AN ABSTRACT OF THE THESIS OF

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William E. Winner

This thesis consists of three manuscripts describing ecophysiological research on the cyanolichen *Lobaria oregana.* The first manuscript includes a re-evaluation of the assumptions underlying past estimates of N fixation by this species and provides an estimate of annual N fixation at the Wind River Canopy Crane (WRCC). Based upon litterfall data, canopy biomass data, N content of lichen tissue, and published growth rates, *L. oregana* fixes 0.4-1.6 kg N_2 ha^{-1} yr^{-1}. The second manuscript presents a series of physiological response curves and a model of N fixation by *L. oregana*. Temperature is the most important parameter controlling nitrogenase activity in hydrated thalli. The model is used to predict annual N fixation at the WRCC and at the H. J. Andrews (HJA) Experimental Forest. *Lobaria oregana* fixes 1.4-1.8 kg N_2 ha^{-1} yr^{-1} at the WRCC, and low winter temperatures often inhibit nitrogenase activity. Temperatures at the HJA are slightly warmer during the winter, and *L. oregana* fixes 2.6-16.5 kg N_2 ha^{-1} yr^{-1} depending on its stand-level biomass. The third manuscript investigates the effects of thallus water content, light, and temperature on CO_2 exchange in *L. oregana*. This species shows a typical photosynthetic response upon rehydration, and like other lichens it becomes light-saturated at low PAR levels. Positive net photosynthesis in *L. oregana* occurs only between 1-12°C. High respiration rates prevent carbon gain at warmer temperatures. The temperature constraints on carbon gain and nitrogen fixation may explain some of the landscape distribution patterns of *L. oregana*.

Ecophysiology of the Cyanolichen *Lobaria oregana*

by

Marie E. Antoine

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Major Professor, representing Botany and Plant Pathology

Redacted for Privacy

Chair of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

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Marie E. Antoine, Author

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This thesis is dedicated to my Mom and my Dad.

Ecophysiology of the Cyanolichen *Lobaria oregana*

Chapter 1: Introduction

The epiphytes in Douglas-fir forests of the Pacific Northwest have been the focus of considerable scientific interest since the development of canopy access techniques in the 1970s (e.g., Denison 1973). Several general trends in epiphyte ecology have become apparent over the years. First, epiphyte biomass and diversity slowly increase as forests age (Neitlich 1993, McCune 1993, Howe 1978). Second, epiphyte groups (e.g., cyanolichens) are vertically stratified in forest canopies (McCune et al. 1997, McCune 1993). Third, cyanolichens are associated with old-growth forests, and their biomass is positively correlated with proximity to streams (Sillett & Neitlich 1996). Fourth, some cyanolichens (e.g., *Lobaria oregana*) are sparse or absent in young forests because of limited dispersal abilities (Sillett et al. 2000, Sillett & Goward 1998).

Cyanolichens have received special attention because they often dominate epiphyte assemblages (Sillett 1995, Neitlich 1993, Pike 1975) and because of their ability to fix N (Denison 1979, Pike 1978). The focus of this study was the cyanolichen *Lobaria oregana,* which can account for 60-80% of total lichen biomass in some forests (Sillett 1995, McCune 1994, Pike et al. 1977). *Lobaria oregana* is foliose lichen whose large yellow-green thalli drape over branches or grow loosely appressed to bark. Although it is not uncommon for the mycobiont to produce apothecia, reproduction by *L.oregana* is primarily vegetative and thallus margins are lined with tiny, easily-detached lobules (Rhoades 1983). It is one of only 3-4% of lichens representing a tripartate symbiosis, and research on the structural and functional characteristics of the mycobiont-photobiont interface has typically focused on lichen symbioses involving only two partners (Honegger 1991). The three-way interaction among the fungus, the green algal biont

(*Myrmecia*), and the cephalodial cyanobiont (*Nostoc*) in *L.oregana* is undoubtedly more complex and remains poorly understood.

I used an ecophysiological approach to study *L.oregana* in hopes of contributing to a better understanding of this important species. My research was conducted at the Wind River Canopy Crane (WRCC) in Washington, where a construction crane allowed non-destructive access to the old-growth forest canopy. *Lobaria oregana* thalli were collected from the canopy at regular seasonal intervals. Following each collection, some thalli were used immediately for physiological measurements under ambient field conditions, and some were air-dried and returned to the laboratory for further analyses.

This thesis presents research I conducted on *L.oregana* and is divided into three chapters. In the first chapter, I re-evaluate the assumptions underlying past estimates of annual N fixation by *L.oregana* (i.e., Denison 1979, Pike 1978). I also estimate annual N fixation at the WRCC site using litterfall data, canopy biomass data, and published growth rates for *L.oregana*. In the second chapter, I develop a model based on the physiological parameters controlling N fixation. Physiological response curves are used to model N fixation by *L.oregana* at the WRCC site over three years and in various sites within the H. J. Andrews Experimental Forest over one year. In the third chapter, I investigate environmental factors controlling CO_2 exchange rates in *L.oregana*, since carbon metabolism is necessarily linked with N fixation. This is a first step towards modeling the effects of water content, light, and temperature on CO_2 exchange in *L.oregana*. More research will be necessary to elucidate the interplay between environmental parameters and the metabolic interactions among the three symbionts.

Chapter 2

Re-evaluation of Nitrogen Fixation by *Lobaria oregana*

Marie E. Antoine and William E. Winner

ABSTRACT

Lobaria oregana is an epiphytic cyanolichen associated with old-growth forests west of the Cascades. This lichen is well known for its ability to fix atmospheric nitrogen, and this study provides an estimate of annual N fixation by *L. oregana* at the Wind River Canopy Crane site in Washington. Seasonal measurements of nitrogenase activity, photosynthesis, and respiration show that there are temperature-dependent changes in *L.oregana*'s physiological activity. N fixation and respiration rates were highest in fall and spring, and physiological activity was inhibited by low winter temperatures. Analysis of litterfall data from the WRCC site suggests that *L. oregana* contributes only 2% of the total N potentially released through decomposition of litter, but this represents a source of new N to the forest ecosystem. Approximately 20 $kgha^{-1}$ of *L. oregana* falls as litter each year at the WRCC site. In order to replace canopy biomass lost as litter and allow for a 15% annual growth rate, we estimate that *L. oregana* fixes 0.4 – 1.6 kg N_2 ha^{-1} yr^{-1} at the WRCC. Site to site differences in lichen biomass and local patterns of temperature and precipitation will determine how much N_2 *L. oregana* fixes in a given forest. Consequentially, generalizations about the importance of *L. oregana* in forest nutrient cycling should be made with caution.

INTRODUCTION

Nitrogen fixation by cyanolichens in Pacific Northwest old-growth forests

Lobaria oregana (Tuck.) Müll. Arg. is an epiphytic cyanolichen endemic to low-elevation forests west of the Cascades. Suitable habitat for this old-growth associated, pollution-sensitive species is greatly reduced by human activities such as clear-cut logging and urban development (Norse 1990). When *L. oregana* is present, however, it is often abundant with canopy biomass frequently exceeding 1 t ha^{-1} (McCune 1993, Neitlich 1993, Pike et al. 1977). *Lobaria oregana* is widely

recognized for its ecological role of fixing atmospheric N_2 into NH_3, a form of N available for plant uptake. Studies done in the 1970s estimate that *L. oregana* may fix up to 4.5 kg N_2 $ha^{-1}yr^{-1}$ (Pike 1978, Denison 1979), accounting for over 50% of the annual new N input to the forest ecosystem (Sollins et al. 1980).

Nitrogen availability limits productivity in Pacific Northwest forests (Miller et al. 1996) and biological N fixation by *L. oregana* provides new N to the forest nutrient cycle. Nitrogenous leachates and *in situ* decomposition of cyanolichens supports complex canopy food webs (Carroll 1979), and decomposition of litterfall releases N to plants on the forest floor (Pike 1978).

The previous estimate that put N fixation by *L. oregana* at 3.5 kg ha^{-1} yr^{-1} was based on several assumptions (Denison 1979). For example, the estimate was made assuming that *L. oregana* was hydrated and constantly fixing N at an optimal rate from September 15 to June 15 (Denison 1979). In addition, both Pike (1978) and Denison (1979) assumed an annual net productivity rate for *L. oregana* of 30% and a steady-state canopy biomass, suggesting that annual litterfall of *L. oregana* exceeds 100 kg ha^{-1}. Finally, a 3:1 conversion ratio was used to estimate N fixation rates from nitrogenase activity measured with the Acetylene Reduction Assay (Denison 1979).

Measurements of nitrogenase activity and gas exchange in *L. oregana* reveal the complexity of a three-way physiological interaction. The fungus receives C and N from symbiotic green algae (*Myrmecia*) and cyanobacteria (*Nostoc*), respectively. N fixation and gas exchange are necessarily linked, since photosynthesis, whether by *Myrmecia* or *Nostoc*, provides electrons and ATP to fuel the energy intensive process of N fixation by the cyanobiont. Fixed N, in turn, is crucial in the construction of rubisco and myriad other metabolic enzymes.

Research objectives

The goals of this paper are to provide an estimate of annual N fixation by *L. oregana* at the Wind River Canopy Crane (WRCC) site, and to evaluate the assumptions upon which past estimates rested. Our overall objectives are as follow:

1. To obtain instantaneous measurements of nitrogenase activity, net and gross photosynthesis, respiration, and hydration for *L. oregana* thalli collected at the WRCC site during winter, spring, summer and fall.
2. To estimate annual input of N by *L. oregana* through litterfall at the WRCC site.
3. To estimate annual N fixation by *L. oregana* at the WRCC site.

METHODS

Study site

Lobaria oregana thalli were collected and fieldwork was conducted at the WRCC site near Carson, Washington. The crane site is located within the Wind River Experimental Forest (45°49'N, 121°55'W) in the Gifford Pinchot National Forest. More information about the WRCC Research Facility is available at www.depts.washington.edu/wrccrf. Winter daily mean temperatures at the WRCC range between –5 °C and 10 °C. There is a pronounced summer drought period with over 90% of the 2.5 m annual precipitation falling between October and May.

The 4 ha crane plot is centered on a Liebherr 550 HC construction crane. The crane is 87 m high, has a horizontal reach of 85 m, and provides access to 10^5 m^3 of old-growth forest canopy. The dominant canopy trees are *Pseudotsuga menziesii* (Mirb.) Franco and *Tsuga heterophylla* (Raf.) Sarg.; *Thuja plicata* Donn. is also common. Stand age is approximately 500 years, and the site displays old-growth characteristics such as the presence of *Taxus brevifolia* Nutt. and an abundance of snags and fallen logs (Franklin and Spies 1991, Norse 1990). The lichen flora at the crane site is diverse with 113 known species. Bryophytes dominate on the ground and in the lower canopy, while the pendulous green algal lichens ("alectorioids") are most abundant in upper canopy and the exposed outer branches (Clement and Shaw 1999, McCune 1997). *Lobaria oregana* and the other less abundant cyanolichens predominate in the middle canopy and are absent above

40 m (McCune 1997). Foliose green algal lichens are distributed throughout the vertical profile of the canopy. A previous survey of the lichen flora at the WRCCRF showed that *L. oregana* is the most abundant N-fixing lichen at the site (McCune 1997). Therefore, an estimate of N input by *L. oregana* will approximate N input from cyanolichens in general at the WRCC site.

Sampling protocol

Sampling was scheduled to determine how physiological processes of *L. oregana* changed with season. At regular intervals during winter, spring, summer, and fall, the crane was used to collect *L. oregana* thalli from the forest canopy. Thalli were collected from two different heights in the canopy, and on two individuals each of *P. menziesii*, *T. heterophylla* and *T. plicata*. Temperature and photosynthetically active radiation (PAR) were recorded at each collection site. Some thalli were used immediately for physiological field measurements and some were air-dried and taken back to the laboratory for further analysis.

Measuring nitrogenase activity

Nitrogenase activity was measured using the Acetylene Reduction Assay (Hardy et al. 1973). The ARA is based on the fact that nitrogenase catalyzes the reduction of C_2H_2 to C_2H_4 more readily than the reduction of N_2 to NH_3. Lichen thalli were weighed, and 1-1.5 g samples were placed into the airtight plexiglass incubation chambers. Acetylene gas was generated by the reaction of calcium carbide with water. Acetylene was injected into the chambers to 10% of the chamber's volume (37 mL). The chambers were shaken vigorously and internal 12 V fans were used to insure air circulation within the chambers during incubation. Chambers were placed in conditions that approximated the ambient temperature and PAR levels of the lichen collection sites, and the lichen samples were incubated for 30-60 min. Empty control chambers were incubated in the same conditions (10% vol/vol acetylene injected) to detect the small background levels of ethylene present in the acetylene gas. After the incubation period, three 2-mL

gas samples were withdrawn from each chamber, and injected into leak-proof autosampler vials. Ethylene levels in the vials were determined with a Varian model 330 gas chromatograph. The amount of C_2H_4 produced was calculated as the average of the 3 gas samples withdrawn minus the average of the controls.

Measuring gas exchange

Gas exchange measurements were made using a LiCor 6400 Portable Photosynthesis System, where infrared gas analyzers detected differences between sample and reference CO_2 levels. All gas exchange measurements were made on the ground within 4 h of collecting lichen samples. The leaf-like thallus of *L. oregana* provided for ease of measurement with the clamp-on cuvette design, and humidity in the cuvette was kept at high levels to prevent desiccation during measurements. Temperature in the cuvette was set at the ambient air temperature at the lichen collection site. Net photosynthesis was measured at ambient light levels (typically 50-100 $\mu E\ m^{-2}\ sec^{-1}$ under cloudy conditions) and at high light levels (1200 $\mu E\ m^{-2}\ sec^{-1}$). Respiration rates were obtained by turning off the light source in the cuvette. Gross photosynthesis was calculated by adding the absolute values of respiration and net photosynthesis.

