Patrick J. Brown and **David A. Dalton,**[1] Biology Department, Reed College, Portland, Oregon 97202-8199

In situ Physiological Monitoring of *Lobaria oregana* Transplants in an Old-growth Forest Canopy

Abstract

Lobaria oregana (lettuce lung lichen) is an abundant nitrogen-fixing cyanolichen in old-growth Douglas-fir forests of the Pacific Northwest. In this study, we used the Wind River Canopy Crane Research Facility to study nitrogenase activity, photosynthesis, and growth to clarify the potential for *Lobaria* to contribute fixed nitrogen to these forests and to better understand the ecological factors that determine the distribution of *Lobaria*. Pendants of *Lobaria* were placed at three positions in the canopy: top (62 m above the ground), middle (39 m), and bottom (2 m). There was a complex pattern of seasonal and spatial variation. Greatest growth was found at the middle position where there was a 19.3% increase in dry weight over the 13-mo study period. Lichens at the bottom position died after transplanting. Nitrogenase activity was consistently higher in the middle position and averaged 115 nmol C_2H_4 g^{-1} hr^{-1} for wet season measurements (February, March, and November) with a range of 0-310 nmol C_2H_4 g^{-1} hr^{-1}. Photosynthesis activity averaged 0.322 mg CO_2 g^{-1} hr^{-1} for the wet season with a range of 0-0.801 mg CO_2 g^{-1} hr^{-1}. Activities were strongly correlated with hydration except that hydration >200% inhibited photosynthesis. Laboratory experiments showed that photosynthesis increased at photosynthetic photon flux densities of up to 1000 µmol m^{-2} s^{-1}. This study supports the conclusion that *Lobaria* is a source of nitrogen input for these forests and addresses how the physiological activities of *Lobaria* respond both spatially and temporally to the extremely variable environment within the canopy.

Introduction

Lobaria oregana (lettuce lung lichen) is an epiphytic cyanolichen endemic to the old-growth forests of the Pacific Northwest. As a tripartite cyanolichen, it represents a three-way symbiosis between an ascomycete, a green alga *(Myrmecia),* and a cyanobacterium (*Nostoc*). While the alga acts as the primary photobiont, supplying the lichen with photosynthetic sugars, *Nostoc* acts to supply nitrogen that it fixes within specialized structures called cephalodia (Nash 1996).

This lichen has received recent attention both for its specificity to old-growth forests and for its ability to provide a significant source of nitrogen to this ecosystem. While *Lobaria* is essentially absent from stands < 80 yr old, it is abundant in old-growth stands where its distribution is heterogeneous but may exceed 1 metric ton ha^{-1} (McCune 1993). On three 400-yr old trees surveyed by McCune (1993)—one individual each of Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and western redcedar (*Thuja plicata*)—total cyanolichen biomass was 1.1, 22.9, and 2.3 kg. Sillett (1995) measured the biomass of 65 epiphytic lichen species, including *Lobaria oregana* and 14 other cyanolichens. Litterfall biomass of *L. oregana* was twenty times greater than the next most abundant cyanolichen, *Lobaria pulmonaria* (lungwort). In a survey by Pike et al. (1977), *L. oregana* represented more than half the total epiphyte biomass. *Lobaria* is absent from young stands

Denison (1979) estimated that *Lobaria* contributes 3-4 kg N ha^{-1} yr^{-1} to old-growth forests. Since cyanolichen abundance is so variable from site to site, however, it is hard to quantify the abundance and nitrogen contribution of *Lobaria* in a typical old-growth forest. Another complicating factor is that no further field measurements of nitrogenase activity in *L. oregana* have been reported since the Denison (1979) study.

The key nutritional role that cyanolichens such as *Lobaria* play in forest ecosystems has inspired several recent studies to define what constitutes suitable cyanolichen habitat. Transplant experiments using weight gain as an indicator of growth have led to mixed results. Sillett (1994) found that *Lobaria* transplants grew less on the forest edge than in the forest interior. Other transplant experiments showed no differences in growth between *Lobaria* transplanted to 35, 100, and 400-yr-old forests (Sillett and McCune 1998). McCune et al. (1997) determined that cyanolichens are concentrated in the light transition zone 13-37 m

[1] Author to whom correspondence should be addressed: E-mail: david.dalton@reed.edu

above the forest floor, where light transmittance declines abruptly from 75% to 25% of the total light striking the top of the canopy.

