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Field acetylene reduction rates of *Lupinus argenteus* along an elevational gradient

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FIELD ACETYLENE REDUCTION RATES OF *LUPINUS ARGENTEUS* ALONG AN ELEVATIONAL GRADIENT[1]

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ABSTRACT.—The effects of elevation and environmental parameters associated with elevation on the dinitrogen fixation rates, $N_2[C_2H_2]$, of a North American lupine species were evaluated. *Lupinus argenteus* Pursh plants growing at five elevations on Francis Peak, Utah, were sampled weekly throughout the growing season. The highest elevation site had the greatest frequency of nodulated plants. Influence of the environmental and physiological variables on fixation activity varied with elevation and the mean time of sampling. Soil moisture, nodule dry weight, and air temperature were important. Less than one-half of the observed variation in fixation rates, $N_2[C_2H_2]$, was accounted for by variation in the associated independent variables. Nevertheless this study documented that nodulated plants of *L. argenteus* were capable of actively fixing nitrogen at a range of elevations on Francis Peak.

Key words: ecology, ecophysiology, legume, nitrogen fixation, nodulation, rangeland, Rhizobium lupini.

Species of *Lupinus* are found from timberline to seacoast in much of the western United States (Phillips 1955). Their importance in the nitrogen economy of range ecosystems has not been extensively investigated. Native legumes are believed to supply biologically fixed nitrogen to the semiarid rangeland plant communities in which they occur, but their role has not been well documented (Farnsworth et al. 1978). Johnson and Rumbaugh (1981) studied the acetylene reduction rates, $N_2[C_2H_2]$, of nodules of 34 legume species growing on mountain grassland sites, big sagebrush–dominated sites, and cultivated former big sagebrush sites in northern Utah. Nodules were present and active in some species even during the driest part of the growing season. However, 13 of the 17 species of lupines native to North America that were growing in a cultivated nursery environment were not nodulated even though the seeds had been inoculated with a commercial peat-base inoculum.

Pegtel (1980) investigated *Lupinus laxiflorus* Dougl., *L. lepidus* Lindl., and *L. polyphyllus* Lindl. on undisturbed sites with serpentine and nonserpentine soils on Mt. Stuart in the Wenatchee Mountains of Washington. All root systems had nodules, and serpentine and nonserpentine populations had the same number of nodules per plant. Additional laboratory research showed that the wild strains of *Rhizobium lupini* all fixed atmospheric nitrogen but that their effectiveness was variable and generally lower than commercially available strains. *L. polyphyllus* had a higher effectivity of nodulation than *L. argenteus* and *L. lencophyllus* in a greenhouse study (Walsh et al. 1983).

McNabb and Geist (1979) measured nitrogen fixation rates ranging from 1 to 10 kg N/ha for four perennial *Lupinus* species native to the Pacific Northwest. There was a significant seasonal variation in nodule biomass of two of the species. The potential for lupine nitrogen fixation was believed to be dependent primarily on nodule biomass. Similarly, whole plant estimates of nitrogen fixation by high-altitude legumes in Montana and Utah were 5 to 15 times greater for *L. argenteus* than for native *Astragalus* and *Trifolium* species (Johnson and Rumbaugh 1986). The difference was ascribed to the greater nodule mass of the lupine.

The objective of this study was to assess the effects of changes in elevation and environmental parameters associated with elevation on nitrogen fixation rates of a native lupine species growing on a semiarid mountain rangeland in the intermountain region of the western United States.

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SITE DESCRIPTIONS AND METHODS

The five study sites were located on west or northwest aspects of Francis Peak in the Wasatch Range of north central Utah. All were within or adjacent to the Davis County Experimental Watershed, which was established in 1930 as a United States Forest Service–administered research area. The climate and vegetation of the watershed is generally representative of a large part of the low- to mid-elevation (1500 to 2800 m) mountain country in the intermountain area. Summers are short, cool, and dry. Most summer storms are convective thundershowers of less than six hours duration (Farmer and Fletcher 1971). The highest average annual precipitation of 1043 mm occurs on the upper part of the watershed. Soil moisture depletion begins immediately after snowmelt and continues to increase at a rapid rate into July (Johnston and Doty 1972). Lupines are most common on Cryoboroll soils with slopes of 8 to 30%. Associated species include *Agropyron cristatum* (L.) Gaertn., *Artemisia tridentata* Nutt., *Bromus carinatus* H. & A., *Carex* sp., *Chrysothamnus nauseosus* (Pall.) Britt., *Madia glomerata* Hook., and *Symphoricarpos* sp.