Calculating thallus hydration levels

Fresh weights of lichen thalli were obtained in the field prior to any physiological measurements. After lichens were used for either the ARA or gas exchange measurements, they were air-dried at room temperature for approximately 48 h and weighed again. Since lichens are poikilohydric organisms that undergo repeated cycles of wetting and drying, "saturation" water content may not be a repeatable parameter (Nash 1996). Therefore, hydration was expressed on an absolute basis, with oven-dried weight as the 0% hydration reference point. Oven drying kills the lichen, so a sub-sample of six "sacrificial" thalli were selected from each collection and then oven-dried for 24 h at 70 °C. The average ratio of oven-dried to air-dried masses calculated for the sacrificial thalli was used

as a multiplier to obtain 0% hydration masses (reference dry mass) for the rest of the thalli in the collection. The following equation was used to calculate thallus hydration levels:

hydration = ((fresh mass – reference dry mass) / reference dry mass) * 100%

Therefore, a thallus that is 100% hydrated has 1 g of water per 1 g of oven-dry thallus.

N content analysis

A sub-sample of nine lichen thalli from each seasonal collection was taken to the Forage Analytical Research Lab at Oregon State University. Crude N content was determined using the Macro Kjeldahl technique (AOAC 1995), in which proteins are extracted by acid digestion, and N is isolated by distillation and titration. Thallus N content is expressed as percent of dry mass.

Calculating N input from L. oregana litterfall

Litter data were used to determine the relative role of *L.oregana*'s N input by decomposition of litterfall. The on-going WRCC Fine Litter Study (D. Shaw, P.I.) was the source of data for estimating cyanolichen litter. In this study, twenty 40 cm x 40 cm litter traps were randomly placed within the crane plot. Litter samples were collected monthly during the snow-free season and then oven-dried, sorted into litter categories, and weighed. The categories of litter include needles (brown and green), deciduous leaves, twigs and cones, cyanolichens, other lichens, and moss. A more detailed description of the WRCC litter study methodology is available on-line at http://depts.washington.edu/wrccrf/craneplot/litter.html.

Cumulative litter mass for October 1997 – September 1998, October 1998 – September 1999, and October 1999 – September 2000 were summed to determine annual litterfall. We used the litter mass data, published N content values for the other six litter components, and N content data for *L. oregana* to calculate the

potential annual input of N from each litter category. The relative importance of each component was calculated as percentage of the total annual N input by litterfall.

Statistical analysis

All physiological data were analyzed using one-way ANOVA, where the independent variable was season, canopy height, or host tree species, and the dependent variable was the physiological parameter. Nitrogenase activity was the mean of 36 samples per seasonal trip, gas exchange data were the mean of n=12, and hydration levels were the mean of n=48. All means are shown plus or minus one standard error. ANOVA with interactions was used to test for significant differences in litterfall among years and months, with the independent variables month and year, and the dependent variable litter mass.

RESULTS

Seasonal differences in physiological activity of L.oregana

Nitrogenase activity, gas exchange rates, and thallus hydration levels for *L. oregana* thalli sampled during winter 1999, spring 2000, fall 2000, winter 2001, and spring 2001 changed with season (Table 2.1). Nitrogenase activity was higher in spring and fall than it was in either winter sample ($p<0.001$), and it was higher in spring 2000 and 2001 than in fall 2000 ($p<0.001$). There were no significant differences in nitrogenase activity between the two winter samples ($p=0.712$) or the two spring samples ($p=0.09$).

In the winter, even when *L. oregana* was fully hydrated, physiological activity was apparently limited by low temperature. In field conditions when temperatures were 0-4 °C, nitrogenase activity and respiration were low, despite hydration levels of over 250%. Physiological activity of *L. oregana* was highest in

spring and fall when lichens were hydrated. Positive carbon gain occurred only in winter and spring when respiration rates were low (Table 2.1).

Estimating N input from L. oregana by decomposition of litterfall

The decomposition of *L. oregana* litter is an important pathway for N in the lichen to be released to the ecosystem. The annual cumulative mass of seven different litter components showed cyanolichens comprised less than 1% of the litterfall within that three-year period (Table 2.2). There was no significant difference in cyanolichen litterfall among the three years ($p = 0.14$), and most of the variation in the litter data was explained by month ($p<0.01$) rather than year. There was a sizeable pulse in cyanolichen litterfall in late 1999 to early 2000, presumably due to winter storms (Figure 2.1).

The average N content of *L. oregana* tissue was 1.85 – 2.14 % on a dry mass basis, and there were no significant differences in thallus N content among seasons ($p=0.09$). Cyanolichen litter was assumed to be composed almost entirely of *L. oregana* (Clement & Shaw 1999). Therefore, approximately 20 kg ha^{-1} of *L. oregana* litter released about 0.4 kg N ha^{-1} yr^{-1} into the forest ecosystem at the WRCC (Table 2.3).

Estimating annual nitrogen fixation by L.oregana

Lobaria oregana makes up 42% of the 1300 kg ha^{-1} of epiphytic macrolichen biomass at the WRCC site (McCune 1997). Each year, around 4% of *L.oregana*'s standing biomass falls to the ground as litter (Table 2.2). In order to maintain a steady-state biomass, *L. oregana* must fix enough N to grow 20 kg ha^{-1} of new lichen tissue. Therefore, 0.4 kg ha^{-1} yr^{-1} represents the minimum annual nitrogen fixation rate by *L. oregana* at WRCC.

Evidence suggests that cyanolichen biomass continues to increase over time (Neitlich 1993, Howe 1978). Therefore, *L. oregana* must fix additional nitrogen beyond that required for maintenance of a steady-state canopy biomass.

Table 2.1. Physiological characteristics of *L. oregana* collected in the old-growth Douglas-fir forest at the WRCC site*

	Winter 1999	Spring 2000	Fall 2000	Winter 2001	Spring 2001
Temperature (°C)	2 - 4	10 - 13	12 - 14	0 - 3	7 - 9
Nitrogenase activity† ($nmol\ C_2H_4\ g^{-1}\ h^{-1}$)	24.1 ± 6.6	171.5 ± 20.4	269.7 ± 22.8	28.1 ± 9.3	115.4 ± 23.8
Net photosynthesis‡ ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)					
ambient light	-0.1 ± 0.1	-0.7 ± 0.3	-0.6 ± 0.2	0.1 ± 0.1	0.3 ± 0.1
high light	0.5 ± 0.2	-0.2 ± 0.2	-0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Respiration ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.9 ± 0.1	2.5 ± 0.4	1.5 ± 0.2	0.4 ± 0.1	0.7 ± 0.1
Gross photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)					
ambient light	0.8 ± 0.1	1.7 ± 0.3	0.9 ± 0.2	0.6 ± 0.1	1.0 ± 0.1
high light	1.4 ± 0.1	2.3 ± 0.3	1.3 ± 0.2	0.8 ± 0.1	1.1 ± 0.1
Thallus hydration (%)	277 ± 14	196 ± 8	207 ± 4	249 ± 6	199 ± 12

* Data are means ± 1 SE.

† Data are means of 36 Acetylene Reduction Assays with 3 replicate gas samples per assay.

‡ Data are means of 12 samples with 6 measurements averaged per sample.

Table 2.2. Cumulative mass of various litterfall components in the old-growth Douglas-fir forest at the WRCC site

LITTER COMPONENT	MASS (kg ha^{-1})†			3-YEAR MEAN (kg ha^{-1})
	1998	1999	2000	
Green needles	75*	131	135	114
Brown needles	2058*	2946*	2257*	2420
Non-conifer leaves	14	27	25	22
Twigs & cones	1334	1562	2155	1684
Cyanolichens	11	18	33	20
Other lichens	75	114	112	100
Moss	15	15	37	22
TOTAL	3582	4812	4753	4383

* significant differences between years ($p<0.01$ with ANOVA)
† WRCCRF Fine Litter Study (Shaw)

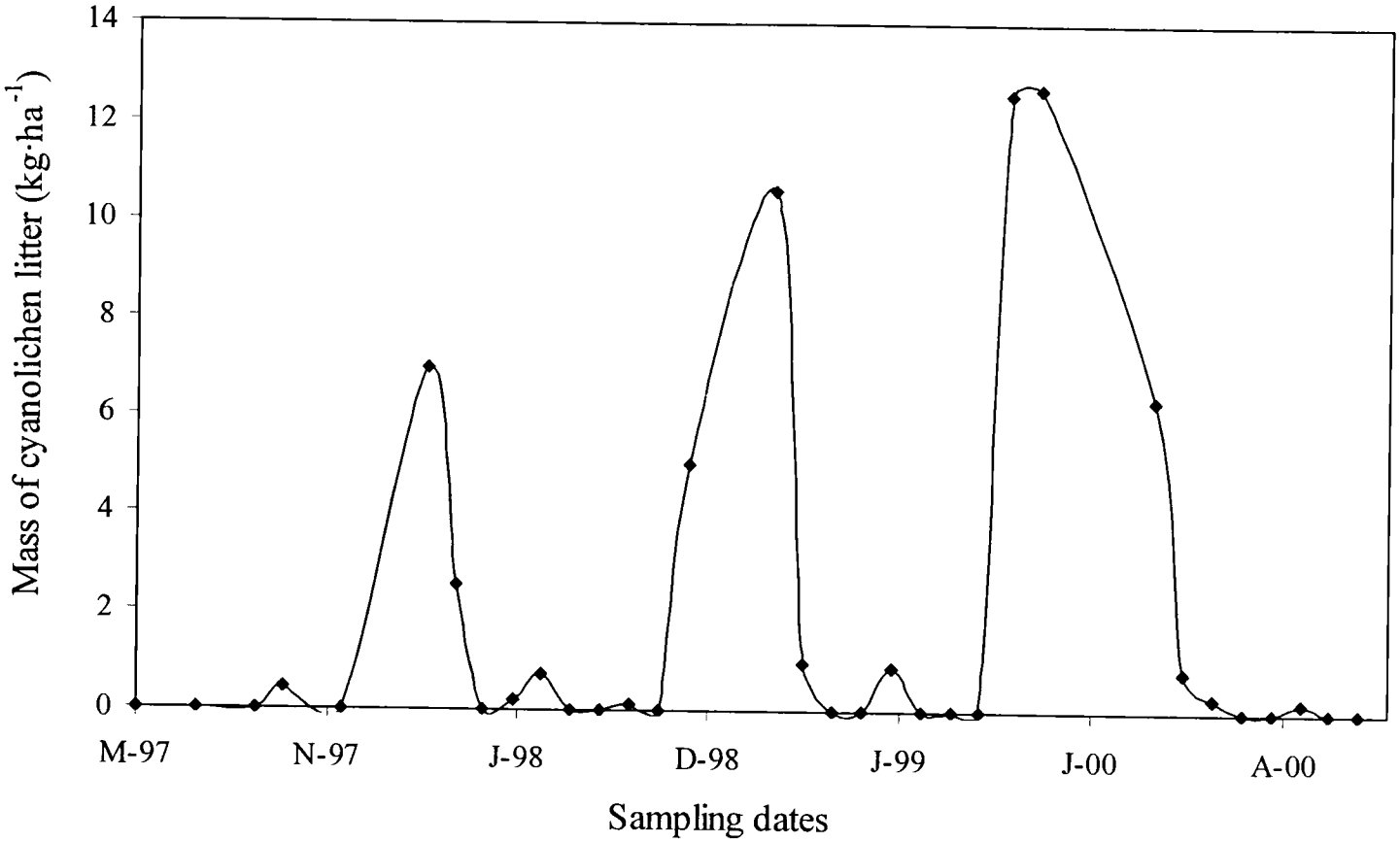

Figure 2.1. Seasonal trends in cyanolichen litterfall over a 3-year period at the Wind River Canopy Crane site

Table 2.3. Potential annual N input through decomposition of litterfall in the old-growth Douglas-fir at the WRCC site

Litter component	N content (% DW)	Potential N input (kg ha^{-1})			Mean annual N input (kg ha^{-1} yr^{-1})	Mean contribution (% of total N input)
		1998	1999	2000		
Green needles	1.4*	1.1	1.8	1.9	1.6	7.9
Brown needles	0.5*	9.5	13.6	10.4	11.1	56.4
Non-conifer leaves	1.0*	0.1	0.3	0.2	0.2	1.1
Twigs & cones	0.3*	4.5	5.3	7.3	5.7	28.9
Cyanolichens	2.0	0.2	0.4	0.7	0.4	2.1
Other lichens	0.5†	0.4	0.6	0.6	0.5	2.5
Moss	1.0†	0.1	0.1	0.4	0.2	1.1
TOTAL	-	15.9	22.0	25.1	19.8	100

* Data are from Sollins et al. (1980).
† Data are from Pike and Tracy (1972).

The best estimate of an annual growth rate for this lichen is 15% (Sillett and McCune 1998). After accounting for the 4% productivity rate required to replace tissue lost as litterfall, 11% of the potential annual productivity remains to contribute to an increasing canopy biomass. The growth of 61.5 kg ha^{-1} of new lichen tissue requires the fixation of 1.2 kg ha^{-1} of nitrogen. Therefore, based on known ecological parameters, *L. oregana* fixes between 0.4 – 1.6 kg N_2 ha^{-1} each year at WRCC (Table 2.4).

Table 2.4. Estimate of annual nitrogen fixation by *L. oregana* in the old-growth Douglas-fir forest at the WRCC site

Canopy biomass (McCune 1997)	546 $kgha^{-1}$
Average annual litterfall (WRCC)	20.4 $kgha^{-1}$
Canopy biomass lost as litterfall (%)	3.7 %
N content of *L. oregana* tissue (% dry mass)	2.01 %
N fixation needed to replace biomass lost as litterfall	0.4 $kgha^{-1}$
Estimated annual productivity of *L. oregana* (Sillett & McCune 1998)	15%
Productivity remaining after litterfall is replaced	11.3 %
Potential annual canopy biomass increase	61.5 $kgha^{-1}$
N fixation needed to allow canopy biomass increase	1.2 $kgha^{-1}$
Estimated annual nitrogen fixation by *L. oregana*	0.4 – 1.6 $kgha^{-1}$

DISCUSSION

Linkage between N fixation and C metabolism

Respiration rates and nitrogenase activity in *L. oregana* were highest when temperatures were moderate. However, net photosynthesis was only observed at cooler temperatures, presumably because low temperatures inhibited the respiratory cost of the fungus (Kershaw 1985). The temporal separation of net C and N gain suggests a mechanism for storing fixed C until warmer temperatures allow N fixation and the capacity for rapid fungal respiration and growth. Evidence for this mechanism may lie in leaching experiments that identified large quantities of ribitol as the primary leachate from *L. oregana* thalli (Cooper & Carroll 1980). Ribitol is the form in which the photobiont, *Myrmecia*, transfers carbon (Ahmadjian 1993). Although ribitol may allow the accumulation of fixed C in the lichen thallus, it is unlikely to be a safe long-term storage compound because it is water-soluble (Honegger 1991). Maximum growth rates for *L. pulmonaria* occur in the spring (Muir & Shirazi 1997), and the data in Table 2.1 suggest that the same may be true for *L. oregana.*

Previous estimates of N input by L. oregana

Although earlier estimates of annual N fixation by *L. oregana* assumed the lichen was constantly and optimally active throughout the wet season, nitrogenase activity and gas exchange rates varied significantly when *L. oregana* was sampled at the WRCC site. These differences in metabolic activity seem to be primarily temperature dependent. Like any other enzyme, nitrogenase operates best within an optimal temperature range, but this optimal range depends on the particular species of cyanobiont and ecological habitat. Measurable N fixation is known to occur at temperatures as low as –5 °C in some arctic species (Kallio et al. 1972). Our observations of negligible N fixation near 0 °C are consistent with Denison (1979). Daily mean temperatures at the WRCC site remain close to 0 °C during December and January. Therefore, N fixation in *L. oregana* does not occur at a constant

optimal rate throughout the winter rainy season at this site because of low temperatures.