We used *in situ* physiological measurements of *Lobaria* in the forest canopy to assess the contribution to the nitrogen nutrition of Pacific Northwest old-growth forests. These studies are of interest because previous work with *Lobaria* has been limited to use of loose thalli under artificial conditions and thus the significance of this species in the nitrogen budget of these forests remains unresolved. *In situ* measurements under natural light and temperature conditions have now become feasible due to the availability of the canopy crane. Such studies can provide insight into the environmental constraints that determine the distribution of *Lobaria* within the canopy as well as its absence from young stands. The specific objectives were: 1) to measure growth at different canopy positions during wet and dry seasons; 2) to measure net photosynthesis and nitrogenase activity at different hydration states, seasons, and canopy positions; and 3) to define photosynthetic light responses in controlled laboratory studies.

Methods

Study Area

This study took place at the Wind River Canopy Crane Research Facility (WRCCRF) in the Wind River Experimental Forest (Trout Creek Division) in the southern Washington Cascades (45° 49' N, 121° 55' W). WRCCRF maintains a Liebherr 550 HC construction crane that sits amid 12 ha of old-growth forest. With a height of 87 m and a horizontal reach of 85 m, the crane provides access to over 10^5 cubic meters of old-growth canopy for research purposes. The crane is operated jointly by the USDA Forest Service and the University of Washington.

Douglas-fir and western hemlock are co-dominant at the WRCCRF site. Other common canopy trees include western redcedar, Pacific silver fir (*Abies amabilis*), grand fir (*A. grandis*) and noble fir (*A. procera*). In the understory, the presence of snags, fallen logs, and the slow-growing Pacific yew (*Taxus brevifolia*) help characterize the site as old-growth. Over 114 species of lichens have been found in the 12-ha crane plot (McCune 1997). Average annual precipitation is approximately 250 cm, of which < 10% occurs between June and September. The average annual temperature is 8.7°C. Although elevation is <400 m, winter snowfall is typically heavy from December through February with an annual average of 233 cm. Monthly averages for temperature and precipitation for the nearby Carson fish hatchery are available online (Desert Research Institute 2001). Microclimatic data at the crane site for the days on which physiological measurements were made are presented in Table 1. A thorough description of the study site, including an annotated species list is available online (University of Washington 2001a).

TABLE 1. Meteorological data at 1200 hours from the WWCCRF on the dates when physiological measurements were made. Source: University of Washington (2001b).

Date	Height (m)	Temp. (°C)	Relative humidity (%)	Precipitation[1] (mm)	PAR[2] (W m^{-2})
23 Feb	2	2.73	100	14.3	-
	70	-	-	-	-
	2-open[3]	3.9	100	-	153
16 Mar	2	3.59	100	1.9	10.4
	70	4.49	61.9	-	-
	2-open	6.29	65.0	-	1154
7 Apr	2	4.09	93.3	1.2	9.9
	70	4.75	58.4	-	-
	2-open	5.82	58.0	-	1137
30 Sept	10	18.8	49.4	0	1167
16 Nov	2	10.3	100	1.0	8.9
	70	10.5	92.3	-	-
	2-open	11.2	98.7	-	130

[1]preceding 24 hr

[2]photosynthetically active radiation

[3]at 2 m height in an adjacent open field

Pendant Construction

Desiccated *Lobaria* litterfall was collected near the crane site on 17 October, 1998. The litterfall was cleaned of debris, cut into thallus pieces roughly 5 cm in diameter, and air dried for 48 hr. Older, interior portions of thallus without growing edges were not used. Thallus pieces were weighed individually and strung onto nylon fishing line (25-lb test) alternately with numbered beads to form pendants of 20 thallus pieces each (Denison 1988). Each numbered bead identified

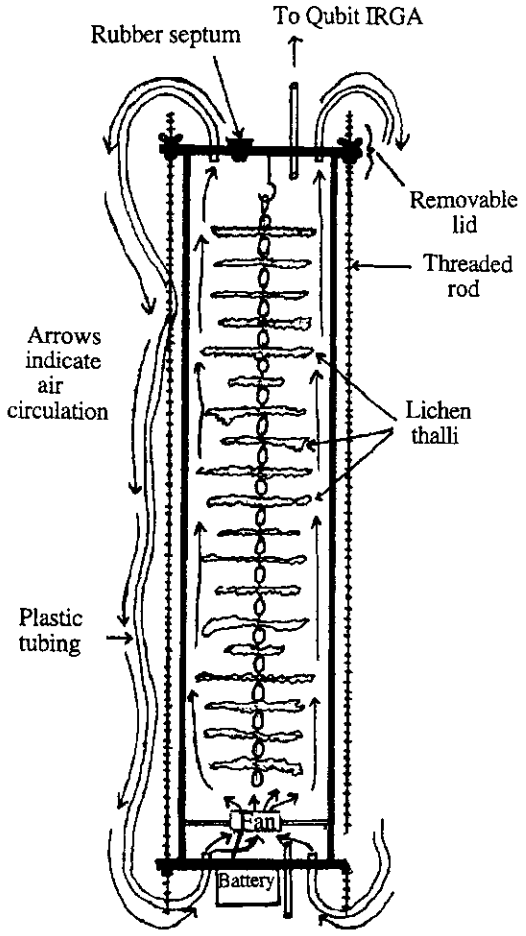

Figure 1. Plexiglas chamber used for physiological measurements of pendants of *Lobaria*. Twenty thallus fragments (~ 5 cm in diameter) were strung onto nylon fishing line and suspended in the chamber.

