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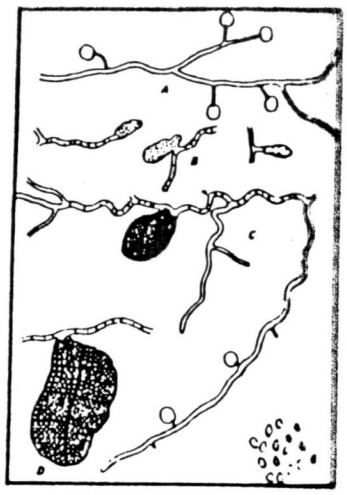
LOBARIA OREGANA, A NITROGEN-FIXING LICHEN IN OLD-GROWTH DOUGLAS FIR FORESTS

William C. Denison
Oregon State University
Corvallis, OR 97331



ABSTRACT

Lobaria oregana (Tuck.) Müll. Arg. occurs on the branches of old-growth Douglas firs in amounts estimated at 10-15 kg dry weight per tree, or approximately 500 kg/ha. This is roughly 5% of the weight of foliage on the Douglas firs. Other nitrogen-fixing lichens occur in much smaller quantities; usually less than 1% of the weight of L. oregana. We found no nitrogen-fixing bacteria or blue green algae on the surfaces of foliage or twigs.

Two methods gave estimates of nitrogen fixed by L. oregana in the range 3-4 kg/ha/yr. Annual growth of the lichen, measured by sequential photographs, averaged 30%, or 150 kg/ha/yr. The nitrogen content of the lichen is 2.1%, amounting to 3.15 kg/ha of nitrogen in each year's new growth. Assuming that all of the nitrogen required for growth is fixed by the lichen, this is a rough estimate of annual fixation. Another method used the acetylene reduction technique to estimate rates of fixation. The average rate of fixation during the wet season (15 September - 15 June) when the lichens are active was 78 nanomoles of nitrogen per gram of dry lichen per hour. Assuming that all fixation occurs during the wet season this amounts to 3.5 kg/ha/yr.

The principal factors influencing the rate of fixation were the moisture content of the lichen and the temperature. Light only affects the rate indirectly. Fixation continues undiminished in

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in the dark, presumably until stored food is exhausted. Transfer of nitrogen from L. oregana to the forest floor occurs by litterfall and decomposition and by leaching from intact lichens. Grazing by invertebrates could not be demonstrated.

INTRODUCTION

The canopy of a mature forest is a complex system which accumulates, stores, and releases nutrients in ways paralleling those of the forest floor. The discovery of nitrogen-fixing lichens in the canopy of western coniferous forests (Pike et al 1972) lead us to develop methods for studying these lichens and their contribution to the nitrogen economy of the forest (Denison et al 1972, Pike et al 1975, Pike, Rydell, & Denison 1977). This paper reports estimates of the amount of nitrogen fixed each year by the major lichen, Lobaria oregana, as well as some general observations on its ecology.

METHODS

The study was conducted at the H. J. Andrews Experimental Forest, Willamette National Forest, on the western slope of the Cascade Mountains 75 km northeast of Eugene, Oregon. We surveyed the epiphytes on 19 large trees in 3 stands of mature, 450-year-old Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) and estimated epiphyte biomass on 8 Douglas firs: 7 large trees 1.25 - 1.50 m dbh and 60-80 m in height; and one smaller tree 1.15 m dbh and 55 m tall.

To sample the trees we climbed them using techniques borrowed from mountaineering (Denison et al 1972, Denison 1973). We climbed a fixed rope using stirrups suspended from jumars, a Swiss climbing aid. A seat sling suspended from one jumar allowed us to work with both hands free. A second rope connecting the climber with a belayer on the ground through a pulley near the top of the tree provided support in emergencies. A moveable spar extending horizontally from the trunk permitted us to move parallel to branches while sampling from them.