The process of converting ARA to N fixation rates can lead to errors in calculating N fixation rates. After measuring nitrogenase activity with the ARA, Denison (1979) used a 3:1 conversion ratio for C_2H_2 : N_2 reduction rates. However, the balanced equation for the nitrogenase reaction is:

$$N_2 + 8e^- + 16ATP + 16H_20 \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i + 8H^+$$

Hydrogen is a by-product of N_2 reduction, so the reduction of N_2 to NH_3 requires 8 electrons. The reduction of C_2H_2 to C_2H_4 requires 2 electrons, which means that for every mole of N_2 reduced, the equivalent of 4 moles of C_2H_2 can be reduced. Therefore the biochemically accurate conversion ratio between the ARA and N_2 fixation for *L. oregana* is 4:1. This is the theoretical conversion ratio. The actual efficiency of nitrogenase can vary with temperature and other environmental factors, resulting in ratios as high as 20:1 or as low as 0.3:1 (Schwintzer & Tjepkema 1994, Liengen 1999, Nohrstedt 1983). Using the 4:1 conversion ratio reduces Denison's (1979) estimate by 25% to 2.6 kg N ha^{-1} yr^{-1}.

Estimating annual input of N by L. oregana litterfall

There are three important sources of N in the forest ecosystem: biological fixation of new N, deposition of new N from the atmosphere, and recycling of N within the ecosystem. At the WRCC site, most of the input of N through decomposition of litterfall represents the recycling of N within the forest ecosystem (Table 2.4). Although *L. oregana* has the highest N content of any of the litter components, its litter biomass is relatively small, so it accounts for a small percentage of the total potential N input through litterfall. However, it is important to note that this 0.4 kg ha^{-1} yr^{-1} is new N being introduced into the forest ecosystem at the WRCC site. The half-life of *L. oregana* litter is 7 months (McCune & Daly

1994) but the N in lichens consumed by banana slugs or deer (personal observation) may the ecosystem more rapidly.

N input from precipitation at the WRCC site is of the same magnitude as the input from cyanolichen litterfall—approximately 0.6 kg ha^{-1} yr^{-1}. This estimate is based on the annual precipitation total at the WRCC site (2500 mm) and N content (1.8 μmol N L^{-1}) of precipitation in western WA (Edmonds et al. 1997). N deposition necessarily accounts for some of the N present in cyanolichen tissues, and using N content of *L. oregana* to estimate N fixation may result in slight over-estimations of annual N fixation.

The input of N from other lichens is also potentially important. The lichens that cannot fix N obtain it from atmospheric deposition, and their high surface area enhances the capture of new N (Knops et al. 1991). Therefore, when considering the role of lichens in forest nutrient cycling, it is important to consider all of the lichens, not just the cyanolichens. Obviously, the relative importance of cyanolichens and other lichens will vary from site to site according to the biomass and composition of the lichen community.

Several studies have shown the leaching of nitrogenous compounds from *L. oregana* (Millbank 1985, Millbank 1982, Pike 1978). Two lines of evidence suggest this leaching from lichen thalli is most important on the local scale, fertilizing the food webs of the surrounding canopy rather than the trees themselves. First, the experiments producing large quantities of nitrogenous leachates typically involved submerging the lichen thalli in water, which would not occur under most natural conditions (Nash 1996). A dry *L. oregana* thallus absorbs 2-4 g of water per g of dry mass, which means that many rainfall events may not saturate the thallus sufficiently to allow accumulation and run-off of water. Second, the N content of *L. oregana* does not vary from season to season. Therefore most fixed N is immediately incorporated into new lichen tissue, rather than accumulating in forms that would leach out.

Estimating annual N fixation by L.oregana

A straightforward approach to evaluating the ecological context of *L. oregana* is to use what is already known about the lichen. The WRCC fine litter study provides an estimate of how much *L. oregana* falls to the ground each year, thus allowing the approximation of the minimum amount of N that *L. oregana* must fix annually simply to maintain a steady-state canopy biomass. Is this steady-state canopy biomass assumption reasonable for a cyanolichen? Franklin and Spies (1991) define old growth as forests where biomass production has reached a steady state, and the WRCC site certainly displays many old-growth characteristics. However, the case of cyanolichens warrants further consideration. Cyanolichens may be present in young forests, but their rate of accumulation is limited by propagule dispersal, and their biomass reaches peak abundance only in very old forests (Sillett et al 2000). So, in estimating annual fixation in the 500-year-old forest at the WRCC site, we must also consider how much N *L. oregana* must fix in order to support a reasonable growth rate.

Lobaria oregana has long been considered an unusually productive lichen species, with annual growth rates exceeding 30% (Rhoades 1977). However, this estimate was based on a set of sequential photographs of only four mature thalli. More recent measurements of mass changes for hundreds of thalli indicate an average annual growth rate of at least 15% (Sillett & McCune 1998, Denison 1988).

Growth rates of individual thalli do not translate directly into annual stand-level productivity rates. If the canopy biomass of *L. oregana* increased by 15% every year, cyanolichens would dominate the ecosystem within a few decades. A net annual biomass gain of only 2-3 % is necessary to explain the rate at which *L. oregana* accumulates as forests age. At the WRCC, around 4% of the canopy biomass falls as litter each year. However, the amount of *L. oregana* tissue that decomposes *in situ* can be several times higher than the amount that reaches the ground as litter (Rhoades 1978). In addition, severe storms result in catastrophic losses of large *L. oregana* thalli, and it may take up to 30 years for canopy biomass

to recover from such events (Rhoades 1978). Therefore, although individual thalli may have a 15% annual growth rate, much of this productivity goes into the maintenance or recovery of steady-state canopy biomass.

It is important to note that the abundance of *L. oregana* at the WRCC is much lower than in many other sites. For example, *L. oregana* biomass exceeds 3000 kg ha^{-1} in some parts of the H.J. Andrews Experimental Forest (Neitlich 1993), and in the 700-year-old stands of the Middle Santiam Wilderness the standing crop of *L. oregana* is estimated to be around 8000 $kgha^{-1}$ (Sillett 1995). Obviously, annual N fixation by *L. oregana* in these forests may be many times greater than the 0.4-1.6 kg N_2 ha^{-1} yr^{-1} at the WRCC site.

Differences in lichen biomass and local patterns of temperature and precipitation determine how much N *L. oregana* is capable of fixing, and an approach allowing site-specific estimates must include a physiological component. The need is for a model that estimates annual N fixation by *L. oregana* based upon a series of physiological response curves and detailed meteorological data. This model will allow the evaluation of *L.oregana*'s ecological importance on a site by site basis.

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Chapter 3

Analysis of Nitrogen Fixation by *Lobaria oregana*: A Physiological Approach

Marie E. Antoine

ABSTRACT

Lobaria oregana is an epiphytic cyanolichen associated with humid old-growth forests in the Pacific Northwest. Nitrogen fixation by *L. oregana* provides an ecologically significant input of new N into the forest ecosystem. This paper presents a model that can be used to quantify N fixation rates by *L. oregana* at any given site, provided that meteorological data and canopy biomass estimates exist for the site. The two physiological parameters controlling nitrogenase activity in *L. oregana* are thallus hydration and temperature. At the Wind River Canopy Crane (WRCC) site, *L. oregana* fixes around 1.6 kg N_2 ha^{-1} yr^{-1}. Maximum N fixation rates occur in the fall and spring, and low temperatures inhibit nitrogenase activity during the winter. At the HJ Andrews Experimental Forest (HJA), *L. oregana* fixes between 2.6-16.5 kg N_2 ha^{-1} yr^{-1}, depending on its stand-level canopy biomass. The WRCC site receives slightly more rain than HJA. However, winter and spring temperatures at HJA are warmer, resulting in consistently higher N fixation rates. The wide range in estimates of total annual N fixation emphasizes the importance of evaluating *L. oregana*'s ecosystem contributions on a site by site basis.

INTRODUCTION

Cyanolichens dominate epiphyte assemblages in humid old-growth Douglas-fir forests in the Pacific Northwest (Sillett 1995, Neitlich 1993, Pike et al. 1975). They are an important component of these ecosystems for many reasons including their ability to fix atmospheric N_2 into forms of nitrogen available for plant uptake (Denison 1979, Pike 1978). Nitrogen-rich leachates and *in situ* decomposition of cyanolichens provide part of the nutritional base for complex food webs in the forest canopy (Carroll 1979). *Lobaria oregana* (Tuck.) Müll. Arg. is the most abundant cyanolichen, often accounting for 60-80% of the total

epiphytic lichen biomass in humid old-growth forests (Sillett 1995, McCune 1994, Pike et al. 1977).

Several attempts have been made to quantify annual N fixation by *L. oregana*. Research at the HJ Andrews Experimental Forest (HJA) in western Oregon suggested that *L. oregana* fixes 3-4 kg N ha^{-1} yr^{-1} (Denison 1979, Pike 1978), contributing over 50% of the annual new N input to the ecosystem (Sollins et al. 1980). A re-evaluation of the underlying assumptions suggests that these studies may have somewhat overestimated annual N fixation in the HJA sites (Chapter 2). At the Wind River Canopy Crane (WRCC) site in southern Washington, annual N fixation is estimated to be between 0.4-1.6 kg ha^{-1} yr^{-1} (Chapter 2). The discrepancy between estimates for different study sites indicates that generalizations about *L. oregana* over its entire range should be made with caution. However, a model based on the physiological parameters controlling N fixation by *L. oregana* allows evaluation of the lichen's ecological importance in any given site, provided that meteorological data and canopy biomass estimates exist for the site.

The goal of this paper is to develop a model of N fixation by *L. oregana* that can be used to estimate annual N fixation at any site, and our objectives are to define the physiological parameters that control N fixation by *L. oregana*, use these parameters to develop a model of N fixation, and use the model to estimate annual N fixation by this species at the WRCC and the HJA.

METHODS

Study site

The Wind River Canopy Crane was used to collect *Lobaria oregana* from the canopy of the Wind River Experimental Forest. WRCC is located within the Gifford Pinchot National Forest (45°49'N, 121°55'W) near Carson, Washington.

The 4 ha crane plot is centered on a Liebherr 550 HC construction crane that provides non-destructive access to 10^5 m^3 of old-growth forest canopy. *Pseudotsuga menziesii* (Mirb.) Franco and *Tsuga heterophylla* (Raf.) Sarg. are co-dominant in the canopy, and *Thuja plicata* (Donn.) is also common. The 500-year-old stand surrounding the WRCC displays old-growth characteristics such as the presence of *Taxus brevifolia* (Nutt.) and an abundance of snags and fallen logs (Franklin & Spies 1991, Norse 1990). The non-vascular epiphyte community is vertically stratified, with peak abundance of *L. oregana* and other cyanolichens occurring in the "light transition zone" of the middle canopy (McCune 1997).

The meteorological data used in this study were recorded at an open met station on the ground near the crane tower. Instantaneous measurements of temperature, relative humidity (RH), precipitation, and photosynthetically active radiation (PAR) were logged every 30 min. Detailed meteorological data from February 1998 to the present are available on-line at www.depts.washington.edu/wrccrf.

Sampling protocol

Lobaria oregana thalli were collected at two canopy heights (15m and 35m) from two individuals each of *P. menziesii*, *T. heterophylla* and *T. plicata* during regular intervals from winter 1999 to spring 2001. Temperature and PAR were recorded at each collection site. Thalli were either immediately used for physiological field measurements or air-dried for use in the laboratory experiments described below.

Physiological measurements

Nitrogenase activity was measured with the Acetylene Reduction Assay (ARA) (Hardy et al. 1973), which is based on the fact that nitrogenase catalyzes the reduction of C_2H_2 to C_2H_4 more readily than it catalyzes the reduction of N_2 to NH_3. Lichen thalli were incubated for 60 min in airtight chambers containing 10% vol/vol acetylene gas. Empty control chambers were also incubated to quantify the

small background levels of ethylene present in the acetylene. Replicate gas samples were withdrawn from each experimental and control chamber, and rates of ethylene production were determined using a Varian model 330 gas chromatograph. The theoretical 4:1 conversion ratio between acetylene reduction and N_2 reduction rates was used (Liengen 1999).

Lichens are poikilohydric organisms and saturation water content may not be a repeatable parameter. Therefore, hydration was expressed on an absolute basis, with oven-dried mass as the 0% reference point. A sub-sample of six "sacrificial" thalli were selected from each seasonal collection, oven-dried for 24 hours at 70°C, and the average ratio of oven-dried to air-dried masses was used as a multiplier to obtain 0% hydration for the remaining thalli. Therefore, a thallus at 200% hydration contains 2g of water per 1g of oven-dry mass.

Field measurements

The nitrogenase activities of 36 lichen samples were measured with the ARA under ambient conditions. Incubation temperatures were documented in order to compile data for a field-based temperature response curve (0-15°C) of nitrogenase activity.

