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ECOLOGICAL ASPECTS OF NITROGEN FIXATION BY *PURSHIA TRIDENTATA*

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SUMMARY

This study examines several aspects of nitrogen fixation by *Purshia tridentata* (Pursh) D.C., a rosaceous shrub widespread in the Central Oregon pumice region, especially as an understory species in *Pinus ponderosa* and *Pinus contorta* forests. Acetylene reduction was used to assay nodule activity in both field and greenhouse plants. The maximum rates were observed at 20°C, although summer soil temperatures were frequently around 15°C, at which a much lower rate was observed. Acetylene reduction by excised nodules was linear for 5 h and then slowly declined, finally ceasing after 19 h. Nodule activity declined in water stressed plants, essentially ceasing in plants with xylem pressure potentials below −25 bars.

Field studies at five sites revealed that nodule activity began in mid-May or early June when soil temperature at 20 cm increased to above 10°C. Activity began later and remained lower until July 20 in plants located under *Pinus contorta*, probably because of the cooler temperatures at this site. Nodule activity at all sites was maximum in June and July. In late July, nodule activity declined sharply, corresponding with moisture stress readings in the −25 bar range. Acetylene reduction rates declined sharply during the night; this decline was even more severe late in the season.

Only 46 per cent of Purshia plants were nodulated. Several possible explanations for this low nodulation are discussed, but the primary reasons appear to be low soil temperature and unfavorable moisture conditions. Previous speculations that Purshia may contribute significant amounts of nitrogen to the ecosystems in which it occurs are disputed using estimates based on seasonal acetylene reduction rates and a determination of nodule biomass at one site. The estimated nitrogen accretion rate was only 0.057 kg N/ha·yr.

INTRODUCTION

The significance of nitrogen fixation by non-legumes has received increasing attention in recent years. The interest centers in part on

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the ability of these plants, in some instances, to contribute large amounts of nitrogen to other members of the biotic community and the subsequent potential for increased productivity of these other species.

Purshia tridentata (Pursh) D.C. (bitterbrush or antelope brush) is in one of three rosaceous shrub genera reported to bear nodules. The other nitrogen fixers in this family are some species of Dryas and Cercocarpus. Nodulation of Purshia was not reported until 1958 when Wagle[36] noted the presence of nodules on both *P. tridentata* and *P. glandulosa*, the only two species in this genus. Nodulated *P. tridentata* plants grew well in low nitrogen soil while Purshia plants without nodules showed signs of nitrogen deficiency[37]. Nitrogen fixation ability of *P. tridentata* was confirmed using ^{15}N (Ref. [38 39]).

Purshia tridentata is widespread in open range and as an understory in *Pinus ponderosa* and *Pinus contorta* forests. The range of the species includes about 138 million ha in 11 western states and southern British Columbia[18]. The general autecology of Purshia has received attention in several reports [24 34].

Although the potential ecological significance of nitrogen fixation by Purshia is occasionally mentioned[21 22] the subject has remained unexplored. This is surprising because Purshia is one of the few non-leguminous nitrogen-fixing plants with any direct economic potential. It is highly desirable as a browse species and is used heavily by both deer and domestic livestock[9 11 20]. Purshia has a fairly high nutritional value, even in winter months, and, of several species tested, was found to be the only natural food capable by itself of sustaining caged deer[5]. It is possible that Purshia's nitrogen fixing capability enhances its nutritional value.

Purshia's widespread occurrence in pine forest suggests that it may be important in the nitrogen budget of these areas. Dickson and Crocker[10] observed nitrogen accretion rates of 56 kg N/ha·yr in young stands of *Purshia tridentata* and *Pinus ponderosa*. At that time, the nitrogen fixing ability of Purshia was not known. The authors were puzzled by this high accretion rate and by the apparently healthy appearance of the pines and suggested that free-living nitrogen-fixing bacteria were involved. Silvester[30] has speculated that Purshia was responsible.

The present study is an attempt to evaluate nitrogen fixation by *Purshia* in the field and to determine the effect of temperature and moisture on the activity of the nitrogen-fixing enzyme system. Variations due to season and time of day are also examined.

DESCRIPTION OF STUDY SITES

Five study sites were selected in the central Oregon pumice region. Soils in this region are a mixture of fairly recent aeolian pumice and glacial outwash.

Total nitrogen levels are modest (.105–.160 per cent) under Purshia communities and carbon-nitrogen ratios are surprisingly high[12]. Due to the abundance of small pores, these soils have favorable moisture retaining properties.

The Cascade Range immediately to the west removes much of the moisture from air masses moving inland from the Pacific. As a result precipitation in the area is slight. Most precipitation occurs as snow. Summer rainfall accounts for only about 12 per cent of the yearly total, and occurs mostly as light, scattered showers[40] which are ineffective for most plant growth. Generally air temperatures show strong diurnal variation with cool nights and warm days in the summer. Summer frosts are not uncommon. Of the five study sites, three were located in the *Pinus ponderosa* zone, one in the *Pinus contorta* zone, and one in a shrub stand dominated by *Artemesia tridentata*.

TABLE 1

Temperature (°C) at study site 1, 3, and 4

Date	Study site	Air temperature				Soil temperature Average at 20 cm
		Average daily high	Average daily low	High	Low	
5/17 to 6/2	1	na*	na	37.0	−6.5	13.0
	3	23.0	−1.0	33.0	−13.0	10.0
	4	21.0	0.5	34.5	−8.0	9.5
6/8 to 6/18	1	na	na	35.0	−3.5	14.0
	3	27.0	0.0	34.0	−7.0	13.0
	4	25.0	3.0	33.0	0.0	9.5
6/19 to 7/2	1	19.0	3.0	24.5	−3.5	13.0
	3	20.0	3.0	24.5	−3.0	12.0
	4	18.0	0.5	24.5	−3.5	9.5
7/3 to 7/20	1	na	na	37.0	−0.5	14.5
	3	31.0	8.5	38.0	0.0	13.0
	4	30.0	8.5	37.0	0.5	13.0
7/21 to 7/30	1	31.5	5.0	37.0	3.0	16.0
	3	32.0	5.0	39.5	1.5	15.0
	4	31.0	4.0	38.0	−3.0	15.0
7/31 to 8/13	1	na	na	35.0	na	16.5
	3	30.0	−0.5	34.5	−5.0	14.5
	4	30.5	2.0	33.5	−0.5	15.0
8/14 to 8/31	1	24.0	3.5	33.0	−1.5	14.5
	3	23.5	1.1	33.0	−6.1	12.0
	4	21.5	3.0	31.5	−3.0	13.5

* Values not available due to thermograph malfunction.