a specific thallus piece, so that the weight of lost pieces could be accounted for. Total pendant dry weights ranged from 15-17 g.

Five air-tight Plexiglas cylinders were constructed to accommodate the lichen pendants (Figure 1) during physiological measurements. Cylinders featured battery-powered fans (Radio Shack 12V DC Brushless) to maintain air circulation around each pendant during closed incubations. Tygon tubing connected each end of a cylinder to the system for measuring CO_2 exchange as described below. Rubber septa allowed gas injection and removal for the acetylene reduction assay. The total volume of each cylinder was 1.15 L.

Transplant Sites

Twenty pendants were constructed for transplantation to four different sites. Five pendants each were transplanted to three different sites in the old-growth canopy: top (62 m above the forest floor), middle (37 m), and bottom (2 m). Top and middle groups were both transplanted using the canopy crane to a single large Douglas-fir with a broken top. Pendants were hung from accessible branches using plastic cable ties. The top group was above the natural vertical distribution limit of *Lobaria*. Lichen genera common to this microhabitat include the alectoreoids (*Alectoria* and *Usnea* spp.), bone lichens (*Hypogymnia* spp.), and rag lichens (*Plastismatia* spp.). The middle group lay within the vertical distribution of many cyanolichens including *Lobaria oregana*, *Lobaria pulmonaria*, and *Pseudocyphellaria* spp. The bottom group was accessible from the ground, and pendants were hung from the lower branches of understory hemlock, yew, and vine maple (*Acer circinatum*) in a microhabitat dominated by bryophytes. *Lobaria* was present here only in the form of litterfall.

The remaining five pendants were transplanted to the understory (2 m) of an even-aged, single-species stand of ~45-yr-old Douglas-fir. This site was located about 8 km north of the WRCCRF site. Lichen diversity in this second-growth is greatly reduced compared to the old-growth site and arboreal cyanolichens are absent. No physiological measurements were taken from this group, but the pendants were retrieved in April 1999 to assess growth.

Measurements of CO_2 Exchange and Nitrogenase Activity

Pendants were transplanted in October 1998. Physiological measurements were taken in February, March, April, September, and November 1999. Measurements were always taken from the top group to the bottom group. Each pendant was removed from the tree, weighed, enclosed within a gas tight cylinder (Figure 1), and then measured for CO_2 exchange. This was followed within 15 min by the nitrogenase activity (acetylene reduction) assay. Cylinders were hung from the edge of the crane gondola to mimic natural light and temperature. Air for the CO_2 exchange assay was pumped up through tygon tubing that dangled several meters below the gondola. This

ensured that the air source was relatively free of human influence, yet representative of the humidity and temperature of each microclimate. After the assays were complete, the pendants were reattached to the tree. A typical measurement session on an individual pendant lasted 30 min.

A CO_2 analysis package from Qubit Systems, Inc. was used for all CO_2 exchange measurements. An infra-red gas analyzer (IRGA) for measuring CO_2 concentration was coupled with light, temperature, or humidity sensors through a serial box interface (SBI) capable of recording two inputs simultaneously. The SBI was connected to a Macintosh Powerbook 3400c equipped with Qubit datalogging software. The IRGA was calibrated using standards containing 0 and 500 ppm CO_2. Net photosynthesis was measured for each pendant as the difference between ambient [CO_2] and the [CO_2] of a pendant-containing cylinder. Typical values for $\Delta[CO_2]$ were 50-100 ppm. Air was pumped through the pendants at a flow rate of ~21 L hr^{-1}. At this rate a stable experimental reading was obtained in ~5 min.

The CO_2 exchange rates of individual *Lobaria* fragments were also tested in the laboratory. A Qubit Plexiglas cuvette (1 x 3 x 3 cm) and a flow rate of 2.7 L hr^{-1} were used for these laboratory experiments. Photosynthetic photon flux density (PPFD) was determined with a portable LI-COR model LI 185B quantum meter.