An outline of methods used in estimating biomass follows; a detailed description is given elsewhere (Pike, Rydell, & Denison 1977). Biomass of L. oregana on branches was obtained from 2 consecutive rounds of data collection: during the first round preliminary estimates were obtained for each branch; during the second round detailed examination and systematic harvesting of the epiphytes provided improved estimates for a small subset (5-6) of the branches. Comparison of first and second round

estimates for those branches subjected to second round sampling provided the basis for correction of the first round estimate for the entire tree. First round estimates were based on quantitative descriptions gathered by the climbers relating both to branch surface area (diameter, length, branch area, etc.) and to cover of Lobaria on each branch. The subset of branches chosen for second round sampling was chosen by an unequal probability scheme which favored sampling larger branches with abundant epiphytes, and which resulted in a sample distributed vertically through the canopy. Both living and dead branches of all sizes were eligible for sampling. Sampled branches larger than 4 cm diameter were divided into 0.5 m lengths and one cylindrat, a sampling unit including the cylindrical surface of a 1 dm long section, was sampled in each 0.5 m length. The surface area included in a cylindrat varied with the diameter of the branch. Lobaria oregana and other non-crustose species were removed from each cylindrat, sorted, dried, and weighed. All branchlets (less than 4 cm diameter) were described (diameter measured; total area and Lobaria cover estimated): one-fifth of them were taken to the laboratory where epiphytes were removed, sorted, dried, and weighed.

We used the acetylene reduction technique (Hardy et al. 1968) to measure rates of nitrogenase activity in the field at intervals of two weeks from September 1976 through April 1978. Most samples were collected and incubated near midday, but on five occasions we continued sampling throughout a 24 hr cycle. Samples were taken from 3 randomly preselected branches at three heights in the canopy, usually 5-6 samples from each branch. At the base of the tree they were cleaned of debris and weighed to a uniform sample weight, typically 3g. Subsequent drying and reweighing in the laboratory provided moisture content. Each sample was placed in a plastic (Cryovac) bag. The bag was gathered and sealed with a rubber band around a short length of rigid plastic tube closed by a rubber septum. The air in the bag was removed with a syringe and replaced by a mixture of 10% acetylene in ambient air. The samples were incubated for one hour, at the end of which time two 5 ml gas samples were removed into Vacutainers. Incubating bags were placed on a log at temperatures resembling those in the canopy, but kept out of direct sunlight. Subsequently the levels of ethylene in the Vacutainer samples was measured by gas chromatography.

SURVEY OF MACRO- AND MICROEPIPHYTES

More than 80 species of macroepiphytes (lichens and bryophytes) were found in the canopy (Pike et al. 1975); the mosses chiefly on the trunk and in the moist, shaded lower canopy, and the lichens more evenly distributed throughout the canopy.

A single species of lichen, Lobaria oregana, dominated the epiphyte community: its biomass, commonly 10-15 kg per tree, was greater than that of all other epiphyte species combined (Table 1).

TABLE 1. APPROXIMATE DRY WEIGHT (kg) OF EPIPHYTES FOR ONE TREE

Epiphyte	Trunk	Branches	Twigs	Total
Lobaria oregana	0.0	3.1	7.1	10.2
Other N-fixing Lichens	0.0	0.02	0.01	0.03
All Other Lichens	0.6	0.7	1.5	2.8
Bryophytes	2.5	2.1	0.05	4.6
Total	3.1	5.9	8.7	17.7

Assuming an average biomass of L. oregana of 12.5 kg per tree and 40 mature trees per hectare, we estimate the biomass of L. oregana in these stands at 500 kg/ha, dry weight. Since L. oregana absorbs 2.7 g of water per g of dry thallus the "fresh" weight of this population, when wet, approaches 2 metric tons per hectare. Put another way, the biomass of L. oregana is roughly 5% of that of the foliage on the Douglas firs.

At least ten other species of nitrogen-fixing lichens were found in the canopy, but none was as abundant as L. oregana. Typically the biomass of these other species was less than 1% that of L. oregana. In each case the nitrogenase activity of these species is due to a symbiotic blue green alga. In some species, such as Sticta weigelii (Ach.) Vain., the blue green alga is the primary photosynthetic alga, but in other species, such as L. oregana, a green alga is the primary photosynthetic alga, and the blue green occurs in nodule-like cephalodia. Table 2 compares the rates of nitrogenase activity of 4 of the most abundant of these other nitrogen-fixing species with L. oregana.