Lichens collected in fall 2000 were randomly assigned to four groups and stored in the dark for 0, 6, 12, or 16 hours prior to the ARA. This experiment was designed to test whether N fixation in hydrated lichens assayed at 5°C occurred at the same rate during night and day.

Desiccation rates of *L. oregana* were measured in the field at 2°C and 98% RH under overcast skies. Thalli were placed on an accessible branch, "saturated" using a spray bottle, and left to dry for 0-90 min. Groups of three thalli were removed and weighed at 10 min intervals.

Laboratory experiments

A lab-based temperature response curve for nitrogenase activity was constructed using thalli collected in spring 2001. Thalli that had been air-dried and

stored for 72 h were re-hydrated for 60 min in distilled water ranging in temperature from 0-20°C. The ARA was used to measure nitrogenase activity during a 60 min incubation period at each 2°C interval (n=3 per temperature level). PAR levels were kept constant at around 100μE m^{-2} sec^{-1} for all assays.

The relationship between thallus hydration level and nitrogenase activity was determined using thalli collected in fall 2000. First, thalli that had been air-dried and stored for 72 h were re-hydrated in distilled water for 0, 5, 10, 30, 60, 120 and 180 min (n=3 per hydration time) to determine how fast re-hydration occurred. Next, lichens that had been stored for approximately one week were re-hydrated to approximately 300, 250, 200, 150, and 50% hydration levels (n=6 per hydration level, actual hydration levels were ± 20% of target hydration level). The ARA was used to measure nitrogenase activity in the lichens at each hydration level. Temperature and PAR level were kept constant at around 13°C and 100 μE m^{-2} sec^{-1}, respectively, for all assays.

Desiccation rates for *L. oregana* were measured in the lab at 80%, 60%, and 40% RH. Thalli were re-hydrated for 60 min, weighed, and placed in the LiCor cuvette where RH was controlled to each experimental level. Thalli were incubated in the cuvette for 10-90 min (n=3 per 10 min interval) and then removed and re-weighed to determine hydration level.

Modeling annual nitrogen fixation

The experiments described above define the physiological constraints within which *L. oregana* operates and determine which of the environmental parameters (i.e., thallus water content, canopy position, light levels, and temperature) are important in modeling annual N fixation. Results of these experiments (see Results) produced the following model assumptions:

1. *Lobaria oregana* must be hydrated to be physiologically active.
2. Nitrogenase activity is not dependent on canopy position or host tree species.
3. Nitrogen fixation rates are independent of light.

4. Nitrogen fixation rates are controlled primarily by temperature.
5. *Lobaria oregana* becomes fully hydrated within 5-10 min of rainfall.
6. When rain stops, *L. oregana* remains physiologically active for approximately 60 min.

Therefore, for the purpose of this model precipitation events were considered ON/OFF switches for physiological activity and temperature controlled the magnitude of nitrogenase activity in physiologically active lichens. WRCC meteorological data provided a record of precipitation and temperature at 30 min intervals for 1998, 1999, and 2000. The average of the lab- and field-based temperature response curves was used for the entire model after a sensitivity analysis was performed with the two separate curves and the 1999 met data.

The temperature response curve was used to generate temperature-dependent N fixation rates (nmol N_2 fixed per kg dry mass of lichen) for each 30 min period. The lichens were assumed to be active only during and 60 min after precipitation events. Daily, monthly, or annual N fixation was simply the sum of all 30 min periods in which N fixation occurred. Rates were scaled up to kg N_2 fixed ha^{-1} by multiplying by estimated standing biomass of *L. oregana.*

Using the model for the HJA

Meteorological data from Climatic Station 2 (CS2MET) at the HJA from May 1998 to April 1999 were obtained on-line from http://ftp.fsl.orst.edu/pub/henshaw/pinto. Temperature and precipitation measurements were recorded at 15 min intervals, and 30 min averages were calculated for use in the model. CS2MET is located within the old-growth forest of Watershed #2 (44°54' N, 122°57' W). Nitrogen fixation rates were calculated using the model parameters described above. Annual N fixation was estimated for sites within HJA with *L. oregana* biomasses of 3523, 2620 and 1587 kg ha^{-1} (Neitlich 1993) and 500 kg ha^{-1} (Denison 1979).

Statistical analysis

One-way ANOVA was used to test for significant differences in nitrogenase activity among treatments. Nitrogenase activity was the dependent variable, and the independent variable was either length of dark exposure, canopy height, or tree species. To test the effect of exposure to darkness on nitrogenase activity, n=9 in each of the four treatment groups (i.e., 0, 6, 12, and 16 hours of darkness). To test the effect of canopy height, n=18 in each of the two treatment groups (i.e., 15m and 35m). To test the effect of host tree species, n=12 in each of the three treatment groups (i.e., *P. menziesii*, *T. heterophylla* and *T. plicata*).

RESULTS

Developing the model parameters

Nitrogenase activity in *L. oregana* increased as thallus water content increased (Figure 3.1). There was no measurable N fixation in lichens that were less than 50% hydrated, and rates remained low at 100% hydration. Beyond this level nitrogenase activity increased rapidly with increasing hydration and reached apparent saturation at around 200%. Although the highest N fixation rates were measured in thalli exceeding 300% hydration, all wet lichens collected from the field had water contents between 150% and 295% (Table 3.1). Field and lab measurements showed that average N fixation rates in fully hydrated lichens peaked at around 75nmol N_2 g dry mass^{-1} hr^{-1}.

Rehydration of desiccated lichens occurred within 10 min of exposure to simulated rainfall, although saturation did not occur for 60 min (Figure 3.2). Since thalli quickly reached the minimum hydration levels necessary for optimal nitrogenase activity (i.e., 150-200%) our model assumed that precipitation events "turned on" N fixation.

The rate at which *L. oregana* desiccates depended on the relative humidity of the surrounding air and the size of the lichen thallus. Thallus size was not

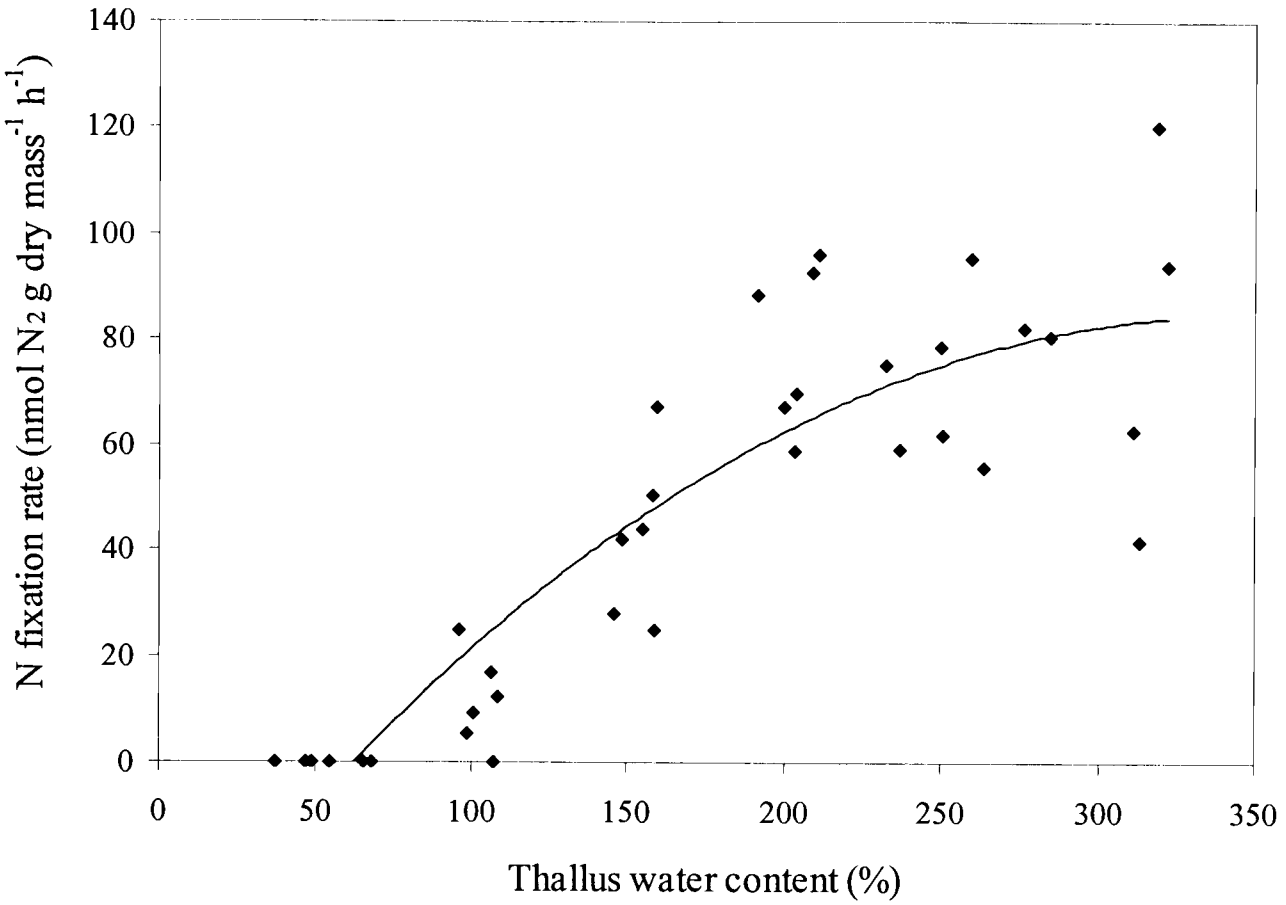

Figure 3.1. The relationship between N fixation rates and thallus water content of *Lobaria oregana*

Table 3.1. *In situ* nitrogenase activity and thallus water content of *Lobaria oregana* in the 500-year-old Douglas-fir forest at the Wind River Canopy Crane site

	WINTER 1999	SPRING 2000	FALL 2000	WINTER 2001	SPRING 2001
TEMPERATURE (°C)	2 - 4	10 - 13	12 - 14	0 - 3	7 - 9
NITROGENASE ACTIVITY† ($nmol C_2H_4\ g^{-1}h^{-1}$)	24.1 ± 6.6	171.5 ± 20.4	269.7 ± 22.8	28.1 ± 9.3	115.4 ± 23.8
THALLUS HYDRATION (%)	277 ± 14	196 ± 8	207 ± 4	249 ± 6	199 ± 12

* Data are means ± 1 SE.

† Data are means of 36 Acetylene Reduction Assays with 3 replicate gas samples per assay.

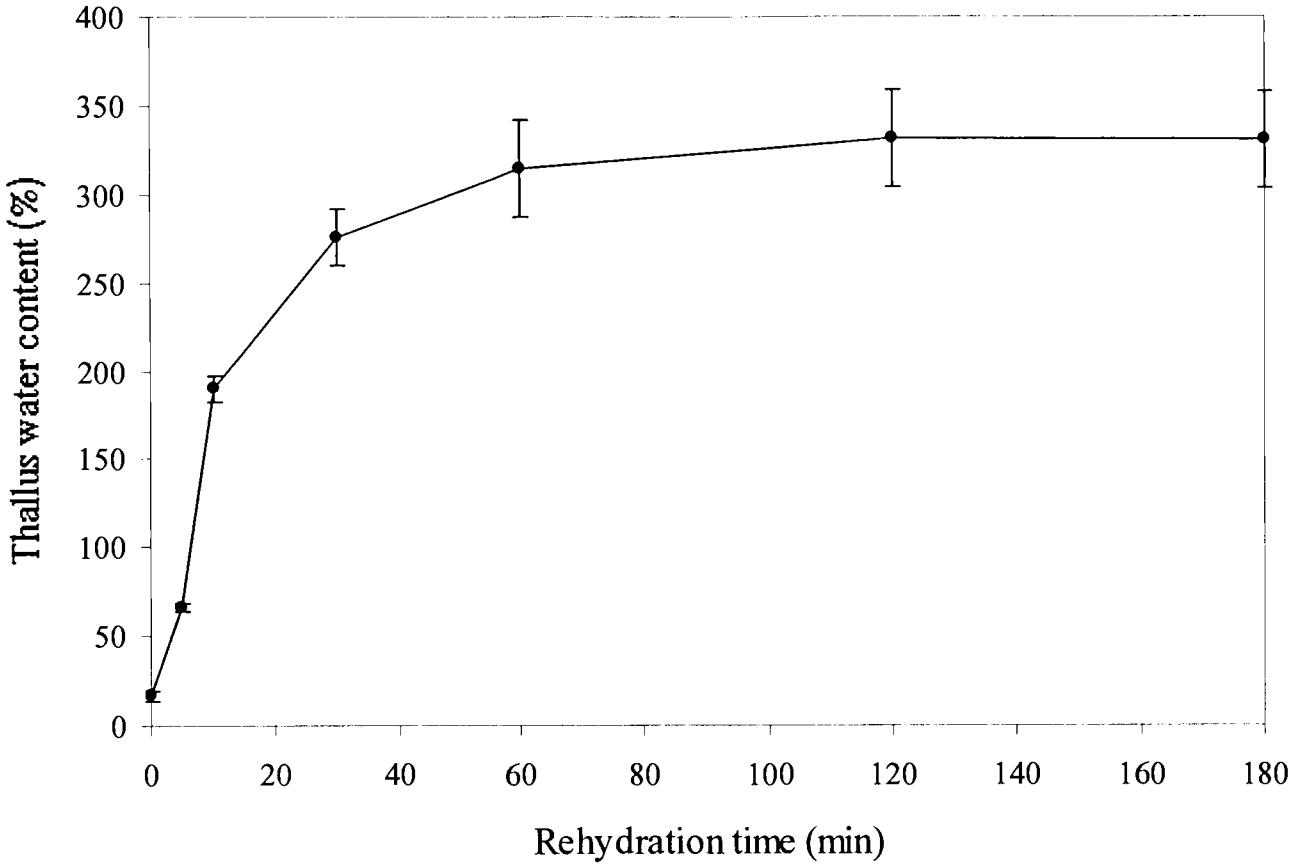

Figure 3.2. Thallus water content of *Lobaria oregana* over different rehydration times

directly considered in our model. We assumed that the faster drying of small thalli and slower drying of large thalli averaged as the desiccation rates of our medium sized (around 2g dry mass) thalli. Even at 98% RH, thallus water contents dropped to below 100% after 60 min, and at lower RH this same water loss occurred in less than half the time (Figure 3.3). Since nitrogenase activity rapidly dropped to zero below 100% thallus hydration (Figure 3.1) we assumed that *L. oregana* fixed N for 60 min after rain stopped and then "turned off".