The acetylene reduction assay was used to measure nitrogenase activity. Acetylene was generated by reacting calcium carbide with water. Sufficient acetylene was injected into the cylinder to achieve a concentration of 10% by volume. The cylinder was shaken vigorously, vented to atmospheric pressure, and the battery-powered fan was engaged. A 1-mL gas sample was then withdrawn by syringe for a time zero reading. After 20 min of incubation, three more 1-mL gas samples were taken. Syringes were inserted into rubber stoppers to prevent leakage during transport back to the laboratory where the ethylene content of each sample was determined with a Varian model 330 gas chromatograph equipped with a 0.318 x 76 cm stainless steel column of Poropak N and a flame ionization detector. The initial gas sample (at t=0) was subtracted from the mean of the three subsequent gas samples (at t=20) for each cylinder to control for the slight (but not negligible) background levels of ethylene that are normally present in acetylene. An additional control consisting of a chamber with 10% acetylene but lichens showed no change in background levels of ethylene during a typical 20-min assay period.

Thallus Weight and Hydration

The initial dry weight of each pendant was measured in October 1998. In April 1999, all pendants were removed from the site, air-dried for 48 hr, re-weighed, and returned to the canopy. In November 1999, all pendants were removed and weighed again after 48 hr of air-drying. This allowed us to measure growth during the wet season (October-April) and dry season (April-November) separately.

The wet weight of each pendant was determined prior to each physiological measurement session. The ~10.5 g weight of the non-absorbent pendant components (beads, fishing line, and a trace of silicone sealant) was subtracted from the total pendant weight to determine the weight of the hydrated lichens, which was then used to calculate hydration as follows:

$$\text{Hydration (\%)} = ((\text{wet wt.}-\text{dry wt.})\ (\text{dry wt})^{-1}\ ) * 100.$$

Missing thallus pieces were noted so that their dry weights could be deducted from the total pendant dry weight.

Oven-dried weights and not air-dried weights are typically used to calculate thallus weight and hydration. However, oven-dried thalli cannot be used for further physiological study. Therefore, sacrificed thalli (Sillett 1994) were weighed after 48 hr of air-drying and again after a further 24 hr of oven-drying at 70°C. Oven-dried thalli weighed ~ 10% less than thalli collected and air-dried in both October and April. This ratio was used to determine theoretical oven-dried weights of pendants for calculations of thallus hydration, so that a hydration level of 100% represents 1 g of water for each 1 g of oven-dried thallus. These calculations were based on the assumption that no dry weight gain had occurred since the last determination of oven-dried weight.

Differences Between Laboratory and Field Measurements

The laboratory measurements of CO_2 exchange and nitrogenase activity differed from field measurements in several ways. First, quantities of lichen for each lab sample were 0.1-0.6 g compared

to 4.5-6.5 g for each field sample. In the laboratory, CO_2 exchange was measured in a 9 cm^3 Qubit cuvette and nitrogenase activity in 15-mL test tubes, whereas cylinders used for both field measurements were 1150 cm^3. Flow rates through the Qubit cuvette were correspondingly lower (2.7 L hr^{-1} versus 21 L hr^{-1} for the field chambers). House compressed air provided a source of CO_2 with a stable concentration for the laboratory experiments. Dry air from the lab outlet was passed through a solution of saturated NaCl to create high humidity levels that were favorable for lichen activity.

Results

Growth of Transplants

Pendants transplanted to the top and middle positions of the old-growth canopy gained weight during the experiment whereas those at the bottom position in the old-growth site lost weight (Figure 2). During the wet season (October to April), both top and middle groups registered nearly identical weight gains (16.2% and 16.4%). During the dry season (April to November), however, the top group suffered a mean weight loss (-4.1%) while the weight gain of the middle group was much reduced but still positive (2.9%). Therefore, the overall weight gain (October to November) of the middle group was greater (19.3% vs. 12.0%).

Transplants to the forest understory (2 m) of both old-growth (bottom) and second-growth (bottom-45 yr) gained significantly less weight than their counterparts in the canopy ($P<0.005$, Figure 2). The old-growth bottom group became discolored soon after transplanting, lost weight (6.1% for the wet season) and developed an unpleasant fishy odor. The second-growth bottom group appeared healthy in April 1999, showed some growth (4.8% for the wet season), but had been heavily grazed. The low weight gain for the second-growth bottom group probably reflects a higher incidence of grazing rather than the lack of suitability of this microclimate for *Lobaria* growth. Both bottom groups were removed from the field in April 1999.