In addition to macroepiphytes, such as lichens and mosses, the canopy serves as substrate for large numbers of microepiphytes: microscopic fungi, bacteria, and algae (Bernstein et al. 1973, Sherwood & Carroll 1974, Caldwell 1978). We expected to find free living nitrogen-fixing bacteria on the foliage of Douglas

TABLE 2. AVERAGE NITROGENASE ACTIVITY OF EPIPHYTIC LICHENS WITH BLUE GREEN ALGAE

Species	Nitrogenase Activity (n-moles C_2H_4 /g/hr)	Number of Samples
Lobaria oregana	350	760
Lobaria pulmonaria	278	51
Peltigera aphthosa	578	22
Pseudocyphellaria anthraspis	388	9
Sticta weigelii	365	2

firs, such as those reported by Jones (1970, 1976) from Douglas fir in Europe. However, repeated attempts to isolate nitrogen-fixing bacteria from foliage or twigs, or to detect nitrogenase activity on fresh samples of foliage and twigs, were completely unsuccessful. Except for the nitrogen-fixing lichens mentioned above the only evidence of nitrogen-fixing microorganisms found on any canopy surface was the occasional isolation of nitrogen-fixing bacteria from moss colonies (Caldwell 1978). We conclude that free-living, foliar bacteria do not play a significant role in nitrogen fixation in coniferous forests west of the Cascades.

TWO ESTIMATES OF THE TOTAL AMOUNT OF NITROGEN FIXED ANNUALLY BY LOBARIA OREGANA

We used two methods to estimate the amount of nitrogen fixed annually by L. oregana: one based on estimated growth rates; the other on field measurements of nitrogenase activity. Both estimates were in the range 3-4 kg/ha/yr.

Rhoades (1977) estimated growth rates of L. oregana thalli from sequential photographs. Rates ranged from more than 100% in very small thalli to approximately 30% per year in those weighing 0.5 g or more. Although there were many more smaller thalli, 95% of the biomass consisted of thalli weighing more than 0.5 g. Thus, for the population as a whole annual new growth may be estimated at 30% of the total biomass, or 150 kg/ha/yr. If one assumes that all of the nitrogen fixed during the year is incorporated in new

growth, with none taken up from, or lost to, the environment, then the nitrogen content of the new growth should equal the amount fixed. The nitrogen content of Lobaria oregana thalli is consistently 2.1% (Pike et al. 1972). Applying this percentage to the 150 kg/ha of new growth produced each year gives an estimate of 3.15 kg/ha/yr of fixed nitrogen.

The second method involves estimating the average rate of fixation over an entire season. Over the entire sampling period (September 1976 - May 1978) rates of nitrogenase activity in L. oregana ranged from 0 to 845 nanomoles of ethylene produced per g of dry thallus per hr, and averaged 160 n-moles/g/hr over the two seasons (15 September - 15 June) when the lichens are normally active. However, the winter of 1976-1977 was the driest on record; on more than half of the sampling days the lichens were dry and inactive, resulting in a very low average rate (97 n-moles C_2H_4 /g/hr) for the season. The following winter, 1977-1978, was a normal wet season, consequently the average rate was higher, 235 n-moles/g/hr. Selecting this rate and assuming the theoretical equivalence of 3 moles of ethylene to one mole of nitrogen, the average rate of nitrogen fixation was 78 n-moles/g/hr. Assuming the biomass of L. oregana was 500 kg/ha, and that fixation occurred only during the 6,552 hr of the wet season (15 September - 15 June) then the estimated total of nitrogen fixed annually was 3.5 kg/ha/yr.

Both estimates rest upon inadequately tested assumptions, but upon different sets of assumptions. It is interesting that the two estimates agree so closely. It seems unlikely that subsequent study will require them to be revised upward or downward by as much as an order of magnitude.

ENVIRONMENTAL FACTORS INFLUENCING NITROGENASE ACTIVITY

The two environmental factors exerting the greatest influence on nitrogenase activity in L. oregana were moisture and temperature. Light apparently influences nitrogenase activity in L. oregana only indirectly.