Sites in the *Pinus ponderosa* zone lay within the Deschutes National Forest along a 24 km transect west from the town of Sisters. The elevation in this area drops gently from 1030 m to 980 m from west to east. Air and soil temperature were measured with recording thermographs at two stations within this area, air temperature at 60 cm under an A-frame shelter, and soil temperature at a depth of 20 cm (Table 1). Sisters has an average January temperature of −0.5°C and an average July temperature of 17.5°C[40]. Soils are generally frozen in winter months and in 1975 remained so in spots until mid-April. Soil temperature, measured at 20 cm, did not exceed 17°C during the summer of this study.

The fourth study site was in the *Pinus contorta* zone. This tree is considered to be a topoedaphic climax species in broad, level depressions in a large area in the south central Oregon pumice region[4]. *Pinus contorta*, which is highly cold tolerant, occupies the bottom of these depressions, while the dominant changes to *P. ponderosa* just a few meters higher[4]. The study area was located approximately 40 km southwest of Bend. Temperature measurements were made as described previously (Table 1).

The fifth study area was located on the western edge of the high desert of eastern Oregon, near the base of Pine Mt. The vegetation was dominated by *Artemesia tridentata*. No temperature records are available; however, the open nature of the stand and the higher elevation suggest cooler night and warmer day temperatures than those recorded under the ponderosa pine. Further information on each of the individual study sites is provided in Table 2. The values for frequency and coverage of Purshia shown in this table were determined on 25 0.2 × 0.5 m microplots[9] at each site.

TABLE 2

Characteristics of study sites

	Study site No.				
Dominant species	1 *Pinus ponderosa-Abies grandis*	2 *Pinus ponderosa*	3 *Pinus ponderosa*	4 *Pinus contorta*	5 *Artemesia tridentata*
Per cent frequency of Purshia	60	60	44	76	4
Per cent coverage of Purshia	19.1	25.2	15.7	20.4	1.5
Elevation	1025 m	1010 m	1005 m	1315 m	1450 m
Precipitation	1200 mm[26]	690 mm[26]	510 mm[26]	510 mm[31]	310 mm[34]
Location	SW¼Sec 31 T13S R9E	NE¼Sec 26 T14S R9E	NW¼Sec 17 T15S R10E	SE¼Sec 19 T20S R10E	NE¼Sec 29 T20S R16E

MATERIALS AND METHODS

General

Acetylene reduction was used to measure nodule activity of both greenhouse and field plants. 8.8 ml vials containing approximately 8–60 mg fresh weight of nodules were sealed with a rubber serum stopper, 0.88 ml of the atmosphere withdrawn with a syringe, and an equal amount of acetylene injected to give a final concentration of 10 per cent. Rubber stoppers were discarded after one use. Acetylene was generated immediately before use by reacting CaC_2 with water.

Incubation times varied from 40–65 min, but all acetylene reduction values were converted to a 60 min basis by linear extrapolation. Incubation temperature was controlled in the laboratory by submerging the vials in a water bath and in the field by burying the vials in the soil at a depth of 20 cm.

Gas samples from laboratory work were immediately analyzed on the gas chromatograph[14]. Gas samples from field work were collected in evacuated blood sample tubes ('vacutainers')[28] and returned to the laboratory for analysis. This required the use of factors to correct for differences in atmospheric pressure between the test sites and the laboratory, and also for slight background levels of ethylene found in the vacutainers.

All nodule weights were determined as fresh weight. Nodules from field samples were placed in formalin-acetic acid-alcohol (FAA) until they could be weighed. Before weighing, all senescent nodule branches and woody tissue proximal to active branches were removed.

Effects of temperature

To examine the effect of incubation temperature on acetylene reduction, approximately 30 nodulated plants, 12 to 40 cm tall, were removed from Study Site 2 in February. The plants were transplanted into pots containing the pumice soil from this site and placed in the greenhouse. All plants were watered twice weekly and allowed to grow 2–3 months before acetylene reduction was measured. To determine the response of nodule activity to different temperatures, unsealed vials containing nodules were partially submerged in a water bath at 20°C for 10 min to equilibrate. The vials were sealed and acetylene introduced. After 30 min, gas samples were withdrawn. Serum stoppers were removed and the vials containing the same nodules placed in another water bath at 5, 10, 15, 20, 25, 30, 35, or 40°C. Vials and nodules were allowed to equilibrate to the 'test' temperature for 10 min with frequent flushing with air to remove traces of the previous gas mixture. The vials were again sealed, acetylene was injected and after 30 min ethylene production was measured. Ethylene production at the 'test' temperature was expressed on a basis relative to that during the initial incubation at 20°C. Recovery was examined by returning the vials and nodules to 20°C, equilibrating, flushing, and measuring acetylene reduction as before.

A brief comparison of relative acetylene reduction rates of field plants was undertaken by incubating 6 samples each of freshly collected nodules in water baths at 15°C and 20°C and collecting gas samples in vacutainers.