Nitrogenase activity in *L. oregana* did not differ among groups of lichens assayed at constant temperature after exposure to 0-16 h of darkness ($p=0.29$). The longest nights in the middle of winter at WRCC do not exceed 16 h. Therefore, we assumed that light does not limit nitrogenase activity.

The average nitrogenase activity for 18 thalli collected in Spring 2000 from the middle (35m) of the canopy was 45±6 nmol N_2 g DW^{-1} h^{-1}. This was not significantly different from the 39±6 nmol N_2 g DW^{-1} h^{-1} average activity measured in 18 thalli collected from the bottom (13m) of the canopy ($p=0.08$). The average nitrogenase activities for groups of 12 thalli collected from *P. menziesii*, *T.heterophylla*, and *T. plicata* were 44± 5, 42±4 and 41±5 nmol N_2 g DW^{-1} h^{-1}, respectively. There was no difference in average nitrogenase activity among thalli collected from different host tree species ($p=0.37$). Therefore, we did not consider canopy position or host tree species in modeling N fixation by *L. oregana*.

Temperature was the parameter that directly controlled nitrogenase activity in hydrated *L. oregana* (Figure 3.4). There was no nitrogenase activity at 0°C in lichens measured in the lab or in the field. Measurable activity began at 1-2 °C and increased linearly with increasing temperature up to 20 °C. The average of the best-fit lines through the lab and field data was chosen to model the relationship between temperature and nitrogenase activity (Figure 3.4). The relationship is:

$$\text{N fixation} = 5.88(\text{temperature}) - 2.83 \quad (R^2 = 0.90)$$

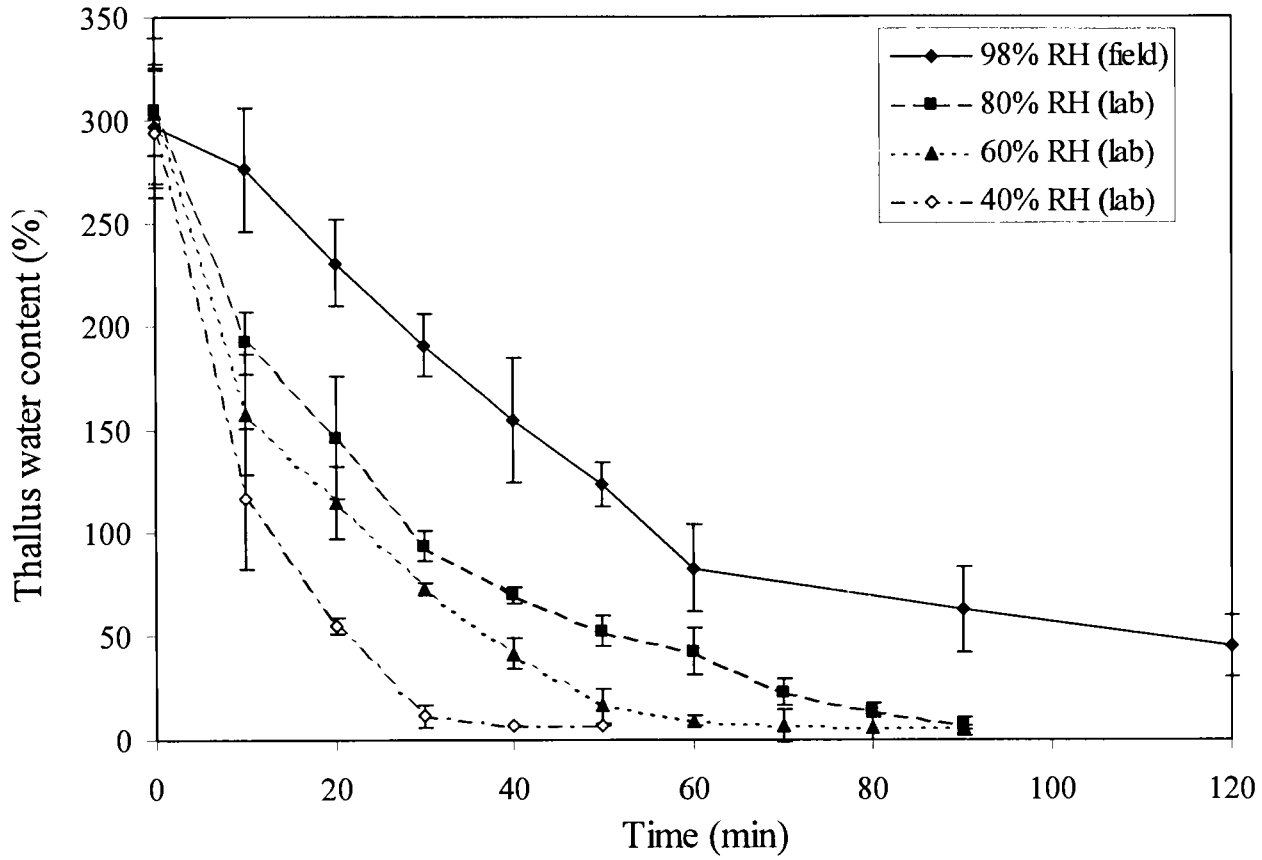

Figure 3.3. Desiccation trends of *Lobaria oregana* at four atmospheric humidity levels

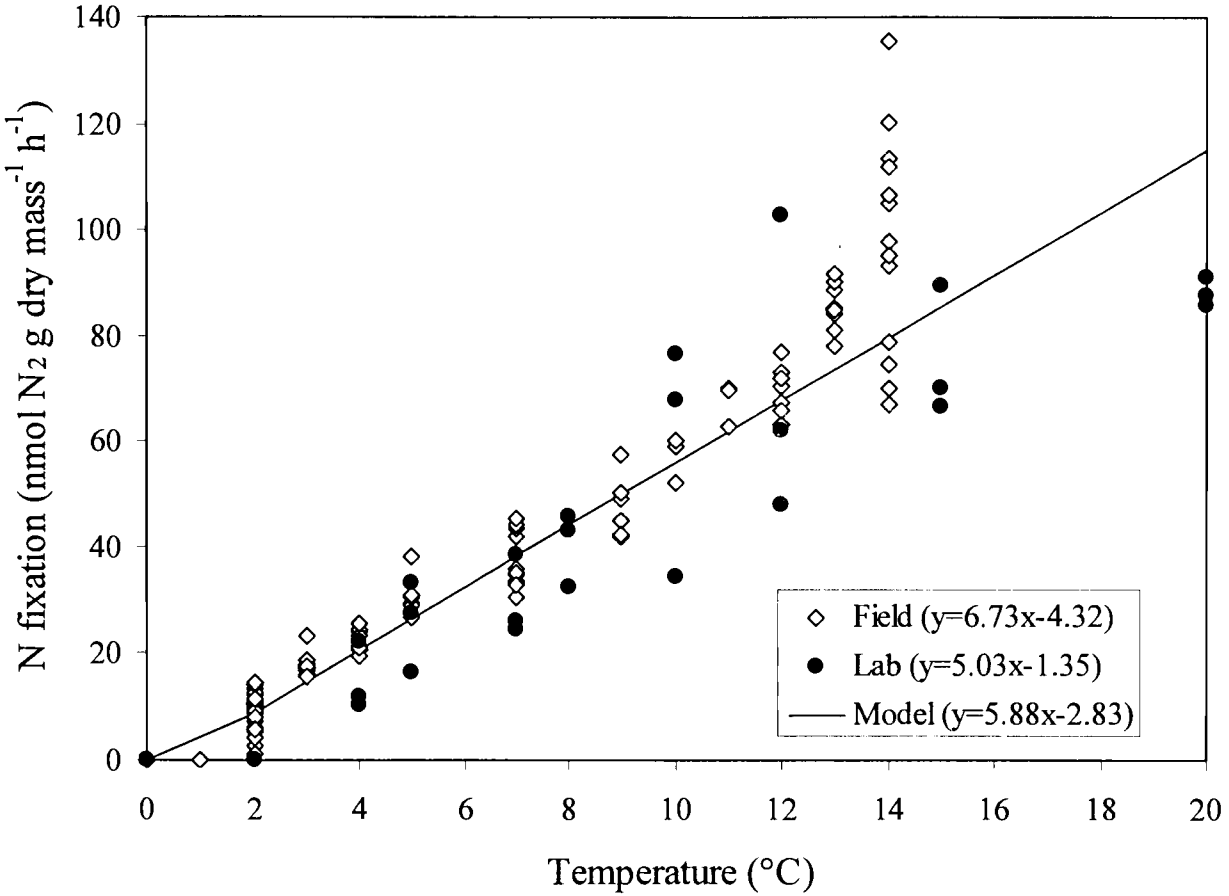

Figure 3.4. Temperature response curves for nitrogenase activity in *Lobaria oregana*

Temperatures at and below 0°C were automatically assigned zero N fixation activity. A temperature-dependent N fixation rate was calculated for each 30-min period with rain or within 1 h of rain.

N fixation by L. oregana *at WRCC*

Before using the average temperature response curve to model N fixation over the entire three-year period, a sensitivity analysis was performed to test the effect of the different slopes of the field- and lab-based curves. Each of the three equations was used to calculate the temperature-dependent N fixation rate for 1999 (Table 3.2). Since the different curves did not affect the annual estimate by more than 10%, we decided that the average of the two curves was an appropriate representation of the relationship between temperature and N fixation rates.

Table 3.2. Differences in estimates of annual N fixation by *Lobaria oregana* at the Wind River Canopy Crane site using three different temperature response curves for nitrogenase activity

N FIXATION EQUATION	DATA SOURCE	N FIXATION ($kg\ ha^{-1}\ yr^{-1}$)	DIFFERENCE FROM MODEL
5.03 (temp) – 1.35	lab	1.29	- 10 %
6.73 (temp) – 4.32	field	1.60	+ 10 %
5.88 (temp) – 2.83	average (model)	1.44	-

Assuming the model parameters described above and a canopy biomass of 550 kg ha^{-1} (McCune 1997), we estimate that *L. oregana* fixes around 1.6 kg N_2 ha^{-1} each year at WRCC (Figure 3.5). The 1999 meteorological data were the

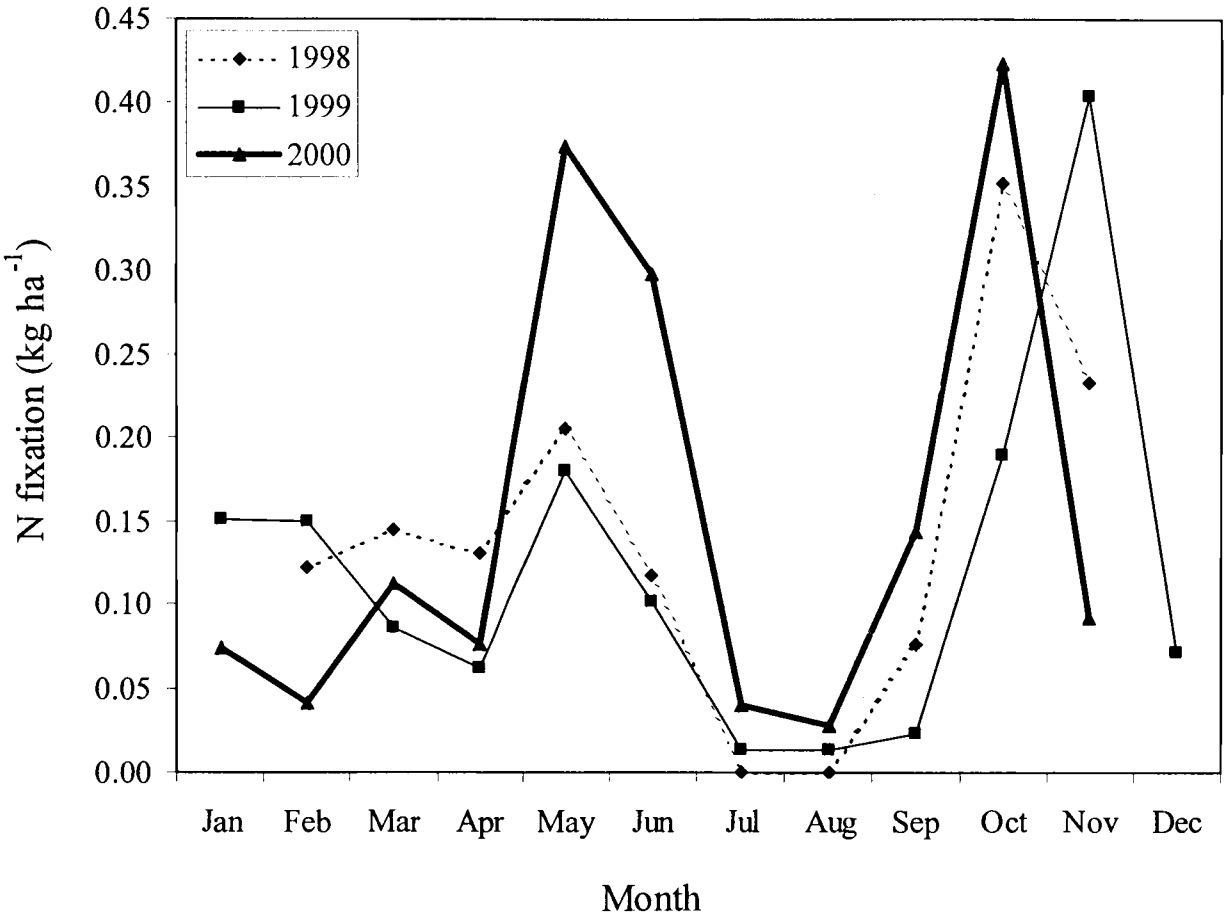

Figure 3.5. Annual trends in N fixation by *Lobaria oregana* at the Wind River Canopy Crane site based on three years of temperature and precipitation data

most complete of the 3 years, and total N fixation for 1999 was 1.4 kg ha^{-1}. Both 1998 and 2000 were missing some meteorological data, so it is likely that total N fixation for these 2 years was underestimated. There were no data for January or December 1998, and the cumulative N fixation value the remaining 10 months was 1.38 kg ha^{-1}. If the monthly total for Dec 1999 and the average of Jan 1999 and 2000 were substituted for the missing data, the 1998 total increased to 1.6 kg N ha^{-1}. Data for December 2000 were also missing, but 2000 had the highest cumulative N fixation value regardless, with 1.7 kg ha^{-1}. If December 1999 data were substituted, the 2000 total increased to 1.8 kg N ha^{-1}.

