegetation program because of their ability to biologically fix dinitrogen and thereby possibly increase soil nitrogen levels, which could reduce the necessity for annual applications of nitrogen fertilizer.

Crockett and Becker (1976) studied the dinitrogen fixation potential of 5 native North American grassland legumes and found that pioneering legumes had potential dinitrogen fixation rates 8 times greater than the species that occupied primarily climax communities. Johnson and Rumbaugh (1986) reported that *L. argenteus* in Utah and Montana had specific nodule acetylene reduction activities between 8 and 37 μmol ethylene g^{-1} nodule dry weight h^{-1}. They also found that lupines growing on disturbed sites had larger nodule mass and higher specific nodule acetylene reduction activity compared to lupines growing in undisturbed sites.

As part of a study on the use of native lupines for revegetation programs (Kenny 1981), a study was undertaken to determine if native stands of *Lupinus argenteus* Pursh, *L. caudatus* Kellogg, and *L. alpestris* A. Nels. growing in disturbed sites in Colorado are capable of biologically fixing atmospheric nitrogen. The first part of the study measured soil nitrogen content around lupine plants and the second measured in situ acetylene reduction.

Authors are research assistant (currently assistant agronomist, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser 99350); and professor, Department of Agronomy, Colorado State University, Fort Collins 80523. Funding from the High Altitude Revegetation Committee is gratefully acknowledged. Contributed from the Colorado Agricultural Experiment Station.
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Materials and Methods

Soil Nitrogen Content

The Kjeldahl method has been used for estimating soil nitrogen accumulation near nitrogen fixing plants (Palaniappan et al. 1979). Two soil samples were collected from a 10 cm depth 10 cm from lupine tap roots and an additional 2 samples were obtained at a 10 cm depth 3 m from lupine plants. As the samples were taken, root fragments, nodules and dead plant fragments were removed. The paired samples were taken in July 1978 from 33 disturbed sites which had lupines as the dominant vegetation. All samples were air-dried and ground to pass a 2-mm sieve. The paired samples from each location were analyzed for exchangeable ammonium and nitrate by steam-distillation (Bremner 1965a) and for total nitrogen by a semimicro-Kjeldahl method (Bremner 1965b). The replicate soil sample nitrogen values were averaged, the pairs grouped by species and the differences between pairs analyzed by a paired *t*-test.

Acetylene Reduction

The reduction of acetylene to ethylene and subsequent measurement by gas chromatography of the ethylene produced is a sensitive, but indirect, measurement of dinitrogen fixation (Burris 1972). Acetylene reduction measurements enable both estimates of potential dinitrogen fixation and comparisons of potential for dinitrogen fixation among plants to be made. Twelve of the 33 sites in the soil nitrogen content study were sampled in early August 1979 when all sites were in mid-flower. Plants were excavated, and a segement of root with intact nodules was cut from the plant, trimmed to within 1 cm of the nodules, and placed into a 60-ml serum bottle. After the bottle was sealed with a rubber serum stopper, a 1-ml volume of acid-washed acetylene was injected into each bottle. The bottles were buried in the soil to simulate nodule depth and to maintain in situ temperature during incubation. Two bottles representing each plant were prepared. To determine any nonrhizobial dinitrogen fixation, bottles containing soil and root segments without nodules were similarly prepared. None of the bottles with soil or root segments without nodules had measurable ethylene production. Samples were incubated on site for 1 hour. The bottles were transferred to a chest filled with dry ice and kept frozen until returned to the laboratory for analysis. To determine any loss of ethylene during transport to the laboratory, known volumes of ethylene were injected into empty stoppered serum bottles and handled similarly to the bottles with nodules. Later analysis of the ethylene-only bottles demonstrated that no loss of ethylene occurred.

Analysis of ethylene production was by gas chromatography using a flame ionization detector. One-half milliliter volumes were withdrawn from the reaction bottle and injected into the gas chromatograph. The operating parameters included an oven temperature of 100° C, 20 cc min^{-1} N_2 carrier gas flow rate, injection port temperature of 185° C, detector temperature of 220° C, and a 1.8-m × 3.2-mm stainless steel column packed with 80–100 mesh Porapak N. Peak heights were measured and ethylene concentration in each sample was calculated by reference to an ethylene standard. After the acetylene reduction assay, nodules were

Table 1. Comparison between soil nitrogen levels in soil samples collected at a 10 cm depth 10 cm from lupine tap roots and 3 m from lupine plants growing on disturbed mountain sites in Colorado.