Time course

A time course study was carried out on greenhouse plants that had been treated as the plants used in the temperature studies. Nodules were excised at approximately 9:00 am and incubated in a vial with acetylene at 22°C. Gas samples were withdrawn at time intervals of 1 — 4 h up to 24 h. At each sampling time two 0.55 ml samples were removed from the vial and 1.1 ml of gas mixture containing 10% acetylene re-injected to maintain normal atmospheric pressure. Values for ethylene production were calculated to include the amount of ethylene that had been removed in all previous samples.

Effects of plant moisture stress

The effects of plant moisture stress on acetylene reduction were examined on both greenhouse plants and field plants. Approximately 50 nodulated plants were removed from Study Site 2 and treated as described for the plants used in the temperature experiments. All plants were watered twice a week for 3 weeks. After this time new leaves were fully emerged and most plants were growing rapidly. The plants were then divided into two groups with approximately equal size distributions. One group was watered as before but the other received no water. After 1, 6, and 14 days acetylene reduction measurements were made at 22°C on 3 to 12 plants in both groups. Water stress was determined in each case with a pressure chamber[29]. To examine recovery from water stress, the xylem pressure potential of 4 additional plants from the unwatered group was measured on day 14 but nodule activity was not examined. These plants were then returned to the greenhouse and watered regularly. Their xylem pressure potential and acetylene reduction activity were measured after an additional 12 days.

Field moisture stress measurements were made throughout the growing season on most plants sampled for acetylene reduction. These readings were taken mostly from 9:00 am to 2:00 pm, but predawn measurements also were made at Study Site 2. All pressure chamber readings were taken on plants that had been uprooted to sample nodules, immediately after the nodules were removed.

On July 30, approximately 60 plants on Study Site 2 were irrigated. These plants were 5–12 years old and in groups of 5–10 individuals resulting from old seed caches. Each group received about 8l. Predawn and midday acetylene reduction and water stress measurements were made on these plants 2 days later and compared to nearby non-watered plants. Similar measurements were made on August 13.

Seasonal and diurnal variation

The variation in acetylene reduction rates throughout a growing season was examined by sampling at 2–4 week intervals from April to September. During each visit, acetylene reduction trials were conducted on 6–8 plants.

Mostly small plants, 10–30 cm tall, were selected. Most plants were 3–12 years old. No more than 2 plants were used from the group resulting from any one seed cache. Acetylene vials were buried in the soil at a depth of 20 cm to control temperature. Initially acetylene reduction measurements were taken any time from 8:00 am to 6:00 pm, but after mid-June, when the extent of diurnal variation was discovered, all measurements were made between 9:00 am and 2:00 pm.

Diurnal variation in acetylene reduction rates was determined at site 2 from mid-June to mid-August by comparing rates on nodules removed at different times of the day. Usually this involved measurement shortly before dawn (3:00–4:00 am) and midday (11:00 am–2:00 pm). In one case additional measurements were taken at 7:30 am and 7:30 pm.

Evaluation of Purshia nodulation

The percent of Purshia plants with nodules was determined by carefully excavating 364 plants from Study Sites 1–4. Any plant whose roots appeared badly damaged or severed was discarded. Each acceptable plant was numbered and the presence or absence of nodules recorded. Plants with only senescent nodules were not considered to be nodulated. A short segment near the base of each stem was clipped off and the annual rings counted under a dissecting microscope.

An evaluation of nodule biomass was conducted at Study Site 2. A small plot (8.12 m^2) was selected at random in an area with high Purshia coverage and all Purshia plants in the plot were carefully excavated. All nodules were excised and weighed.

Greenhouse seedlings were grown from seed from 12 different sources from several Western states. Germination was promoted by soaking seed in a 10% thiourea solution for 5 min[19]. After drying, the seeds were planted in 20 cm plastic pots containing a mixture of wet pumice soil collected from two areas in central Oregon where nodulated Purshia occurs. Soil in all pots was watered to saturation once weekly. After one month, the seedlings were thinned to 6 or fewer per pot and a nitrogen-free fertilizer solution[13] was applied once. After 3 months, at least five seedlings from each seed source were harvested, acetylene reduction at 22°C measured on intact root systems, and per cent N of foliage determined using the Kjeldahl technic[8].

RESULTS AND DISCUSSION

Effects of temperature

The results of comparative acetylene reduction measurements made on the same nodules at two different temperatures are presented in Fig. 1. This procedure was adopted because of the limited number of greenhouse plants available and the high variability in acetylene reduction activity between nodules from different plants.

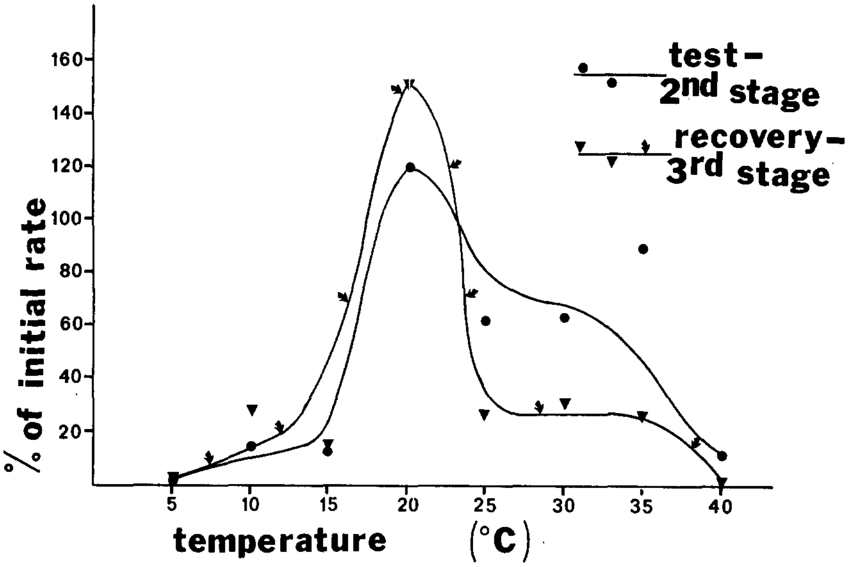

Fig. 1. Response of acetylene reduction rates to temperature treatments. Arrows distinguish the recovery response line. See text for explanation. Each point is the mean of 3 trials.