The thallus moisture content of samples of L. oregana ranged from 0.03 - 2.70 g water per g of dry thallus. Samples with water contents below 0.7 g/g exhibited little or no nitrogenase activity. Those with higher water contents showed a rapid increase in activity with increasing water content, reaching effective saturation at a water content of 1.5 g/g. Only a few samples (10%) had water contents in the transitional range (0.7 - 1.5 g/g): the remainder were either above 1.5 g/g (50% of samples) or below 0.7 g/g (40% of samples). Apparently under natural conditions

L. oregana becomes saturated or dries out very rapidly, spending most of the time either dry or above saturation.

The level of nitrogenase activity in moist thalli is controlled primarily by temperature. Activity ceased at or near 0° C, increasing rapidly with increasing temperature to just below 15° C. Although temperatures above 30° C were recorded at the research site during the study period, we encountered only dry lichens when temperatures were above 15° C. Within the range 0° - 15° C nitrogenase activity increased rapidly with increasing temperature. A linear regression of nitrogenase activity against temperature for samples with water contents above 1.5 g/g yielded the equation:

$$N = 99t + 0.14 \quad (r^2 = 0.50)$$

where N = nitrogenase activity expressed as n-moles C_2H_4 /g/hr, and
t = incubation temperature (° C)

Although it seems unlikely that the relationship between temperature and nitrogenase activity is, in fact, linear, attempts to fit these data to other curves yielded less satisfactory results ($r^2 < 0.45$).

Light, unlike moisture and temperature, appears to have little or no immediate effect on nitrogenase activity in L. oregana. Samples incubated at light levels ranging from 100-3,800 lux showed no correlation between levels of illumination and rates of acetylene reduction. Samples incubated at night in the dark showed nearly the same range of activity, a similar mean, and a similar standard deviation as parallel samples incubated in the light during the day (Table 3.). We found no evidence of a consistent nocturnal depression in nitrogenase activity in L. oregana. This finding contrasts sharply both with published descriptions of lowered nocturnal rates in lichens belonging to the genus Peltigera (MacFarlane et al. 1976) and with our own experience of lowered nocturnal rates in Peltigera membranacea. We assume that nitrogen fixation in L. oregana, as in other organisms, requires energy derived from photosynthesis, and is thus secondarily light-dependent, but we did not study the effect of prolonged light deprivation in this species.

TRANSFER OF NITROGEN FROM LOBARIA OREGANA TO THE FOREST FLOOR

Transfer of nitrogen from Lobaria oregana thalli in the canopy to the forest floor occurs: 1) by litterfall of whole thalli or fragments, followed by decomposition; 2) by leaching from intact thalli; and 3) by decay and dissolution of dead and dying thalli in the canopy. Grazing by invertebrates appears to be rare or

TABLE 3. RATE OF ACETYLENE REDUCTION BY LOBARIA OREGANA IN DAYLIGHT AND AT NIGHT

Conditions*	Rates of Acetylene Reduction (n-moles C_2H_4 /g/hr) Minimum	Maximum	Average	Standard Deviation	Number of Pooled Samples#
Daylight	0	698	320	±188	20
Night (dark)	0	678	352	±199	9

*Moisture content and temperature were not controlled. However dry samples with moisture contents below 0.7 g/g were excluded.

#Each pooled sample consisted of three or more samples collected and incubated at the same time.

nonexistent. The possibility that the thalli may release ammonia or volatile amines has not been investigated. Lobaria litterfall is episodic, coinciding with major storms, usually during the winter. Lobaria thalli which fall early in the winter often remain green and have significant nitrogenase activity for some time. They usually turn brown and decay rapidly following warm, wet periods in April or May. Estimates of the amount of Lobaria litterfall based on sampling from the ground have been consistently lower than estimates of new growth (Rhoades 1978). Although dead and decaying thalli have been observed in the canopy, no systematic attempt has yet been made to measure the extent of nitrogen loss by this process. Freshly collected L. oregana thalli which were irrigated with rainwater in the laboratory yielded nitrogen in the leachate, both as soluble nitrogen and as insoluble particulates, chiefly microbial cell mass (Pike 1978).

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