Species	Number of sites sampled	Exchangeable Ammonium		Nitrate		Total Nitrogen[1]	
		Soil Sample Distance from Lupine Tap Roots					
		10 cm	3 m	10 cm	3 m	10 cm	3 m
		mg kg^{-1}					
L. argenteus	24	32.7 ± 34.4	19.9 ± 25.1	7.6 ± 5.8	5.3 ± 4.6	1056 ± 1001	1016 ± 1032
Paired t-value		4.70**		6.22**		0.86NS	
L. alpestris	5	38.2 ± 9.1	20.4 ± 9.1	11.3 ± 5.1	7.7 ± 3.1	1060 ± 609	885 ± 491
Paired t-value		9.18**		2.97*		1.60NS	
L. caudatus	4	20.7 ± 10.5	9.7 ± 8.2	6.8 ± 1.4	4.6 ± 2.6	1325 ± 983	1241 ± 859
Paired t-value		4.13*		3.55*		0.96NS	

[1]Mean ± Standard Deviation. * and ** indicate significant difference between pairs for nitrogen level at P = 0.05 and P = 0.01, respectively. NS indicates no significant difference between pairs for nitrogen level.

removed from the root segments, washed, dried at 60° C, and weighed.

Results and Discussion

The nitrogen analysis results from 33 disturbed habitat soil collection sites are summarized by lupine species in Table 1. The sites with a range in elevation between 2,130 and 3,350 m were located throughout the Rocky Mountain region of Colorado. The sites were road construction disturbances, abandoned mine tailings, open meadows created by past fires, ski slope construction or logging operations, and a sand dune. Most of the soils were loam or sandy loam in texture. Lupines were the dominant vegetation with grasses and sometimes small shrubs growing at the sites. A complete description of each site is listed in Kenny (1981). The sand dune soil was the only one without measurable exchangeable nitrogen. Exchangeable ammonium levels ranged between 0 and 117.5 mg kg^{-1}, nitrate between 0 and 97.4 mg kg^{-1}, and total nitrogen between 69 and 4,808 mg kg^{-1}. The highest nitrogen levels were on a ski slope that had been fertilized. Significant differences ($P \leq 0.05$) in exchangeable nitrogen were detected between the paired samples, with exchangeable ammonium being 13.8 mg kg^{-1} higher and nitrate being 2.7 mg kg^{-1} higher in samples obtained adjacent to lupine roots compared to samples obtained at a 3-m distance. No significant differences were found between the paired samples for total nitrogen. The higher inorganic nitrogen levels around lupines could be due to either their large tap roots, which could extract nitrogen from greater soil depths than plants without tap roots, or the lupines using symbiotically fixed atmospheric nitrogen to meet their nitrogen needs, thereby extracting less inorganic nitrogen from the soil than other plants. For whatever reason, the soil around lupines had more inorganic nitrogen than soil 3 m from lupine plants, suggesting that lupines increase the level of inorganic soil nitrogen.

A site description, the field conditions at the time of sampling, and the mean ethylene production rates for 8 of the 12 sites sampled for acetylene reduction are presented in Table 2. Not all the nodules from a plant were collected, so it is not possible to calculate an acetylene reduction rate on a per plant basis. At the other 4 sites, no measurable ethylene production was recorded. The soil at these 4 sites was dry. Soil moisture for these 4 sites was estimated by feel and appearance to be about 40% of field capacity whereas soil moisture at the other sites was estimated to be 75% or more of field capacity. Dry soil conditions are known to depress dinitrogen fixation (Sprent 1973). The acetylene reduction rates ranged between 0.9 and 42.0 μmol ethylene g^{-1} nodule dry weight h^{-1}. The North Sand Hills site had the highest rate of acetylene reduction and the nodules found were individual small nodules. The nodules collected at the other locations were large interconnected masses of small nodules. Pate (1977) suggested that such perennial nodules probably exhibit low dinitrogen fixation per unit of nodule mass. The difference in size may account for the higher rates of acetylene reduction by the North Sand Hills nodules. Except for the Climax site, the highest rates of acetylene reduction were from those sites which were sampled on a sunny day. Nodules sampled on a cloudy day had low rates of acetylene reduction, which indicates that photosynthetic activity likely influences the rate of acetylene reduction, but carbohydrates stored in the plant or nodules might be available for dinitrogen fixation during times of low photosynthetic activity. Trinick et al. (1976) found that several lupine species have no diurnal rhythm for acety-

Table 2. Field measured acetylene reduction by native lupines growing on disturbed mountain sites in Colorado.