The rate for nodules held at 20°C for all three stages increased after each 30 min period. The rate during the second stage (the test stage) at 20°C was 119 per cent of the initial rate. The rate during the third (recovery) stage increased to 151 per cent. Nodules treated identically in the first stage at 20°C and then incubated in the second stage without the addition of acetylene showed no release of ethylene. These increases in ethylene production are puzzling and may be due to the release of bound ethylene in the presence of acetylene or a delay in temperature equilibration.

The results indicate a sharp maximum of acetylene reduction around 20°C. The rate at 15°C was only 12.1 per cent of the initial rate at 20°. A similar drop is evident in field grown samples (Table 3). Reversing the treatments for greenhouse plants (15°C first followed by 20°C) also resulted in a much higher rate at 20°C. This sudden drop from 20 to 15°C cannot be explained by enzyme kinetics. The apparent Q_{10} from 10° to 20°C is too large – greater than 8.2. Cold injury may account for this drastic decrease. Although 15°C is in the normal range of soil temperature, the sudden 5°C shift may have some harmful effect. Perhaps a more plausible explanation in-

TABLE 3

The effects of incubation temperature on acetylene reduction by nodules from plants at study site 2 on June 21

Temperature	Acetylene reduction (μmoles/g·h)*		
	Mean	High	Low
20°C	1.32	3.17	0.27
15°C	0.04	0.09	0.01

* 6 replicates at each temperature.

volves reduced gas diffusion in and out of the nodule brought about by cooler temperatures and condensation of water vapor on the nodule surface. A slight film of water around nodules greatly reduces acetylene reduction rates[17].

The decrease in nodule activity at higher temperatures may be due to heat inactivation of enzymes or increased drying of the nodule.

The effects of temperature on Ceanothus nodule activity have been examined using nitrogen 15[38]. The optimum temperature was found to be around 23°C. The rate at 15°C was 37.5 per cent of that at 23°C. Unfortunately, no assays were made between 15° and 23°C. Akkermans[1] reported optimum temperatures for acetylene reduction by Alnus nodules near 20°C. Wheeler[43] reported a value near 25°C also for Alnus. The decline in rates at 15°C was much less severe in both cases than that observed for Purshia.

It seems likely that the rates measured at 15°C are artificially low. Soil temperature at the study sites was generally around 15°C throughout the summer, but substantial acetylene reduction was measured in vials buried in the soil at this temperature. Nevertheless, soil temperature appears to be below the optimum for nitrogen fixation. Cool soil temperature may be responsible for the delay until late May before nodule activity begins (see later sections), especially at Study Site 4 which is located in a cold air drainage basin. Soil temperatures were 2 to 6°C cooler in the spring and early summer than at other study sites (Table 1). Nodule activity began later and was generally lower throughout the summer (Fig. 4).

Time course

Acetylene reduction was linear through the first 5 h and then declined slowly, essentially ceasing after 19 h (Fig. 2), presumably

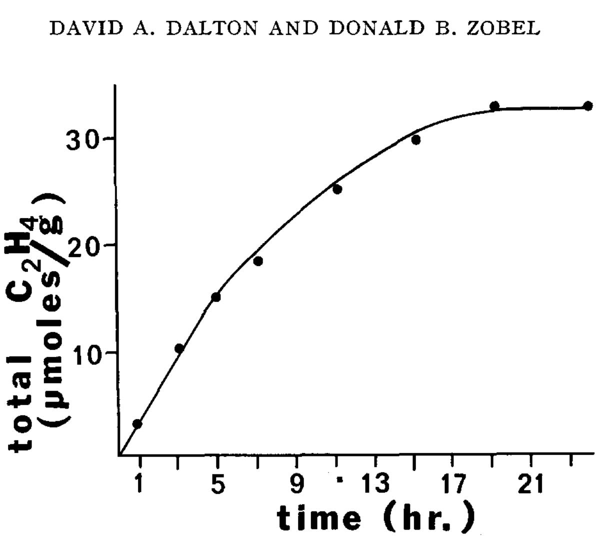

Fig. 2. Time course of acetylene reduction by excised *Purshia tridentata* nodules. Mean of 3 trials.

due to the exhaustion of carbohydrate reserves. The average rate during the first 5 h was 3.5 µmoles C_2H_4/g·h. Varying incubation times were used throughout this study and the initial linearity of acetylene reduction allowed for the easy conversion of all results to a standard 60 min basis.

Effects of plant moisture stress

Greenhouse plants under low to moderate water stress had highly variable rates of acetylene reduction that were not correlated well with water stress (Fig. 3). Extremes ranged from 0 to 10.5 µmoles ethylene/g·h with the average being 3.69 for all plants above —20 bars, 2.25 for plants between —20 and —25 bars, and 0.11 for all plants below —25 bars. Recovery of highly stressed plants from this low activity was good after 12 days with regular watering, provided the initial stress was not too severe (Table 4). One plant with a xylem pressure potential below —68 bars lost many of its leaves and was the only plant in which nitrogenase activity did not recover, although it might have eventually.