Hydration Effects on Physiological Activity

Some of the variation in physiological activity of *Lobaria* can be explained by hydration alone without regard to other factors such as season, temperature, or light. In general, both net photosynthesis (NP) and nitrogenase activity (acetylene reduction) increased markedly with increases in hydration (Figure 3A-E). In the case of NP, hydration levels >200% were less favorable than lower levels (Figure 3B). This decline at high hydration levels was not observed for nitrogenase activity (Figure 3E).

Seasonal Variation in Physiological Activity

Hydration status, photosynthesis, and nitrogenase activity were measured during five canopy crane visits made over 10 mo from February through November 1999 (Figure 4). This sampling frequency

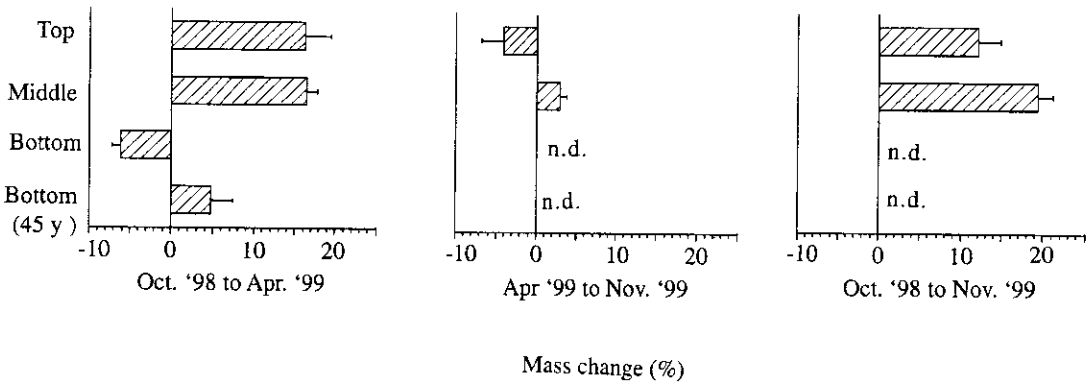

Figure 2. Seasonal weight gains in *Lobaria* pendants which were transplanted to either Top (A, 62 m above the ground) or Middle (B, 39 m above the ground), positions in the old-growth canopy at the Wind River Canopy Crane Research Facility or to the bottom (2 m) position of a 45-yr-old stand of Douglas-fir. Each bar represents the mean of 5 replicates ± 1 SE.

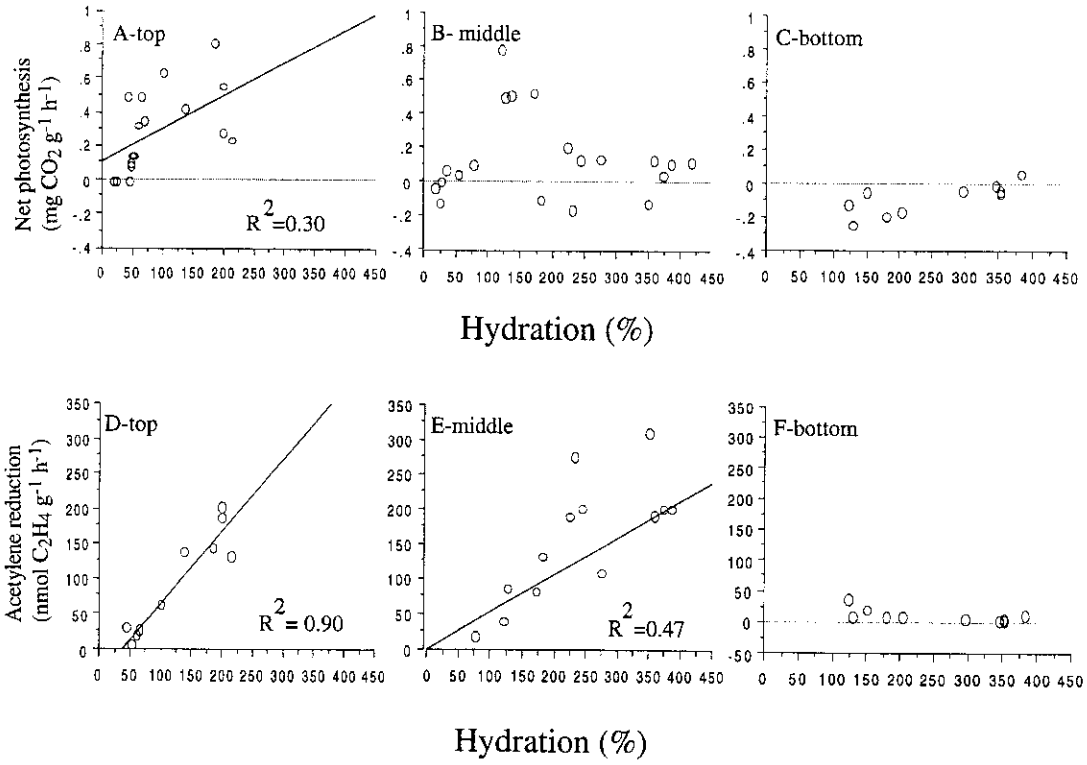

Figure 3. Effects of hydration on rates of photosynthesis (A) and nitrogenase activity (B) by pendants of *Lobaria* transplanted to the top (A, D), middle (B, E) or bottom (C, F) position of an old-growth canopy. Measurements were made between February and November 1999.