Several seasonal trends in N fixation activity were apparent (Figure 3.5). First, peak N fixation occurred in fall. Second, N fixation rates during cold, wet winter months were consistently low. Fixation rates increased during spring as temperatures became more moderate. The high annual N fixation total for 2000 was due to the warm, wet weather in May, when *L. oregana* was hydrated for extended periods, and warm temperatures allowed optimal N fixation rates.

The 1999 meteorological data were used for further analysis of the relationships among rainfall, temperature, and N fixation rates. Seasonal trends in minimum and maximum temperature explained *L. oregana*'s N fixation capability at different times of the year (Figure 3.6). Plotting the rates at which fixed N and rainfall accumulated clearly showed these temperature-dependent seasonal trends (Figure 3.7). Although close to 50% of the annual total rain fell between January and March, only 30% of annual N fixation occurred during that same period. During the summer, there were very few precipitation events, but warm temperatures allowed significant N fixation during the short windows of activity. In the fall, over 50% of annual N fixation occurred, as *L. oregana* was able to take advantage of plentiful rain and moderate temperatures.

Analyzing N fixation on rainy days throughout the year demonstrated the importance of temperature in determining seasonal trends in N fixation (Figure 3.8). Although *L. oregana* was hydrated for 12 h periods on each chosen day,

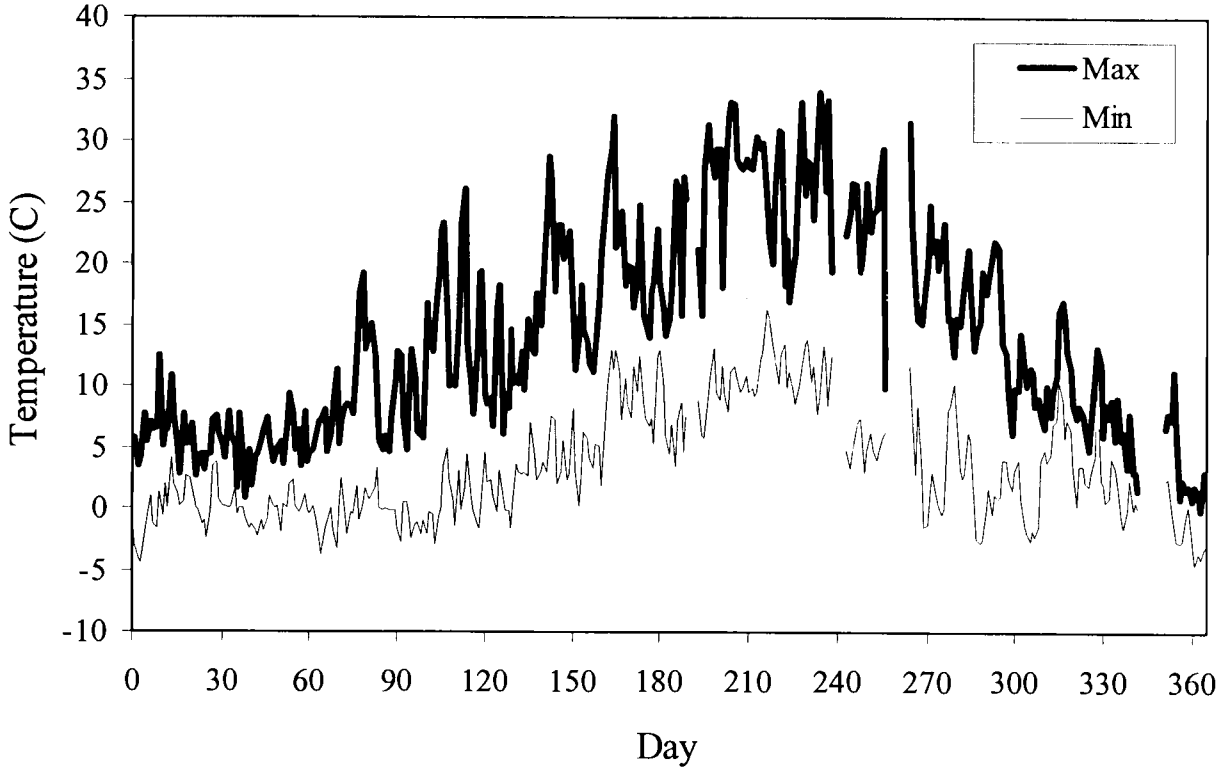

Figure 3.6. Seasonal trends in daily maximum and minimum temperatures during 1999 at the Wind River Canopy Crane site

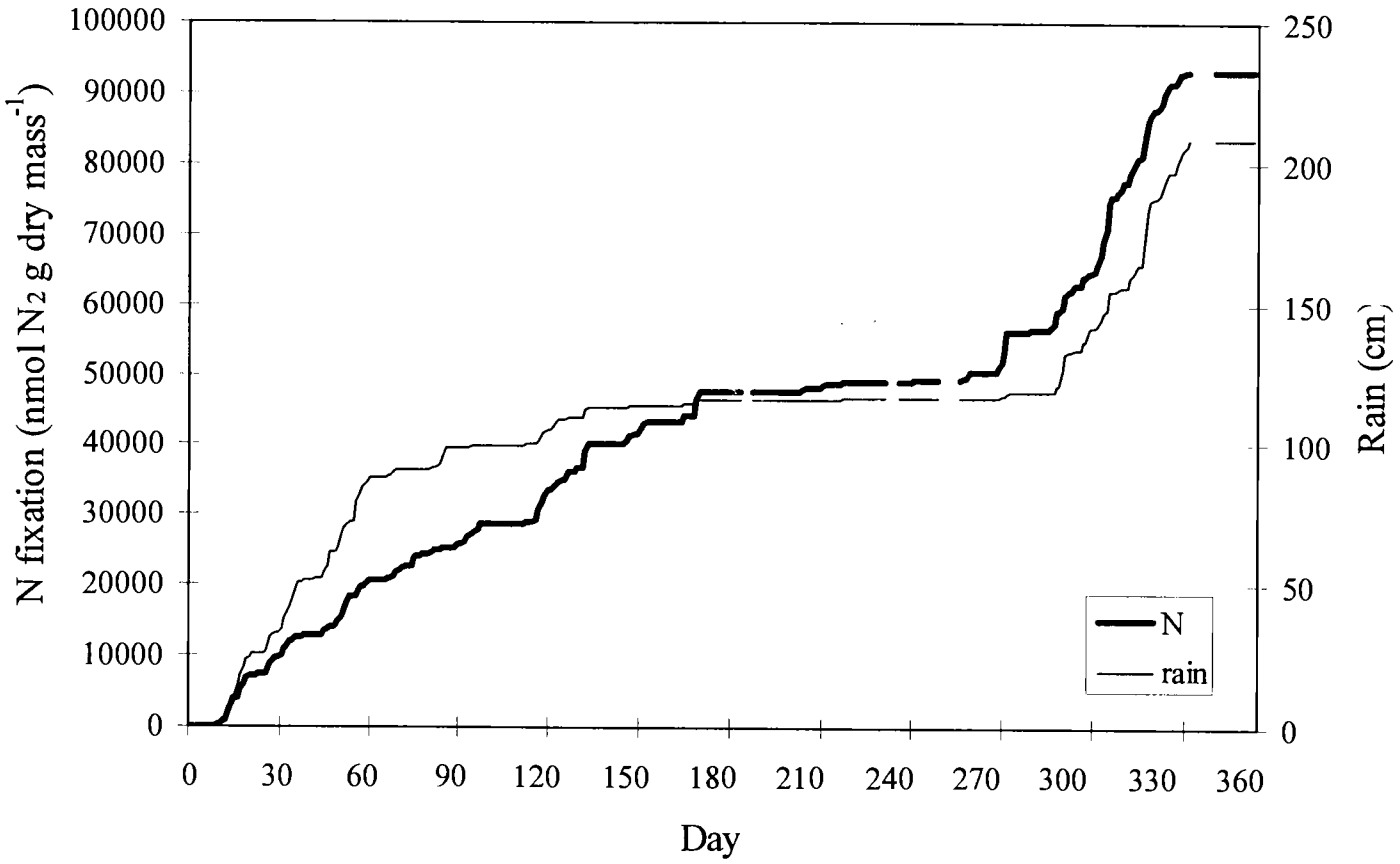

Figure 3.7. Cumulative N fixation and rainfall during 1999 at the Wind River Canopy Crane site

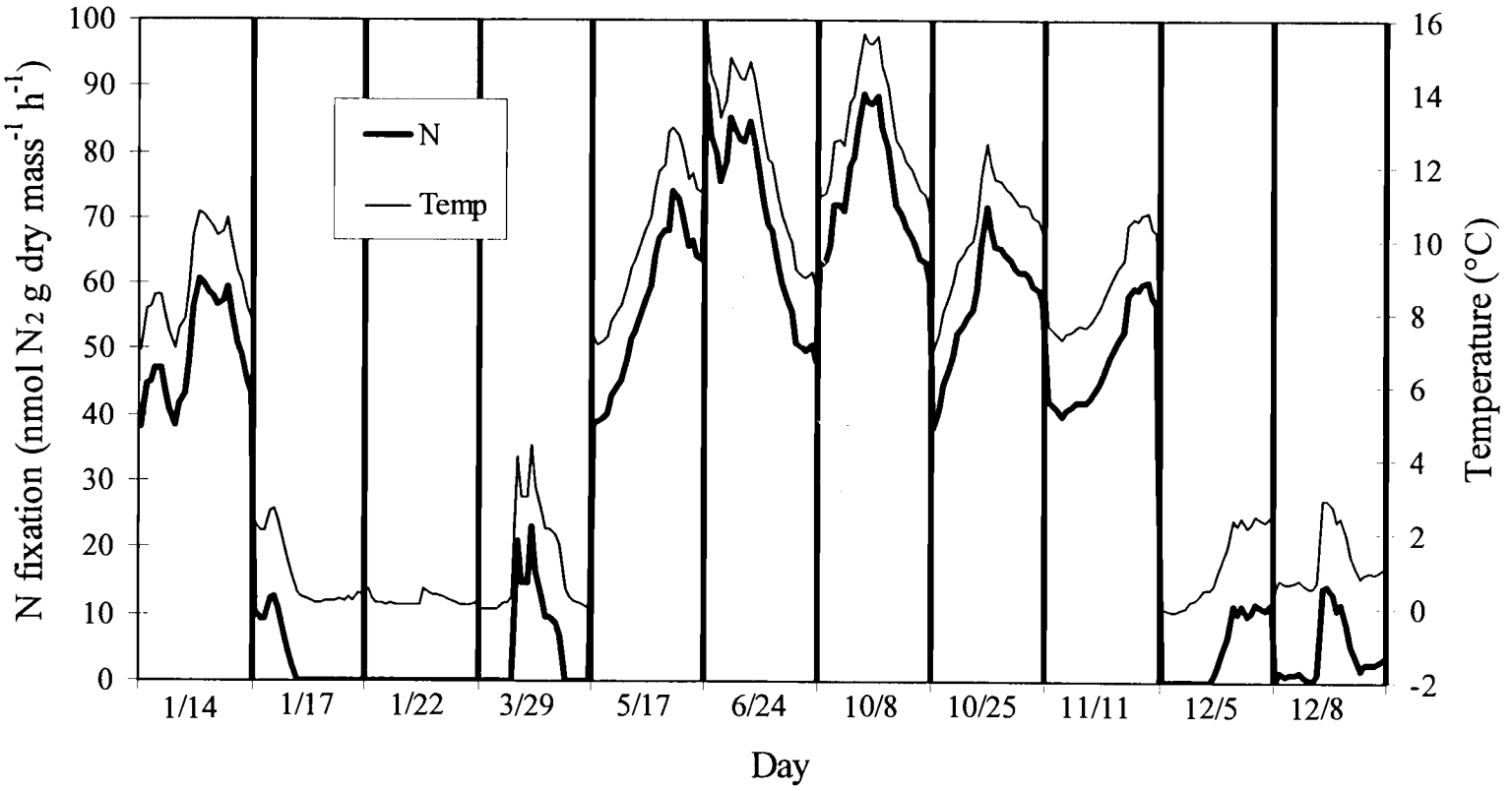

Figure 3.8. Trends in temperature and N fixation by *Lobaria oregana* during 12-h rainy periods in 1999 at the Wind River Canopy Crane site

daily totals for N fixation ranged from 0 to over 3000 nmol g DW^{-1} depending on diurnal temperature variation.

N fixation by L. oregana *at HJA*

To illustrate the utility of the model, we used meteorological data from HJA to estimate annual N fixation by *L. oregana* in a forest other than WRCC. These results were compared with the same one-year time period for WRCC (Table 3.3). December 1999 meteorological data were substituted for the missing December 1998 data at WRCC. Although more rain fell at WRCC from 5/98 to 4/99, over 1.7 times as much N was fixed at HJA. Several factors contributed to this difference. First, temperatures during the rainy spring and fall were generally warmer at HJA than at WRCC. This resulted in consistently higher nitrogenase efficiency per unit rain at HJA (Figure 3.9). Second, the summer drought was more pronounced at WRCC than at HJA. At WRCC there was no rain during July or August and very little during September, while at HJA some rain fell in July and the rainy season began in September. A third factor contributing to greater N fixation at HJA was that, on average, winter temperatures there were more moderate than at WRCC. At WRCC, N fixation during wet winter days was often zero simply because temperatures were too low to allow nitrogenase activity (Figure 3.8). Although average temperatures were only several degrees warmer at HJ A, the difference was enough that N fixation was consistently higher throughout the winter (Figure 3.9). Only in December were temperatures equally cold and N fixation rates equally negligible at both sites.