Midday moisture stress measurements from all sites, and predawn readings from Study Site 2, are presented in Table 5. Water stress corresponded somewhat with differences in annual precipitation between the sites. Moisture stress variation due to different sampling

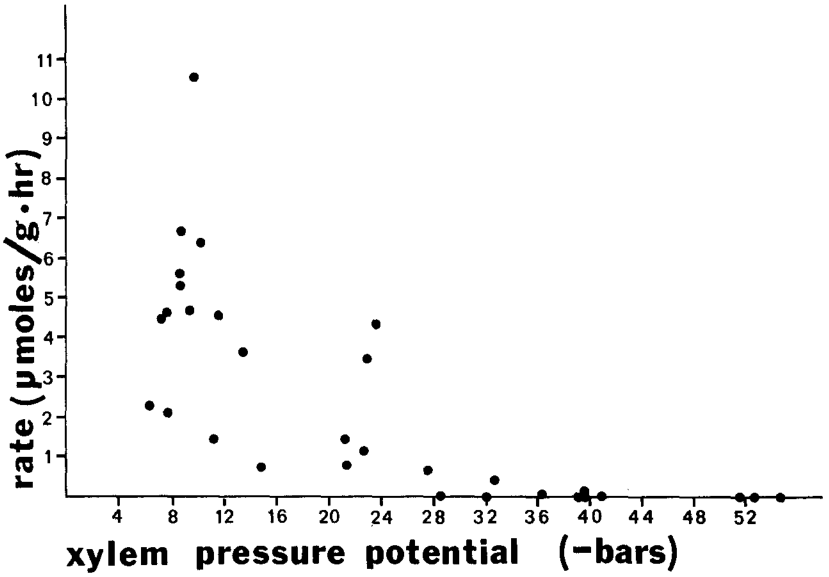

Fig. 3. Acetylene reduction by nodules from plants at different moisture stress levels. Field plants were transplanted into the greenhouse for 3 to 6 weeks.

times during the day undoubtedly accounts for some irregularities, but the general drying trend throughout the growing season can be seen. The drop in acetylene reduction rates in late July (see next section) corresponds roughly with the water stress in the —25 bar range which reduced nodule activity in greenhouse plants. The predawn readings of xylem pressure potential also began to decrease at

TABLE 4

Nodule activity by greenhouse plants subjected to water stress and then provided with water for 12 days

Xylem pressure potential (—bars)		Acetylene reduction
Initial	Final	(µmoles/g·h)
45.5	10.9	2.96
44.4	11.3	3.05
57.6	15.4	2.29
>68	19.7	0

TABLE 5

Xylem pressure potential (−bars) at each study site. Data for Sites 1, 3, 4, and 5 are for midday (900–1400 h). Means of 6–8 samples

Date	Study site					
	1	2-predawn	2-midday	3	4	5
5/17–18	10.9	nd*	7.2	8.9	6.7	12.6
6/7–8	13.5	nd	12.8	9.7	11.3	15.4
6/18–19	12.5	nd	12.5	8.4	rain	nd
7/1–2	14.5	6.6	16.0	13.1	5.9	15.9
7/20–21	13.6	6.0	23.7	20.0	24.8	nd
7/30–31	13.7	11.3	26.5	24.6	22.4	22.2
8/13–14	17.1	15.2	24.1	26.0	19.4	nd
9/4–5	20.7	14.4	23.7	24.2	19.5	nd

* Not determined

this time, indicating failure of the plants to recover from daytime water loss. The continuation of some nodule activity into September may have been due to the unusually wet summer in 1975. August precipitation exceeded 1.5 cm on all sites except possibly no. 5.

Irrigated field plants showed a significant increase in both midday and predawn acetylene reduction only 2 days after watering (Table 6). At this time, predawn xylem pressure potentials were slightly higher, but midday readings were actually slightly lower on the watered plants. After an additional 2 weeks, the same water stress

TABLE 6

Comparison of water stress and nodule activity of irrigated and non-irrigated plants in the field. Water was applied on only one day (July 30)

Date	1 August		13 August	
Plant treatment	Irrigated	Non-irrigated	Irrigated	Non-irrigated
Xylem pressure Potential (−bars)*				
Predawn	9.2	11.3	13.4	15.4
Midday	29.1	26.5	30.3	24.1
Acetylene reduction (μmoles/g·h)*				
Predawn	0.22	0.05	0.03	0.04
Midday	0.67	0.28	0.22	0.44

* Means of 6 samples

patterns were repeated, with higher predawn and lower midday readings on the irrigated plants. Nodule activity of watered plants had declined sharply after this 2 week period. Predawn acetylene reduction rates were very low, and midday rates were even lower than those of the unwatered plants. The lower midday xylem pressure potentials and the lower acetylene reduction rates of the watered plants were unexpected. The addition of water may have interrupted the normal processes of water conservation, such as stomatal closure, to the point that more severe water stress resulted. The plants appeared to suffer uncontrolled water loss during the day with good recovery at night due to the extra soil moisture. At first, perhaps there was enough water to compensate for the loss, but eventually the water stress resulted in a decrease in acetylene reduction rates below those of unwatered plants, in which normal water conservation had not been disrupted.

The reduced nitrogen-fixing activity of nodules brought about by water stress is probably related to reduced carbohydrate supply. The reserve of carbohydrate, primarily sucrose, in the nodules drops rapidly in Alnus plants placed in the dark[43]. This drop in sucrose levels corresponds to a sharp decrease in acetylene reduction rates. The same effects might be achieved by drought induced stomatal closure which would halt photosynthesis and the flow of carbohydrates to the nodules.

This explanation fits well with the data for predawn moisture stress (Table 5). After July 20, inability of the plants to replenish their water supply at night probably resulted in stomatal closure and, consequently, reduced nodule activity.

Water stress leads to structural changes within legume nodules, and if severe enough, will result in the nodule being shed[32]. Reduced acetylene reduction rates resulting from water stress have been reported[23 32] and attributed to reduced respiration in the nodule due to structural changes that reduce O_2 diffusion[25]. Rates in moderately stressed nodules could be completely restored by increasing the pO_2, but severely stressed nodules recovered only partially. There are considerable structural differences between legume and non-legume nodules but it is possible that water stress may also reduce O_2 diffusion in non-legume nodules as well. The critical region appears to be the peripheral uninfected cortex[33] which is similar in both types of nodules.