provides valuable snapshots of physiological conditions, but can not capture the full effects of daily or monthly variation in weather. The group in the canopy middle (39 m) remained consistently more hydrated than the group in the canopy top (62 m) throughout the wet season (Figure 4A, P=0.0007). During the dry season, hydration was uniformly low for both groups, but declined from April (44.9%) to September (22.2%). The hydration values in the old-growth bottom group were similar to those of the middle group in February and March. When considered along with the severe dark coloration and odor of decay, it was apparent that the lichens at this position were necrotic and consequently no further measurements were taken at this position.

The responses of net photosynthesis (NP) were complicated by the decreases observed at hydration levels >200%. Consequently, the middle position was sometimes too wet for optimal photosynthesis (e.g., February, Figure 4B), whereas such high hydration levels were not observed at the top position. Photosynthetic rates for top-February, top-November, and mid-March were roughly comparable with values near 0.5 mg CO_2 g^{-1} hr^{-1}. Net carbon loss occurred both in situations of high hydration (September) and low hydration (November) in the middle canopy. Pendants at the old-growth bottom position showed net loss of CO_2 for both February and March.

Nitrogenase activity was detected only during the wet season (Figure 4C). Nitrogenase activity was consistently higher in the middle group, likely as a consequence of the shading and higher moisture content in this microclimate. The maximum rate observed was 222 ± 32 nmol C_2H_4 g^{-1} hr^{-1} (March, middle, n = 5). The lichens in the top group were more exposed to wind and direct sunlight that resulted in rapid desiccation and thus lower activity of nitrogenase. Pendants at the bottom