Study sites with adequate numbers of *L. argenteus* plants for sampling throughout the growing season were selected at five elevations. These populations were located at mean elevations of 1478, 2027, 2256, 2454, and 2713 m. Vegetation along the elevational gradient varied from a desert shrub association near the base of the mountain to a subalpine meadow at the highest site. One to five representative plants were chosen at approximately weekly intervals at each elevation throughout the growing season. Sampling began on 4 June 1980 at the lowest elevation and was terminated on 21 October 1980 at the highest elevation.

Leaf water potential was measured on each plant by standard pressure chamber techniques (Scholander et al. 1965), the shoot was cut at the soil level for dry biomass weight, and the root system was excavated. Excised root segments with attached nodules were used for the acetylene reduction procedures. The techniques described by Waughman (1971) and used by Westermann and Kolar (1978) and Johnson and Rumbaugh (1981) were followed with the exception of limiting the incubation period to one hour. The hour of sampling was recorded as well as the numerical day of the calendar year. Sampling was limited to the hours between 10:00 a.m. and 4:00 p.m. each day. Care was exercised to shield exposed nodules and incubation chambers from direct sunlight. Air temperature was measured with a mercury thermometer and soil temperature with a bimetallic dial thermometer with a 10-cm probe. Soil samples for moisture determinations were collected from 0–20 cm and 20–40 cm depths.

RESULTS AND DISCUSSION

Air and soil temperatures tended to be lower at the higher sites along the gradient (Table 1). Soil moisture throughout the depth of the profile tested was higher at the 2713-m site than at the four lower elevations. The 1478-m area was situated on a west-facing slope, and soils at this site were deeper than at the other locations. The lupine plants had large, woody tap roots, and nodules were located only on lateral roots. The study area at 2027 m sloped sharply toward the south and was shaded a considerable part of the day by the mountain peak. This location also was the only one of the five sites with trees that shaded the lupine plants. These shading factors probably resulted in the soil temperatures being lower than for sites immediately higher and lower on the mountain side. The 2256-m elevation appeared to be the most xeric of all the study areas. Lupines were growing intermixed with a dense population of *Artemisia tridentata*. The soils at the 2454- and 2713-m elevation sites were shallow and rocky. *L. argenteus* grew in almost pure stands with only a few plants of other forbs as associated species. Plants at the highest site were more uniform in size than at lower elevations, they had less-extensive root systems, and it was easier to find nodules than at the other four study areas.

Plants at the extreme elevations were smaller than those at intermediate elevations (Table 1). Shoot weight was more closely associated with soil moisture than with mean temperatures. The simple correlation of aboveground plant weight with soil moisture in the 20–40 cm zone was $r = -.27$ ($P < .01$). All other coefficients were of lower magnitude. Shoot weight was positively associated

TABLE 1. Environmental and physiological variables measured at five sites on Francis Peak, Utah, during 1980. Acetylene reduction activity, $N_2[C_2H_2]$, of nodulated *Lupinus argenteus* plants is expressed as μ moles of ethylene/h/g nodule fresh weight.

Variable	Site elevation (m)					Variable mean	L.S.D. (0.05)
	1478	2027	2256	2454	2713		
Number of plants (N)	67	62	21	81	76	—	—
Frequency of nodulated plants (%)	45	44	24	32	97	53	—
Air temperature (C)	22.4	22.5	20.9	18.8	13.6	17.8	3.0
Soil temperature (C)	17.7	13.8	15.1	17.5	10.7	13.8	2.3
Soil moisture 0–20 cm (g/kg)	70	63	47	76	105	87	24
Soil moisture 20–40 cm (g/kg)	74	70	93	84	125	101	26
Leaf water potential (−MPa)							
Nodulated plants	8.3	10.4	11.2	12.2	9.1	9.7	1.8
Nonnodulated plants	8.2	9.8	16.8	13.1	8.7	11.4	2.0
Shoot weight (g)							
Nodulated plants	17.0	25.7	4.5	9.8	9.5	13.5	12.5
Nonnodulated plants	15.0	50.7	41.8	16.8	12.9	27.2	20.4
Root weight (g)							
Nodulated plants	57.2	45.4	4.7	14.3	6.5	23.6	41.6
Nonnodulated plants	22.3	64.2	65.4	26.4	12.6	38.6	28.5
Nodule weight (g/plant)	0.04	1.21	0.10	0.10	0.43	0.42	N.S.
Day of year	157	167	170	213	230	202	18.7
Hour	11.1	13.3	11.2	13.6	11.4	12.0	0.6
$N_2[C_2H_2]$ activity (μ moles/h)	8.4	4.4	6.6	2.7	11.4	8.2	4.3

Difference between means required for statistical significance ($P < .05$).
NS = not significant.

with air and soil temperatures, leaf water potential, day of year, and root weight, but negatively related to the other variables.