Seasonal and diurnal variation

Nodules sampled from early February to mid-April failed to produce any detectable ethylene. During this period soil incubation temperatures generally ranged from 0 to 4°C. Nodules incubated at room temperature for up to 48 h also remained totally inactive.

The first signs of nodule activity appeared on May 18 when small amounts of ethylene were produced by some nodules from Study Sites 2, 3, and 5 (Table 7). At this time leaves were fully emerged, but anthesis was not reached for another 7–10 days. By June 7, nodules from Site 1 showed moderate activity, but those from Site 4 produced only traces of ethylene (Table 7).

Throughout June some nodules from each of the 5 sites retained very low or no activity, but average values increased over those in May. Generally, the individual nodules with the highest activity (some as high as 6–8 μmoles C_2H_4/g·h) were also measured in June, making this the month the period of greatest variability between nodules. By the end of July, nodule activity at all sites decreased sharply (Table 7). This low rate continued throughout August and early September.

The late starting date for nodule activity is apparently due to the cool temperature encountered in this region. Substantial activity was detected only after soil temperatures exceeded 10°C. The delay in initiation of nodule activity at Study Site 1 and especially Site

TABLE 7

Seasonal patterns of midday acetylene reduction rates (μmoles C_2H_4/g·h) Means of 6–8 samples

Date	Study Site				
	1	2	3	4	5
April 19–20	0	0	0	0	0
May 17–18	0	.49	.13	0	.01
June 7–8	1.32	2.34	2.17	.01	4.97
June 18–19	4.63	3.11	.68	.88	nd*
July 1–2	2.11	2.24	5.11	1.12	1.33
July 19–20	4.14	1.80	1.00	1.83	nd
July 30–31	.31	.28	1.15	.68	.34
August 13–14	.51	.44	.25	.22	nd
September 4–5	.47	.28	.04	.38	nd

* Not determined

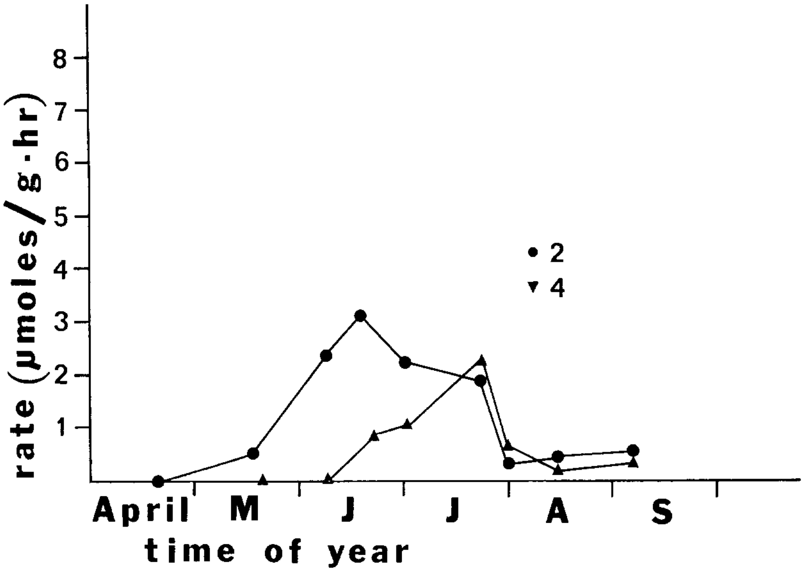

Fig. 4. Comparison of seasonal patterns of midday acetylene reduction rates at Study Sites 2 and 4. Means of 6 to 8 samples.

4 is tied closely to the lower soil temperatures in May and June at these sites (Table 1). The cool temperatures at Site 4, and to some extent Site 1, were due to cold air drainage. Due to the open nature of the canopy on Site 3, temperature extremes frequently exceeded those of other sites, but moderately warm soil temperatures were maintained and nodule activity began by mid-May.

Soil temperatures at Study Site 4 remained substantially cooler until late July. Consequently, acetylene reduction rates were lower and never reached the high levels measured on other sites (Fig. 4). After July 20, when soil temperature at this site did finally reach levels comparable to those of other sites, nodule activity was short-lived due to moisture conditions (Table 5) and by July 30 activity had considerably declined at this site as well as the others.

This decline in rates at the end of July at all sites corresponds to xylem pressure potential readings in the range of —25 bars (Table 5) which were observed to greatly reduce rates in greenhouse plants.

Strong diurnal patterns occurred in nodule activity (Fig. 5). Acetylene reduction rates were low during predawn hours. Rates began to climb shortly after sunrise, eventually reaching a midday maxi-

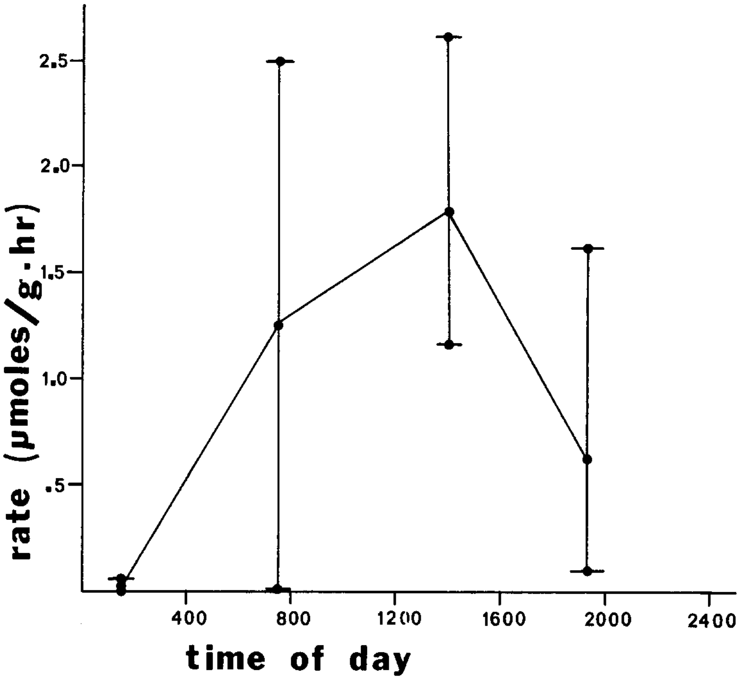

Fig. 5. Variation in acetylene reduction rates of nodules sampled at several times during 24 h. All determinations were made on June 20–21 at Study Site 2. Vertical lines indicate the extremes observed at each determination. Means of nodules from 6 to 8 plants.

mum. Activity declines in the late afternoon. In addition, diurnal patterns showed a seasonal change. Predawn rates declined much more rapidly from June to August than did midday rates when the two rates are comparable on a percentage basis (Fig. 6).