The estimates of cumulative N fixation in Table 3.3 assumed that the canopy biomass of *L. oregana* was the same at HJA as at WRCC. However, there are sites in HJA where *L. oregana* is much more abundant in the canopy. The model provides N fixation rates per kg of lichen, so cumulative fixation rates were easily calculated for sites with different amounts of *L. oregana* (Table 3.4). Obviously, in sites where *L. oregana* reached peak abundance (3+ kg ha^{-1}) the magnitude of annual N fixation increased accordingly. Based on site-specific

Table 3.3. Comparison of total precipitation and estimated annual N fixation by *Lobaria oregana* at the HJ Andrews Experimental Forest and the Wind River Canopy Crane site

TIME PERIOD	SITE	PRECIPITATION (mm)	TOTAL N FIXED (nmol N_2 g^{-1})	N FIXATION RATE (g N_2 kg^{-1} yr^{-1})	ANNUAL N FIXATION* (kg ha^{-1})
5/98 – 4/99	HJA	1460	167,672	4.7	2.6
5/98 – 4/99	WRCC	1840	97,630	2.7	1.5

* Estimates assume 550 kg ha^{-1} of *L. oregana* canopy biomass.

Table 3.4. Estimated annual N fixation by *Lobaria oregana* in different areas of the HJ Andrews Experimental Forest

SITE DESCRIPTION	SOURCE	BIOMASS OF *L. OREGANA* (kg ha^{-1})	ANNUAL N FIXATION BY *L. OREGANA* (kg ha^{-1})
Mesic	Neitlich 1993	3523	16.5
Riparian	Neitlich 1993	2620	12.3
Xeric upland	Neitlich 1993	1587	7.5
Watershed 10	Denison 1979	500	2.5

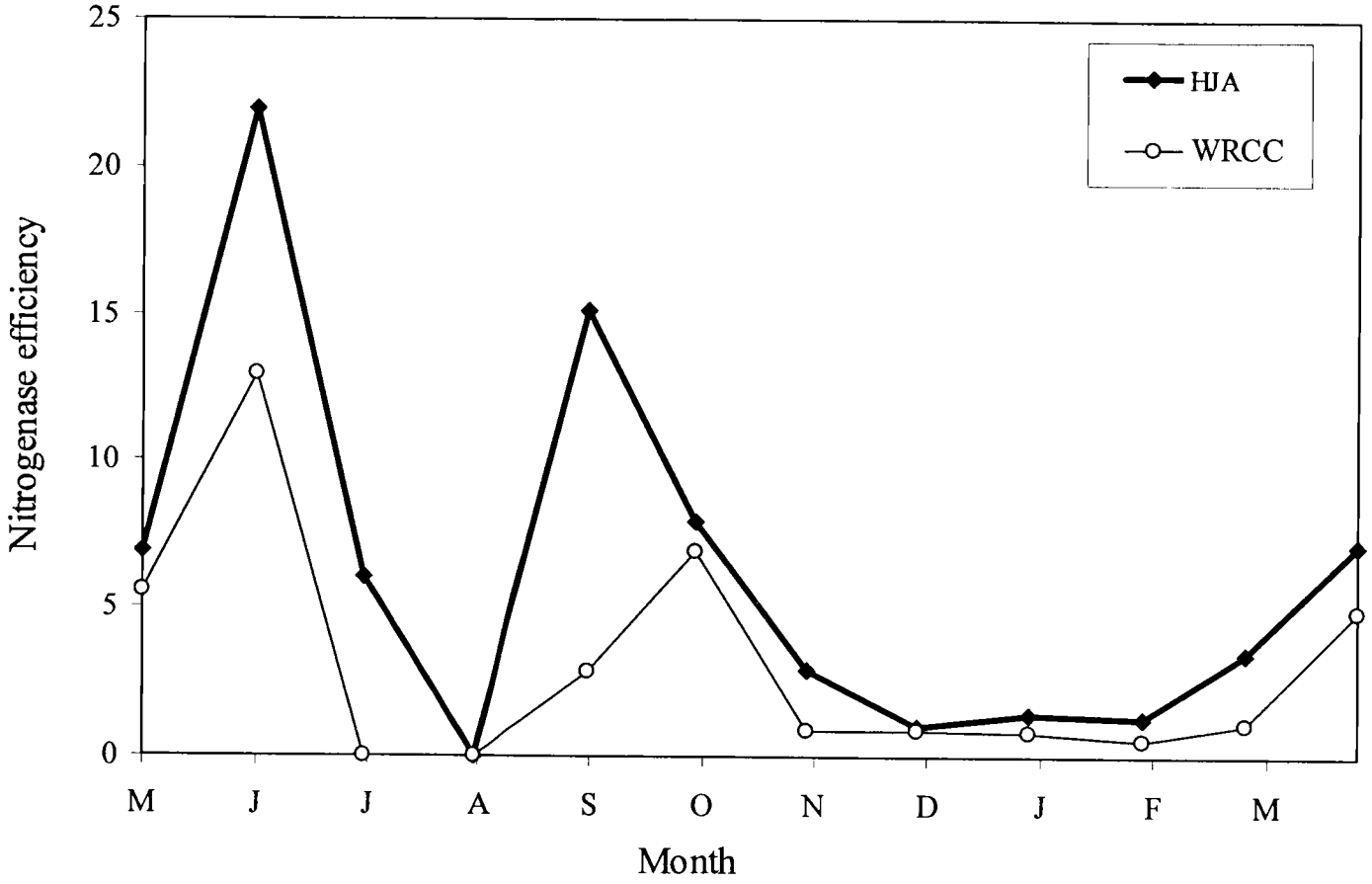

Figure 3.9. Seasonal trends in nitrogenase efficiency (N fixed per unit rain) over a one-year period at WRCC and HJA

biomass estimates for *L. oregana*, annual N fixation ranged from 2.6 to 16.5 kg ha^{-1} in different areas of HJA.

DISCUSSION

Model parameters

In order to model a process as ecologically and physiologically complex as annual N fixation by *L. oregana*, several simplifying assumptions are necessary. Since *L. oregana* is poikilohydric, thalli must be hydrated in order to be physiologically active. Precipitation data provide an approximation of when *L. oregana* is hydrated, with the assumption that each rainfall event activates all of the lichen biomass throughout the canopy. Synchronous hydration of all *L. oregana* thalli is probably not always the case, but the task of monitoring trends in thallus hydration with different amounts of rain and at different canopy positions is not possible. Similarly, it is unlikely that thalli of different sizes or at different positions in the canopy desiccate at exactly the same rate. Smaller thalli dry out faster than larger thalli (Gauslaa & Solhaug 1998), and lichens in the exposed upper canopy probably desiccate more rapidly than lichens in the lower canopy. Quantifying the distribution of different thallus size classes throughout the canopy, and corresponding differences in desiccation rates was simply not practical in this case.

The assumption that all *L. oregana* had the same N fixation capacity per g dry mass is not the case because some variation exists among thalli sampled in the field (Figure 3.4). The cyanobiont in *L. oregana* occurs inside cephalodia. The number of cephalodia and the number of nitrogen-fixing cells (heterocysts) within cephalodia depends on factors such as thallus age, size, and cyanobiont availability (Jordan 1970). Obviously, it would be difficult to account for this thallus-to-thallus variation in cephalodia characteristics within the entire standing biomass of *L. oregana* at any given forest.

Annual N fixation by L. oregana

Our model represents the first attempt to predict annual N fixation by *L. oregana* using the physiological parameters controlling nitrogenase activity. Further research to quantify the effects of thallus size and canopy position on hydration and nitrogenase activity would increase the accuracy of the model. However, the estimates of annual N fixation provided with this model are in striking agreement with an estimate made using only ecological parameters for the same site (Chapter 2). Around 20 kg ha^{-1} of *L. oregana*'s 550 kg ha^{-1} canopy biomass falls as litter each year, and the N content of *L. oregana* tissue is 2%. Therefore, to replace the biomass lost as litter and allow an overall annual growth rate of 15% (Sillett & McCune 1998), *L. oregana* must fix around 1.6 kg N ha^{-1} yr^{-1}. Our model of *L. oregana*'s physiological activity gave an annual N fixation range of 1.4 to 1.8 kg ha^{-1} yr^{-1}.

The convenience of a model based on physiological parameters is that it can be applied to any site with meteorological records and estimates of *L. oregana* biomass. A necessary assumption is that lichens collected from WRCC are physiologically analogous to those throughout the species' range. This assumption is supported by the fact that the range of nitrogenase activity measured in *L. oregana* at the WRCC was similar to that measured by Denison (1979) at the HJA. However, since morphological and physiological differences may occur within species across their range, future use of this model should include tests of the model assumptions at sites other than the WRCC.

We used temperature and precipitation data for an old-growth forest at the same elevation (around 400m) as WRCC to model N fixation at HJA. Nitrogen fixation capacity by *L. oregana* is apparently greater in the warmer HJA, although only tentative conclusions can be drawn from comparing a single year at each site. The WRCC site received more rain, but cooler temperatures resulted in nitrogenase efficiency (N fixed per unit rain) that was consistently lower than at HJA.

Evaluating N fixation by *L. oregana* at HJA is particularly interesting because it was the site of the only other research quantifying N fixation by this

species. Using two different techniques, Denison (1979) estimated that *L. oregana* fixes approximately 3.5 kg N ha^{-1} yr^{-1} in a site with 500 kg ha^{-1} standing biomass. One approach involved intensive *in situ* measurements of nitrogenase activity using the ARA and subsequent projection of the mean rate across the entire rainy season. At the time the study was done, the accepted conversion ratio between ARA results and N_2 fixation rates was 3:1. However, this ratio has since been shown to be biochemically inaccurate because of the evolution of H_2 that obligately accompanies the nitrogenase reaction (Liengen 1999). Application of the theoretical conversion ratio of 4 mol C_2H_2 to 1 mol N_2 reduces Denison's estimate from 3.5 to 2.6 kg ha^{-1} yr^{-1}. Our model predicts annual N fixation rates of 2.5 kg ha^{-1} for the same site. The agreement between the two estimates is striking considering they each rest upon a different set of simplifying assumptions.

The second approach Denison (1979) used to estimate annual N fixation by *L. oregana* assumed an annual productivity rate of 30%— a growth rate based on a set of sequential photographs of only 4 mature thalli (Rhoades 1978). Recent growth experiments measuring actual biomass gain indicate that the annual growth rate of *L. oregana* is 15-23% (Sillett & McCune 1998). These growth rates suggest that *L. oregana* fixes 1.6–2.4 kg N ha^{-1} yr^{-1} in sites with 500 kg ha^{-1} canopy biomass.

Comparisons of annual N fixation by *L. oregana* between WRCC and HJA illustrate the importance of analyzing the ecological context of *L. oregana* on a site by site basis. Nitrogen fixation by *L. oregana* occurs within a set of physiological constraints, and local patterns of temperature and precipitation define its N fixation potential in any given area. Abundance of *L. oregana* in the canopy determines the magnitude of annual N fixation. Epiphytic cyanolichens are most abundant in mid-elevation humid old-growth forests, and their biomass increases with proximity to major streams (Sillett & Neitlich 1996, Howe 1978). Canopy biomass of *L. oregana* exceeds 3 t ha^{-1} in some sites HJA (Neitlich 1993), resulting in high rates of annual N fixation.

Previous studies estimated that cyanolichens contribute between 19% and 68% of the annual litterfall and throughfall N budget at HJA (Pike 1978, Neitlich 1993, respectively). Our estimates of annual N fixation suggest that *L. oregana* contributes between 11% and 69% of the N budget, depending on its local abundance. It is important to note that other N-fixing lichens such as *L. pulmonaria* and *Pseudocyphellaria* spp. make up 10-15% of the total cyanolichen biomass (Sillett 1995, Neitlich 1993). Therefore, estimates of N fixation by *L. oregana* alone underestimate total N fixation by epiphytic cyanolichens.

Lobaria oregana and many other N-fixing lichens are associated with old-growth forests (Sillett & Goward 1998), habitat that has become increasingly rare due to clear-cut logging and development. *Lobaria oregana* is capable of growing in young forests, but it is often sparse or absent from these stands because of dispersal and establishment limitations (Sillett & McCune 1998). The availability of propagule sources (i.e., retained trees) is more essential for the propagation of *L. oregana* than the particular substrates and microenvironments found only in old-growth forests (Sillett et al. 2000). This leads to specific management implications for the conservation of *L. oregana* and other ecologically important cyanolichens.

The temperature constraints on N fixation in *L. oregana* may help to explain the fact that this species drops out of epiphyte assemblages in higher-elevation forests (Sillett & Neitlich 1996). Perhaps rainy-season temperatures are simply too low to permit sufficient N fixation for the growth or survival of *L. oregana*. Placing *L. oregana* transplants into higher-elevation forests with meteorological data-loggers can test this hypothesis.

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Chapter 4

CO_2 Fixation in the Cyanolichen *Lobaria oregana*: Effects of Water, Light, and Temperature

Marie E. Antoine and William E. Winner

ABSTRACT

Lobaria oregana is an epiphytic cyanolichen that is generally recognized for fixing large quantities of nitrogen in Pacific Northwest old-growth Douglas-fir forests. Carbon fixation by *L. oregana* has received little attention, but an understanding of parameters controlling CO_2 exchange may elucidate both the physiology and ecology of this species. This study explores the effects of thallus water content, light, and temperature on photosynthesis and respiration rates in *L. oregana* collected at the Wind River Canopy Crane site. The CO_2 exchange response to rehydration was typical of the triphasic response curve seen in other lichens, although no depression of photosynthesis occurred at high water contents. At 2°C and 10°C photosynthesis became light saturated at PAR levels of 100-200 $\mu E\ m^{-2}\ sec^{-1}$. Lichens assayed at 20°C did not reach compensation point even at the highest PAR levels. Field-based temperature data suggest that positive net photosynthesis in *L. oregana* is only physiologically possible between 1-12°C. At higher temperatures, the respiratory demands of the mycobiont exceed the photosynthetic capabilities of the photobionts. The temperature constraint on CO_2 exchange may have implications for the landscape distribution patterns of *L. oregana*.