Plants for which nodules could not be found weighed more than those with nodules. Average shoot weight of the 145 plants without nodules was 27.2 g in contrast to 13.5 g for the 162 nodulated plants. This was probably a function of plant age. Young, actively growing plants of *L. argenteus* were better nodulated than more mature plants. Nodulated plants averaged 45, 44, 24, 32, and 97% at the five sites along the gradient, with the highest elevation having the greatest proportion of plants with nodules. Differences in shoot weight of nonnodulated and nodulated plants at the highest and lowest elevations were not statistically significant ($P > .05$), whereas differences between the means for the three intermediate elevation sites were significant ($P < .05$). Nodules of *L. argenteus* are perennial, and nodule number was assumed to be relatively stable throughout the duration of the study.

Differences in root biomass also were noted, but these could have been caused by incomplete excavation of the root systems of plants for which nodules were easily found. Pegtel (1980) observed that all root systems of *L. laxiflorus*, *L. lepidus*, and *L. polyphyllus* had functioning nodules. Although roots were excavated to as deep as 100 cm in the present study and the roots carefully examined, nodules might have been overlooked or the excavation might not have been deep or extensive enough to expose all nodules. Rocky soil impeded the digging of some plants, especially at the 2454-m study area. Holter (1978) was of the opinion that determination of root biomass and number of nodules was impractical in the field. He believed that acetylene reduction rates related to these quantities would not be useful and that rates should be expressed on a per plant, per shoot, or per gram of aboveground biomass basis.

Dinitrogen fixation rates of other native perennial lupine species were lower for older plants and plants growing on droughty sites than for younger plants and plants growing on more favorable sites (McNabb and Geist 1979). Acetylene reduction activity for *L. argenteus* on Francis Peak was much greater for plants at the 2713-m elevation than at the four lower sites. The rate of 11.4 μ moles of

TABLE 2. Simple correlations (r) of environmental and physiological variables with acetylene reduction rates, $N_2[C_2H_2]$, of nodulated *Lupinus argenteus* plants. Rates are expressed as μ moles of ethylene/h/g nodule fresh weight.

Correlated variable	Study sites		
	All	Early group	Late group
Elevation (m)	0.06	0.15	−0.15
Air temperature (C)	−0.04	−0.08	0.49**
Soil temperature (C)	−0.10	−0.15	0.36**
Soil moisture 0–20 cm (g/kg)	0.16*	0.21*	−0.31*
Soil moisture 20–40 cm (g/kg)	0.20*	0.20*	−0.28*
Leaf water potential (−MPa)	−0.18*	−0.14	0.08
Shoot weight (g)	0.05	0.03	0.30*
Root weight (g)	−0.03	−0.01	−0.04
Nodule weight (g)	0.10	−0.04	0.38**
Day	0.00	−0.18	−0.13
Hour	−0.28**	−0.01	−0.04

*$P < .05$.
**$P < .01$.

TABLE 3. Rank of importance of independent variables, standardized multiple regression coefficients (Beta), and multiple correlation coefficients (R) from the stepwise multiple regression analysis of acetylene reduction rates, $N_2[C_2H_2]$, of nodulated *Lupinus argenteus* plants. Rates are expressed as μ moles of ethylene/h/g nodule fresh weight.

Independent variable	Study area grouping					
	All		Early		Late	
	Rank	Beta	Rank	Beta	Rank	Beta
Elevation (m)	6	0.12	5	0.30	7	−0.22
Air temperature (C)	7	0.15	9	0.13	1	0.28
Soil temperature (C)	11	0.01	3	−0.25	6	0.55
Soil moisture 0–20 cm (g/kg)	8	−0.08	1	0.15	10	−0.09
Soil moisture 20–40 cm (g/kg)	2	0.23	10	−0.08	8	0.29
Leaf water potential (−MPa)	5	−0.17	11	−0.02	5	−0.30
Shoot weight (g)	4	0.09	7	0.10	3	0.27
Root weight (g)	10	0.01	8	0.05	4	−0.36
Nodule weight (g)	3	0.13	4	−0.10	2	0.41
Day	9	0.06	6	−0.20	11	−0.01
Hour	1	−0.22	2	−0.18	9	−0.10
R	.38		.36		.74	

ethylene per hour per g of fresh nodule weight at the 2713-m elevation was 52% greater than the average rate of the 88 plants from the lower elevations.