Diurnal variation in nodule activity has been reported for soybeans[3] and for 4–5 month old greenhouse seedlings of Myrica and Alnus[42]. These fluctuations, at least in Alnus, are due to the depletion of carbohydrate levels in the nodules in the dark[43]. Akkermans[1], however, observed no substantial diurnal fluctuations in field-grown Alnus and Hippophae. The percent of predawn acetylene reduction rates relative to midday rates (32.8) of Purshia nodules in early June compares well with the values provided by these other reports. The substantial decline in predawn rates later in the season suggests that carbohydrate levels were also decreasing throughout

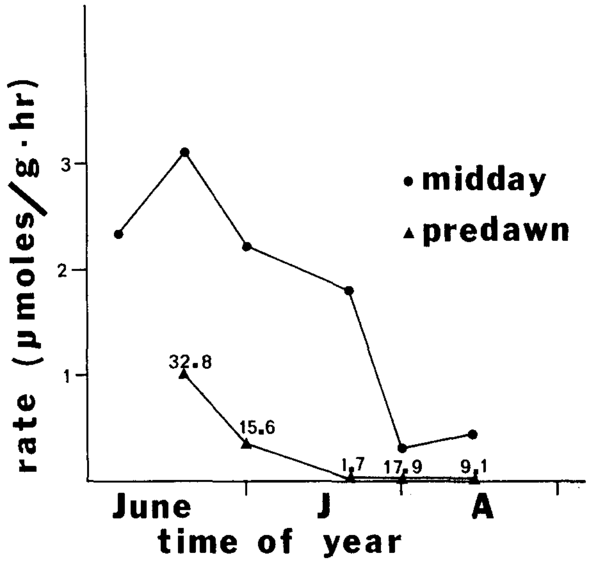

Fig. 6. Seasonal pattern of midday and predawn acetylene reduction rates at Study Site 2. Numbers above each predawn point are the per cent of predawn rates relative to the midday rates of the same day. Means of nodules from 6 to 8 plants.

the season, becoming more completely exhausted each night. This decline of carbohydrate levels is probably also due to reduced photosynthesis brought about by moisture stress.

The variability in average acetylene reduction rates between sampling times from June to mid-July such as observed at Sites 1 and 3 (Table 7) might also be due to fluctuation in carbohydrate levels. Soybean nodule activity declines on a cloudy day[3]. Light rainfall and changes in temperature also might affect the daily carbohydrate level and nodule activity of Purshia although a direct comparison of thermograph data and nodule activity failed to show a strong relationship. Other factors which account for part of this variability are random sampling variability and the fact that all acetylene reduction measurements were not made at the same time of day.

Degree of nodulation

All plants grown from seed in the greenhouse had nodules by the age of 3 months, although two-month-old seedlings often had not formed nodules. All of these nodules reduced acetylene with the ex-

TABLE 8

Percentage of plants with nodules. Plants were sampled at Study Sites 1–4

Age of plant (years)	Total number of plants examined	Percentage of plants with nodules
2–5	162	43
6–10	122	47
11–15	30	53
>15	50	50
all ages	364	46

ception of one group of plants, from seeds collected at Bryce Canyon, Utah. Nodules from 4 2-month-old, 12 3-month-old and 4 4-month-old seedlings from this seed source were examined and no trace of ethylene was produced by any. There was no visible distinction between the nodules of these plants and the active nodules of plants from other sources. This source was, however, distinguished by a low growth habit of older plants. Purshia plants from this source may be genetically different from the other Purshia plants used and perhaps are not adapted to the endophyte found in central Oregon soils.

In the field, there is no consistent trend of nodulation with plant age (Table 8), but all sites except no. 2 had a much lower percentage of young plants (2–5 years old) with nodules. Plants younger than 2 years almost never had nodules. Even though plants greater than 15 years old were just as likely to have nodules as plants 5–15 years old, the nodules on older plants were generally small and only a few occurred on any one plant. Even with the much smaller root system of younger plants the average nodule biomass per plant was greater.

Under normal conditions, nodulation percentages of other nitrogen-fixing non-legumes are generally high, close to 100 per cent[6]. Unfavorable environmental conditions such as moisture stress and low soil temperature probably are responsible for the incomplete nodulation of Purshia plants in the study areas.

The effects of moisture stress on the process of nodulation have not been thoroughly investigated. Moisture availability has been generally accepted as affecting nodule initiation and longevity since Wilson's[44] observations in which up to 57 per cent of nodules on bean plants were shed under moderate water stress. Purshia nodule clusters generally become senescent and are shed before they reach a large size. Although there is no direct evidence, moisture probably is important in controlling the loss of old Purshia nodules as well as the formation of new ones.