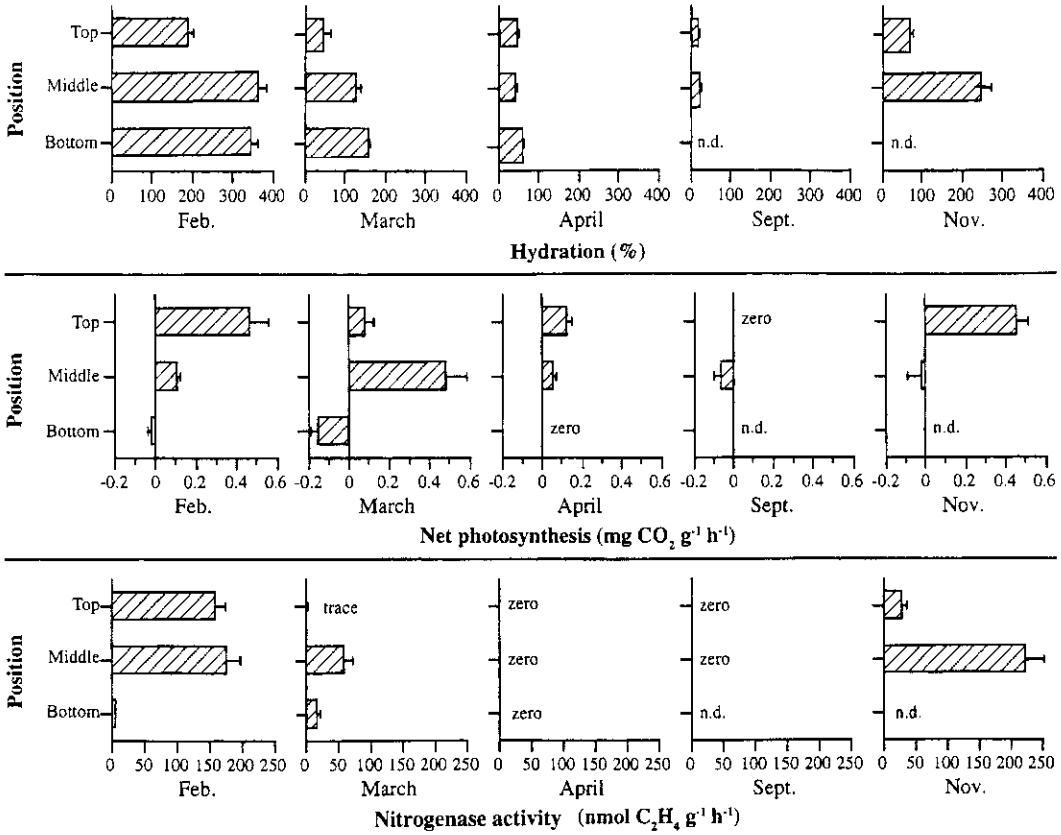

Figure 4. Seasonal variation in hydration, rates of photosynthesis, and nitrogenase activity in pendants of *Lobaria* which had been transplanted to various positions (top, middle, or bottom) in an old-growth canopy. Each bar represents the mean of 5 replicates ± 1 SE.

position showed very low activity in February and March.

CO_2 and Light Limitations of Photosynthesis

Laboratory studies at ambient [CO_2] (350 ppm) indicated that NP in *Lobaria* followed a light saturation curve that reached 50% of maximum at only 100 µmol m^{-2} s^{-1}. Near saturation was reached around 400 µmol m^{-2} s^{-1} and further increases in PPFD up to 800 µmol m^{-2} s^{-1} resulted in only a slight increase in NP. Thallus pieces at different hydration levels or concentrations of CO_2 showed different maximum rates but followed the same pattern of light saturation. Higher concentrations of CO_2 led to elevated rates of NP at all light levels tested (Figure 5). Even in the absence of light, net CO_2 loss was lower at high [CO_2]. Thalli failed to achieve net carbon gain, even if light saturated, at a [CO_2] of 250 ppm.

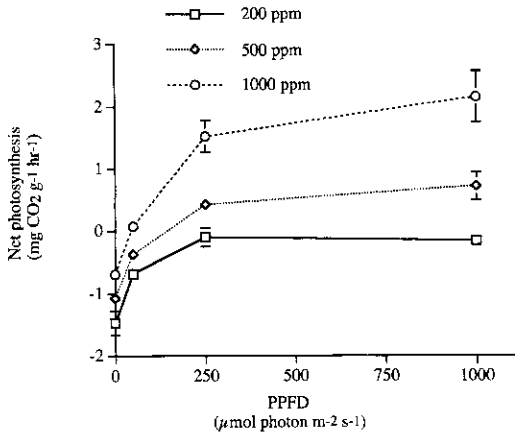

Figure 5. Effects of increasing PPFD on photosynthesis in *Lobaria* pendants. Measurements were at room temperature under controlled laboratory conditions with different concentrations of CO_2 as indicated. Each data point is the mean of 5 replicates ± 1 SE.

Limiting Factors to Nitrogenase Activity

The effects of drying and re-wetting were examined with *Lobaria* samples that were harvested directly from branch surfaces using the canopy crane and returned to the laboratory. Fresh epiphytic material was used because lichens from litterfall had highly variable and unreliable rates of nitrogenase activity. Thalli that had been dried and then rewetted showed considerably less nitrogenase activity (mean of 145 nmol C_2H_4 g^{-1} hr^{-1}) than those that were assayed immediately (mean of 477 nmol, C_2H_4 g^{-1} hr^{-1}, n = 12 , P <0.0001). Rates of nitrogenase activity in the laboratory (18-887 nmol C_2H_4 g^{-1} hr^{-1}) were much higher than those obtained at similar hydration levels in the field (Figure 4C). This discrepancy, which was probably due to differences in light, temperature, humidity, and specific assay conditions, demonstrates the importance of making physiological measurements under natural conditions and as unobtrusively as possible. Although *in situ* field measurements may be under sub-optimal conditions, they are a more realistic indication of the true role of *Lobaria* in these forests.

Discussion

Growth

Mean annual weight gains of 12.0% (top) and 19.3% (middle) are comparable to other reported annual growth rates for transplanted *Lobaria*. Denison (1988) reported growth rates of 43-67% over 3 yr, or 14-22% per yr. McCune et al. (1996), using loops rather than pendants, found an annual growth rate of 4-11%. Sillett (1994) performed reciprocal transplants on *Lobaria* from tree crowns in the forest interior and the forest edge and found annual growth of 6-14%. A further study by Sillett and McCune (1998) reported annual growth between 15-18% for forests of three age classes from 35 to 700 yr old.