Species and site	Elevation	Site description	Sampling conditions				Ethylene production[1]
			Time	Temperature		Sky	
				Air	Soil		
	(m)		(MDT)	—(° C)—			μmol g^{-1} nodule dry wt h^{-1}
L. argenteus							
North Sand Hills	2926	Sand dune	1315	26	17	Clear	29.6 ± 7.6
Dumont Lake	2896	Roadside with grasses	0915	18	11	Clear	16.9 ± 9.4
Blue River	3048	Roadside with grasses	1230	18	14	Cloudy	7.4 ± 0.5
Tennessee Pass	3048	Clearing in lodgepole pine re-growth forest	1800	22	14	Cloudy	6.7 ± 2.4
Hoosier Pass	3109	Roadside with grasses	1200	17	10	Cloudy	4.3 ± 2.2
Climax	3353	Molybdenum tailings	0945	22	11	Clear	1.2 ± 0.4
L. alpestris							
Hideaway Park	2682	Roadside with grasses	1530	26	17	Clear	18.0 ± 0.4
Hot Sulfur Springs	2347	Roadside with grasses	1730	21	15	Clear	16.6 ± 5.5

[1]Mean ± Standard Deviation.

lene reduction; only "long periods of dull weather" and extreme shading reduced overall activity in lupine nodules. Since previous weather conditions at our sites were unknown, further study is warranted to determine any diurnal response to acetylene reduction in these lupine species. The one-time measurements reported in Table 2 indicate that additional studies are necessary to estimate the contribution of biologically fixed atmospheric nitrogen to the lupine's nitrogen requirements during the growing season since soil moisture, nodule size, and sunshine were shown to influence acetylene reduction.

Lupines are colonizers of disturbed soils and other eroded areas. *L. alpestris* and *L. argenteus* reduce acetylene and therefore have the potential for biological dinitrogen fixation. The native lupines studied increased mineral nitrogen concentrations in the soil around their roots. It is likely that their improvement of the soil's nitrogen status prepares the way for later succession. This suggests a role for lupines in revegetation and reclamation of disturbances. Plummer et al. (1968) suggested that native lupines were well adapted for game range improvements, but an end use of a reclaimed area might also include grazing by domestic livestock. Lupines contain alkaloids poisonous to livestock (Keeler et al. 1977), but the Colorado study (Kenny 1981) and a study by Davis (1982) showed significant variation between lupine collection sites for alkaloid content. In the Colorado study, lupine accessions ranged in total alkaloid content from less than 1.0 g kg^{-1} dry weight to 23.2 g kg^{-1} dry weight. It should therefore be possible to select low alkaloid germplasm for use in reclamation programs.

Literature Cited

Bauer, A., W.A. Berg, W.L. Gould. 1978. Correction of nutrient deficiencies and toxicities in strip-mined lands in semiarid and arid regions. p. 451-466. *In:* F.W. Schaller and P. Sutton (eds.), Reclamation of Drastically Disturbed Lands. Amer. Soc. Agron., Madison, Wis.

Bremner, J.M. 1965a. Inorganic forms of nitrogen. p. 1179-1237. *In:* C.A. Black (ed.), Methods of Soil Analysis Part 2, Chemical and Microbiological Properties. Amer. Soc. Agron., Madison, Wis.

Bremner, J.M. 1965b. Total nitrogen. p. 1149-1178. *In:* C.A. Black (ed.), Methods of Soil Analysis part 2, Chemical and Microbiological Properties. Amer. Soc. Agron., Madison, Wis.

Burris, R.H. 1972. Nitrogen fixation—assay methods and techniques. p. 415-431. *In:* A. San Pietro (ed.), Methods in Enzymology 24B. Academic Press, New York.

Crockett, J.J., and D.A. Becker. 1976. Nitrogen fixation in some prairie legumes. Amer. Midl. Natur. 96:133-143.

Davis, A.M. 1982. The occurrence of anagyrine in a collection of western American lupines. J. Range Manage. 35:81-84.

Johnson, D.A., and M.D. Rumbaugh. 1986. Field nodulation and acetylene reduction activity of high-altitude legumes in the western United States. Arctic and Alpine Res. 18:171-179.

Keeler, R.F., L.F. James, J.L. Shupe, and K.R. VanKampen. 1977. Lupine induced crooked calf disease and a management method to reduce incidence. J. Range Manage. 30:97-102.

Kenny, S.T. 1981. *Lupinus argenteus* and allies: potential for domestication and improvement for revegetation uses. PhD. Diss. Colorado State Univ., Fort Collins.

Palaniappan, V.M., R.H. Marrs, and A.D. Bradshaw. 1979. The effect of *Lupinus arboreus* on the nitrogen status of china clay wastes. J. Appl. Ecol. 16:825-831.

Pate, J.S. 1977. Functional biology of dinitrogen fixation by legumes. p. 473-517. *In:* R.W.F. Hardy and W.S. Silver (eds.), A Treatise on Dinitrogen Fixation. Section III: Biology. Wiley, New York.

Plummer, A.P., D.R. Christensen, and S.B. Monsen. 1968. Restoring big-game range in Utah. Utah State Dep. Fish and Game Pub. 68-3.

Sprent, J.I. 1973. Growth and nitrogen fixation in *Lupinus arboreus* as affected by shading and water supply. New Phytol. 72:1005-1022.

Trinick, M.J., M.J. Dilworth, and M. Grounds. 1976. Factors affecting the reduction of acetylene by root nodules of *Lupinus* species. New Phytol. 77:357-370.