Summer soil temperature at 20 cm in Purshia communities in this study never exceeded 17°C and was usually lower. Soil temperatures were probably slightly below normal due to the cool, wet summer of 1975; however, even in normal years, soil temperature throughout this region would still be quite cool. In *Ceanothus velutinus*, which like Purshia grows in the central Oregon pumice region, only 10 percent of the plants formed nodules when grown at a soil temperature of 15°C and no nodules were formed at 10°C[45]. However, all plants grown at 22°C developed nodules. The nodules that did form at 15°C required 70 days to develop, compared to less than 45 days for those at 22°C. If Purshia nodulation behaves similarly, then nodule initiation might not begin until spring soil temperatures exceeds 10°C – approximately mid-May or early June. Since summer soil temperature is generally around 15°C, few nodules would form and those that did might require much of the summer for full development. This would mean that the final stages of nodule development would occur late in the growing season when moisture conditions are unfavorable.

Another factor which affects nodule initiation is the distribution of the endophyte in the soil. If a nitrogen-fixing plant invades an area where it has not previously grown, nodule formation may be scarce until the soil population of the endophyte is built up. The endophyte for Ceanothus persists in Oregon soils up to 200 years after the host plant disappears[46]. However, all of the Purshia communities in this study are climax and Purshia has been present on each site for a number of years, as proven by the occurrence of some individuals over 25 years old at each sampling area. Consequently, soil populations of endophyte should be at sufficient levels to insure universal nodulation. Even in case of a fire, Purshia should become reestablished before soil endophyte populations decline seriously. Several other observations suggest that it is not endophyte distribution that is limiting nodulation. Frequently, 1 or 2 plants in an old seed cache group will be nodulated, while other plants in the same group will have roots intertwined with the nodulated roots, but no nodules of their own. Finally, as noted earlier, nodulation was complete in plants grown in identical soil brought into the greenhouse.

Foliage nitrogen and nitrogen accretion

Kjeldahl analysis of leaves from 14 field plants, 7 with and 7 without nodules, revealed an average nitrogen content of 1.5 ± 0.3 per cent for non-nodulated and 1.7 ± 0.3 per cent for nodulated plants. Foliage levels of 3 month old greenhouse seedlings (all nodulated) ranged from 1.3 to 2.5 per cent with the average being 1.8 per cent. Nitrogen levels were higher in seedlings with nodules that were more active.

An effort was made to estimate the total contribution of nitrogen to the ecosystem by Purshia at Study Site 2. This site had a longer season of nodule activity and more nodules than the other sites. Consequently, this estimate, using nodule biomass per unit ground surface area and seasonal acetylene reduction rates, should represent an upper limit of accretion rates within the sites studied. Similar methods have been used to describe nitrogen accretion rates of 56–130 kg N/ha·yr by *Alnus glutinosa* with a dry weight root nodule biomass of 444 kg/ha[1 2]. However, the Purshia root nodule biomass at Site 2 was calculated to be only 1.91 kg/ha on a *fresh* weight basis.

Nodules were assumed to function at the observed midday rates for 12 h and the predawn levels for 12 h each day. Predawn rates previous to June 19 (the earliest date predawn readings were taken) were considered to be identical to the relative activity observed on this day. Similarly, predawn rates after August 13 were taken as 9.1 per cent of the midday rate. Acetylene reduction values from each sampling date were treated as a constant over a time span extending from half way to the previous sampling date to half way to the next sampling date. Nodule activity after September 4 was considered negligible since acetylene reduction rates were very low and in years with normal precipitation, probably would have approached zero by this time. The conversion factor for 'C_2H_2 reduced' to 'N_2 fixed' of 2.4 was used, based on reports for Alnus, the only non-legume for which this factor has been calculated (Hardy *et al.*[16] based on data from Akkermans[1] and Russell and Evans[27]).

These calculations result in an accretion estimate of only 0.057 kg N/ha·yr. This extremely low value arose from several factors. Nodules were active for only a short time during the year, with substantial acetylene reduction rates occurring only from early June to late July. Even during this period, nodule activity was slight during night hours. Soil temperatures were consistently below the level required for maximum nodule activity. Most important was the very small nodule biomass for a given ground surface area that arose from the small size of nodules and the low percentage of plants with nodules. The biomass estimate was probably below the actual value, due to loss of nodules in the process of excavation, despite careful digging. The percentage of nodules lost in this way was probably low; even assuming a substantial loss, the estimated nitrogen accretion rate would still be low. Similar low nitrogen accretion values due to low nodule biomass have also been reported for Hippophae[1].

Such a small nitrogen accretion rate has negligible ecological significance. Based on Youngberg and Dyrness[47] data on pumice soil nitrogen (.105–.160 per cent) and bulk density (0.7 g/cm^3) and assuming all nitrogen is in the top 15 cm of soil, Purshia would be increasing soil nitrogen by only 0.005 per cent or less each year. This accretion rate is also small when compared to probable input of nitrogen in precipitation. Yearly inputs of dissolved nitrogen of from 0.36 to 1.12 kg N/ha/100 cm of precipitation occur in the western Oregon Cascades[15]. Extrapolated to Study Site 2, this would suggest a probable yearly input of 0.25 to 0.77 kg/ha.

The occurrence of nodules may have a direct impact on Purshia plants. Survival of plants, especially those less than 15 years old, may be improved by the presence of nodules, considering the low soil nitrogen levels and the intense competition from other plants in the same seed cache. As noted earlier, the younger plants generally have larger nodules.

Although it appears that nitrogen-fixation by Purshia is of limited ecological importance in the central Oregon pumice region, this conclusion may not apply throughout the entire range of the species. Wagle and Vlamis[37] compared Purshia grown in the greenhouse in soil from two sites in northern California and reported that nodulation occurred in basaltic soil, but no nodules were formed in pumice soil. Soil type was not the only difference between these two sites and the present study indicates that pumice soil in itself is not inhibitory to nodulation. Considering the varying degree of nodulation of Purshia in the field, as reported both here and by Wagle and Vlamis, and the importance of local environmental conditions, a wide range of sites should be studied before the ecological impact of the nitrogen added from fixation by Purshia is totally discounted.

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