Photosynthesis and Nitrogenase Activity

The hydration response observed for net photosynthesis (Figure 3A-C) closely supports the optimal range of 75-175% reported by Sundberg et al. (1997) for *L. pulmonaria*. The values for nitrogenase activity shown here (Figure 3D-F) are close to those reported by Denison (1979), the only other study to investigate nitrogenase activity by *Lobaria* in the field. Denison reports an average nitrogenase activity of 160 nmol C_2H_4 g^{-1} hr^{-1} for two winter wet seasons (September-June), with rates ranging from 0-845 nmol C_2H_4 g^{-1} hr^{-1}. In comparison, canopy transplants in this study averaged 115 nmol C_2H_4 g^{-1} hr^{-1} for wet season measurements (February, March, and November), with a range of 0-310 nmol C_2H_4 g^{-1} hr^{-1}. The correlation between nitrogenase activity and hydration (R^2= 0.81 if the bottom group is excluded) is remarkable, considering that measurements were taken across a wide range of light and temperature regimes.

The rate of photosynthesis averaged 0.322 mg CO_2 g^{-1} hr^{-1} for the wet season (top and middle positions combined) with a range of 0-0.801 mg mg CO_2 g^{-1} hr^{-1}. There are no values in the literature for rates of photosynthesis by *L. oregana*; however, our values closely match the data reported for *L. pulmonaria* by Sundberg et al. (1997) who reported a maximum rate of 2.18 μmol CO_2 m^{-2} s^{-1} which we calculate to be equivalent to 0.81 mg CO_2 g^{-1} hr^{-1}.

There is a narrow range of conditions in which NP and nitrogenase activity are both carried out at appreciable rates (Figure 4B-C, top-February). In November, NP and nitrogenase activity were almost mutually exclusive. These results underscore that lichen health cannot be maintained under static conditions, since no single condition is optimal for all physiological functions.

While both light and [CO_2] potentially limit the growth of *Lobaria* in its natural habitat, high light levels provide no obvious benefit and may be harmful (Gauslaa and Solhaug 1999). Based on the laboratory results shown here (Figure 5), *Lobaria* is able to take advantage of supraambient [CO_2] such as might result from transitory CO_2 fluxes. The magnitude and occurrence of such [CO_2] fluxes in forest canopies is the subject of current research at WRCCRF.

In lichens, the ability to fix N_2 recovers more slowly following desiccation than does NP (Kershaw 1985). The data shown here do not establish how long after rewetting this effect lasts in *Lobaria*. However, future field measurements of nitrogenase activity in lichens should address not only factors such as hydration and temperature, but also the time since the last dehydration event.

Lobaria contributes to the nitrogen budget of temperate rain forests of the Pacific Northwest. Nutrient cycling in these forests is generally efficient with little loss of nutrients from the system, however, precise nitrogen budgets are difficult to construct. This conservation of nutrients, along with the great age and stability of these forest communities, means that even the modest amounts of N_2 fixed annually by *Lobaria* can account for a substantial proportion of newly accrued, available nitrogen. We estimate the rate of nitrogen accretion to be approximately 2 kg N ha^{-1} yr^{-1} based on our observed value for the annual growth rate (19.3 %, middle position), the concentration of nitrogen in *Lobaria* thalli (2.1%, Denison 1979), and the typical biomass of cyanolichens in these forests (546 kg/ha) (Sillett and McCune 1998). We did not attempt to estimate nitrogen accretion on the basis of our acetylene reduction data because the conversion factor of acetylene reduced to N_2 fixed often varies substantially from the theoretical values of 3:1 or 4:1. However, our estimates of nitrogen accretion based on growth and biomass are similar to previous estimates (Denison 1979) in which a ratio of 3:1 was assumed.

Other potential sources of nitrogen are even more meager since other N_2-fixing symbioses (e.g. legumes, alder) are not common in these old-growth forests . The leafy liverwort *Porella*, which is abundant in these forests and forms a N_2-fixing symbiosis with *Nostoc* (Dalton and Chatfield 1985), may contribute to nitrogen accretion, but this issue has not been examined. Furthermore, precipitation in the Pacific Northwest contains little dissolved fixed nitrogen. We estimate nitrogen input from precipitation to be only about 0.6 kg N ha^{-1} yr^{-1} based on the annual precipitation total for this site and the nitrogen content of 1.8 µmol N L^{-1} in precipitation in western Washington (Edmonds et al., 1991). Finally, we note that Edmonds et al. (1991) also observed that the [NH_4^+] of stem flow was nearly sevenfold higher than the initial concentration in precipitation. They attributed this increase to leaching of nitrogen fixed by *Lobaria*. If this interpretation is correct, than our estimate of the rate of nitrogen accretion based on weight changes may be a gross underestimate since leached nitrogen is not accounted for in this method. Our studies support earlier evidence for the importance of N_2 fixation by *Lobaria* in these forests and also illustrate strikingly the difficulties of accurately quantifying this process due to the complexity of seasonal and positional (within-canopy) variations.

Acknowledgements

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