Simple correlations (Table 2) indicated that soil moisture, leaf water potential, and hour of sampling were significantly ($P < .05$) associated with reduction activity. An examination of the means in Table 1 led to grouping of the study areas into two categories according to mean time of sampling. Nodules from the 2027- and 2454-m elevations were collected later in the afternoon than those from the 1478-, 2256-, and 2713-m areas. The early group mean sampling time and standard error were 11.3 ± 0.01 hours, but those of the late group were 13.4 ± 0.01 hours, or more than 2 hours later in the day. Soil moisture was the only factor correlated with activity of nodules from plants in the early group. Air and soil temperatures, shoot and root weights, as well as soil moisture, were correlated with activity of nodules from plants in the late group. *Trifolium pratense* L. and *Medicago lupulina* L. fixed very little nitrogen when soil moisture was reduced to 8% or less (Holter 1978). Table 1 shows that soil moisture averaged less than that in the surface to 40 cm depths at the four lower study areas on Francis Peak.

Causal relationships among the variables were examined by forward stepwise multiple regression. When all available data were considered, hour of day at which the plants were sampled and soil moisture below 20 cm were the two most influential independent variables determining acetylene reduction rates (Table 3). They jointly generated a multiple

correlation coefficient, R, of .27 and explained 7% of the variation in per plant nitrogen fixation activity, $N_2[C_2H_2]$. Peak activity occurred from 11:00 a.m. to 12:00 noon when the rate averaged 12.0 μ moles of ethylene per hour per g nodule fresh weight. Samples taken the preceding hour produced a reduction rate of only 7.2 μ moles. Mean rates then dropped to 8.4, 5.0, 3.1, 2.6, and 3.5 μ moles for subsequent one-hour intervals in the afternoon.

Hour of sampling and leaf water potential were positively and significantly ($P < .05$) associated, $r = .47$. The impact of hour on acetylene reduction activity probably was due to increased drought stress as the day progressed. This seemed to be particularly true at the 2713-m elevation, which was sampled at four times. One plant sampled at 10:00 a.m. had a leaf water potential of −0.96 MPa, whereas 47 plants sampled at 11:00 a.m. averaged −0.78 MPa. Drought stress then increased markedly, as the 19 plants sampled at 12:00 p.m. had an average leaf water potential of −1.13 MPa. The stress increased still further for the 7 plants sampled at 1:00 p.m., which averaged −1.24 MPa.

Air temperature changed from a low of 15.4 C at 11:00 a.m. to a high of 26.7 C at 4:00 p.m. Air temperature was positively correlated with acetylene reduction activity and was the single most important independent variable when data from the late grouping of study areas were considered (Table 2). Nodule weight was the second most influential variable for the late group and also was important for the early group (Table 3). This was true even though the activity was expressed on a gram nodule fresh weight basis. The degree of hydration of nodules apparently played a role in the nitrogen fixation rates of these plants.

The other independent variables were of lesser value in predicting nitrogen fixation activity. Shoot and root biomass were strongly related to acetylene reduction in four legume species studied by Holter (1978). These were of lesser importance in this study of *L. argenteus*. However, variation in the 11 independent parameters measured and listed in Table 3 explained only 14% of the observed variation in activity. The remaining 86% of the variation in fixation was caused by factors not accounted for in this study, but which must have been, in some way, related to the time of sampling. Separation of the study areas into two groups on the basis of mean sampling time resulted in much stronger predictive relationships for the late group. The multiple correlation coefficient was $R = .74$ (Table 3), and the predictive function explained more than half the observed variation in acetylene reduction rates. For plants in the early group, factors other than those measured in this study profoundly influenced the dinitrogen fixation rates of *L. argenteus* plants on the slopes of Francis Peak. Although acetylene reduction rates of *L. argenteus* could not be accurately predicted from the variables evaluated in this study, plants of *L. argenteus* were nodulated and capable of reducing acetylene along an elevational gradient on Francis Peak.

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