INTRODUCTION

Cyanolichens are dominant components of humid, mid-elevation, old-growth Douglas-fir forests (Sillett 1995, Neitlich 1993, Pike et al. 1975). *Lobaria oregana* (Tuck.) Müll. Arg. alone can account for 60–80% of the total epiphytic lichen biomass in these forests (Sillett 1995, McCune 1994, Pike et al. 1997). This cyanolichen is well known for its ability to fix ecologically significant quantities of nitrogen (Denison 1979, Pike 1978). Depending on local temperature and

precipitation patterns and site-specific canopy biomass, *L. oregana* fixes 0.4-16.5 kg ha^{-1} of nitrogen each year (Chapter 3).

Lobaria oregana is associated with old-growth forests because of dispersal limitations (Sillett et al. 2000), and its biomass within these forests is positively correlated with proximity to major streams (Sillett & Neitlich 1996). The factors determining *L. oregana*'s prevalence in mid-elevation forests are largely unexplored, but an ecophysiological approach may help explain this landscape distribution pattern. Analysis of environmental factors controlling nitrogen fixation in *L. oregana* showed that nitrogenase rates are temperature-dependent (Chapter 3). At higher elevations, consistently low temperatures during the rainy season may prohibit sufficient nitrogen fixation for growth and survival of *L. oregana*.

Temperature may also be important in controlling carbon fixation rates, but the physiological effects of thallus water content and light intensity must be considered too (Sundberg et al. 1997). This is the first study to investigate physiological constraints on carbon metabolism in *L. oregana*. Our objectives are to obtain instantaneous seasonal measurements of photosynthesis and respiration for *L. oregana* thalli at the Wind River Canopy Crane site and to determine the effects of thallus water content, light, and temperature on CO_2 exchange rates in *L. oregana*.

METHODS

Site description

The Wind River Canopy Crane was used to collect *L. oregana* thalli from the Wind River Experimental Forest, which is located within the Gifford Pinchot National Forest near Carson, Washington (45°49'N, 121°55'W). The crane provides access to 10^5 m^3 of old-growth forest canopy, where *Pseudotsuga menziesii* (Mirb.) Franco and *Tsuga heterophylla* (Raf.) Sarg. are co-dominant. *Thuja plicata* (Donn.) is also common, and the site displays old-growth characteristics such as the

presence of *Taxus brevifolia* (Nutt.) and abundant snags and fallen logs (Franklin & Spies 1991, Norse 1990). Over 90% of the annual 2.5 m of precipitation falls between October and May. Detailed information about the Wind River Canopy Crane Research Facility is available online at www.depts.washington.edu/wrccrf.

There are 113 documented lichen species at the crane site, and the non-vascular epiphyte assemblage is vertically stratified (Clement & Shaw 1999, McCune 1997). Bryophytes dominate on the ground and in the lower canopy, while the pendulous green algal lichens ("alectorioids") are most abundant in upper canopy and the exposed outer branches. *Lobaria oregana* and other cyanolichens are concentrated in the 'light transition zone' of the middle canopy, and foliose green algal lichens are distributed throughout the vertical profile (McCune 1997).

Sampling protocol

Sampling was scheduled to determine how CO_2 exchange in *L. oregana* changed seasonally. The crane was used to collect *L. oregana* thalli from the canopy at regular intervals from winter 1999 to spring 2001. Thalli were collected from two different heights in the canopy and on two individuals each of *P. menziesii*, *T. heterophylla*, and *T. plicata*. Temperature and photosynthetically active radiation (PAR) levels were recorded at each collection site. Some thalli were used immediately for physiological field measurements and some were air-dried and taken back to the laboratory for further analysis.

Physiological measurements

Gas exchange measurements were made using a LiCor 6400 Portable Photosynthesis System. The LiCor 6400 is an open gas exchange system, and photosynthesis is computed using the airflow rate, lichen surface area, and the incoming and chamber CO_2 concentrations measured by infrared gas analyzers. The leaf-like thallus morphology of *L. oregana* allowed convenient use of a clamp-on cuvette, and humidity in the cuvette was kept at high levels to prevent desiccation during measurements.

Water contents of *L. oregana* thalli were expressed on an absolute basis, with oven-dried mass as the 0% reference point. Oven-dried thalli could not be used for physiological measurements, so a sub-sample of six 'sacrificial' thalli was randomly selected from each seasonal collection. These thalli were oven-dried for 24 h at 70 °C, weighed, and the average ratio of oven-dried to air-dried mass was used to calculate oven-dried masses for the remaining thalli. Therefore, a thallus at 100% hydration had 1 g of water per 1 g of oven-dried mass.

Field measurements

CO_2 exchange rates in *L. oregana* were measured in the field immediately following collection and weighing of the thalli. Temperature in the LiCor 6400 cuvette was set at the ambient air temperature recorded from the lichen collection site, and data were compiled to construct a field-based temperature response curve for CO_2 exchange. Net photosynthesis was measured at ambient light levels (typically 50-100 $\mu E\ m^{-2}\ sec^{-1}$ under cloudy conditions) and at high light levels (1200 $\mu E\ m^{-2}\ sec^{-1}$). Respiration rates were obtained by turning off the light source in the cuvette. CO_2 exchange rates were measured in 12 *L. oregana* thalli during each seasonal sampling trip.

Laboratory measurements

The relationship between thallus hydration and CO_2 exchange was investigated using thalli collected in Fall 2000. First, lichens that had been air-dried and stored for 72 h were re-hydrated for 0, 5, 10, 30, 60, 120, and 180 min (n=3 per hydration time) to determine how fast certain hydration levels were reached. A spray bottle of distilled water was used to simulate the rehydrating effect of rainfall. A second group of thalli were used for the CO_2 exchange measurements. Thalli were rehydrated for 0, 5, 10, 30, 60, and 120 min (n=3 per hydration time) and CO_2 exchange rates were measured at ambient and high light levels and in the dark. Temperature in the LiCor cuvette was kept constant at 10°C.

Light saturation curves were obtained using lichens collected in Winter 2001. Thalli were re-hydrated with distilled water for 60 min at 2, 10, or 20°C (n=6 per temperature) and 100 $\mu E\ m^{-2}\ sec^{-1}$. The automatic light curve program on the LiCor 6400 was set to measure net photosynthesis at 0, 20, 40, 60, 80, 100, 150, 200, 400, and 1200 $\mu E\ m^{-2}\ sec^{-1}$. At each light level, photosynthetic activity of the thallus was allowed 2-4 min to stabilize before the LiCor logged 3 replicate measurements.

RESULTS

Field measurements

Lobaria oregana thalli were weighed immediately after collection to determine their fresh water contents, which were then used to calculate thallus hydration levels (Table 4.1). Thalli were significantly more hydrated in both winter samples than they were in the spring or fall samples ($p<0.01$). Rain fell during or throughout all of the sampling trips.

The highest respiration rates and air temperatures observed in the field occurred during the Spring 2000 and Fall 2000 sampling periods (Table 4.1). Warm temperatures (10-14 °C) and high respiration corresponded with net carbon loss (i.e., negative net photosynthesis). Thalli measured under ambient light conditions (50 $\mu E\ m^{-2}\ sec^{-1}$) during Winter 1999 were close to the compensation point with almost no net CO_2 exchange. However, when those thalli were exposed to high light levels (1200 $\mu E\ m^{-2}\ sec^{-1}$), net photosynthesis rose to the highest levels measured in the field. Net carbon gain was also observed under ambient and high light levels during Winter and Spring 2001.

Water content

Lichens can be stored for up to a month in the air-dried state, and upon rehydration, behave as if they had undergone a natural desiccation period in the

Table 4.1. CO_2 exchange and thallus hydration levels of *Lobaria oregana* measured in the field during different seasons at the Wind River Canopy Crane site. All data are means of n=12 ± 1 SE.

	WINTER 1999	SPRING 2000	FALL 2000	WINTER 2001	SPRING 2001
TEMPERATURE (°C)	2 - 4	10 - 13	12 - 14	0 - 3	7 - 9
NET PHOTOSYNTHESIS (μmol CO_2 $m^{-2}s^{-1}$)					
ambient light	-0.09 ± 0.11	-0.73 ± 0.28	-0.58 ± 0.15	0.12 ± 0.09	0.25 ± 0.04
high light	0.52 ± 0.15	-0.19 ± 0.22	-0.20 ± 0.14	0.32 ± 0.11	0.32 ± 0.05
RESPIRATION (μmol CO_2 $m^{-2}s^{-1}$)	0.91 ± 0.08	2.45 ± 0.37	1.52 ± 0.22	0.44 ± 0.05	0.71 ± 0.07
THALLUS HYDRATION (%)	277 ± 14	196 ± 8	207 ± 4	249 ± 6	199 ± 12

field (Antoine, unpublished data). The rate at which lichens lose water depends on thallus size (Gauslaa & Solhaug 1998) and atmospheric relative humidity, but *L. oregana* thalli typically desiccate within two hours following a rain (Chapter 3).

Laboratory experiments were conducted to determine the rate at which desiccated *L. oregana* thalli became rehydrated. Within 10 min of rehydration, thalli reached water contents of 200-250% (Figure 4.1). This was the hydration range typically seen in fresh *L. oregana* sampled in the field on rainy days. Water contents increased to approximately 300% after 60 min rehydration and reached 325% after 120 min. This represented the apparent "saturation" point for *L. oregana*, and there was little change in water content after 180 min.

The effect of thallus water content on CO_2 exchange was also investigated in the laboratory. Upon rehydration, there was a short burst of respiratory activity that lasted approximately 5 min, and then photosynthesis increased steadily (Figure 4.2). The water compensation point was reached after 20 min, or at hydration levels of approximately 50-200%. Photosynthetic rates reached a plateau after 60 min of rehydration (approximately 300% water content). There was a very slight decrease in net photosynthesis of thalli with water contents of 325%.

Light

The effect of PAR on CO_2 exchange in *L. oregana* was measured at 2, 10, and 20°C (Figure 4.3). *L. oregana* became light saturated at PAR levels of approximately 100 $\mu E\ m^{-2}\ sec^{-1}$ (2 °C) or 200 $\mu E\ m^{-2}\ sec^{-1}$ (10 °C). The thalli assayed at 20°C did not reach compensation point even at the highest PAR levels.

Temperature

Data collected in the field were compiled to form a temperature response curve for CO_2 exchange in *L. oregana* (Figure 4.4). There was little physiological activity as temperatures approached 0°C. Under ambient light conditions (50-100 $\mu E\ m^{-2}\ sec^{-1}$), the temperature compensation point was slightly above 2°C. Positive net photosynthesis occurred between approximately 2 and 10°C. Average

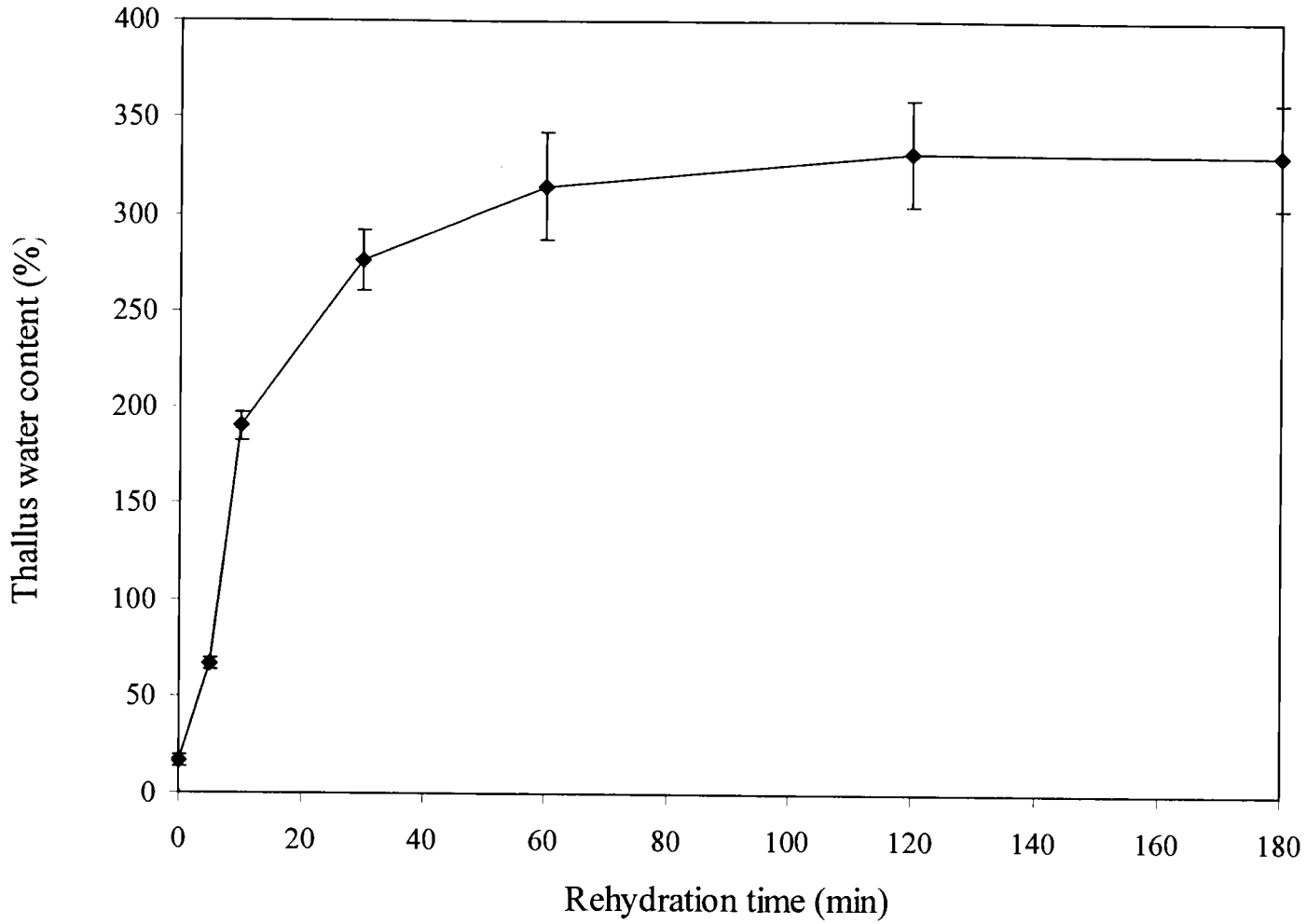

Figure 4.1. Thallus water content of *Lobaria oregana* during rehydration. Data are means of n=3 ± 1 SE.

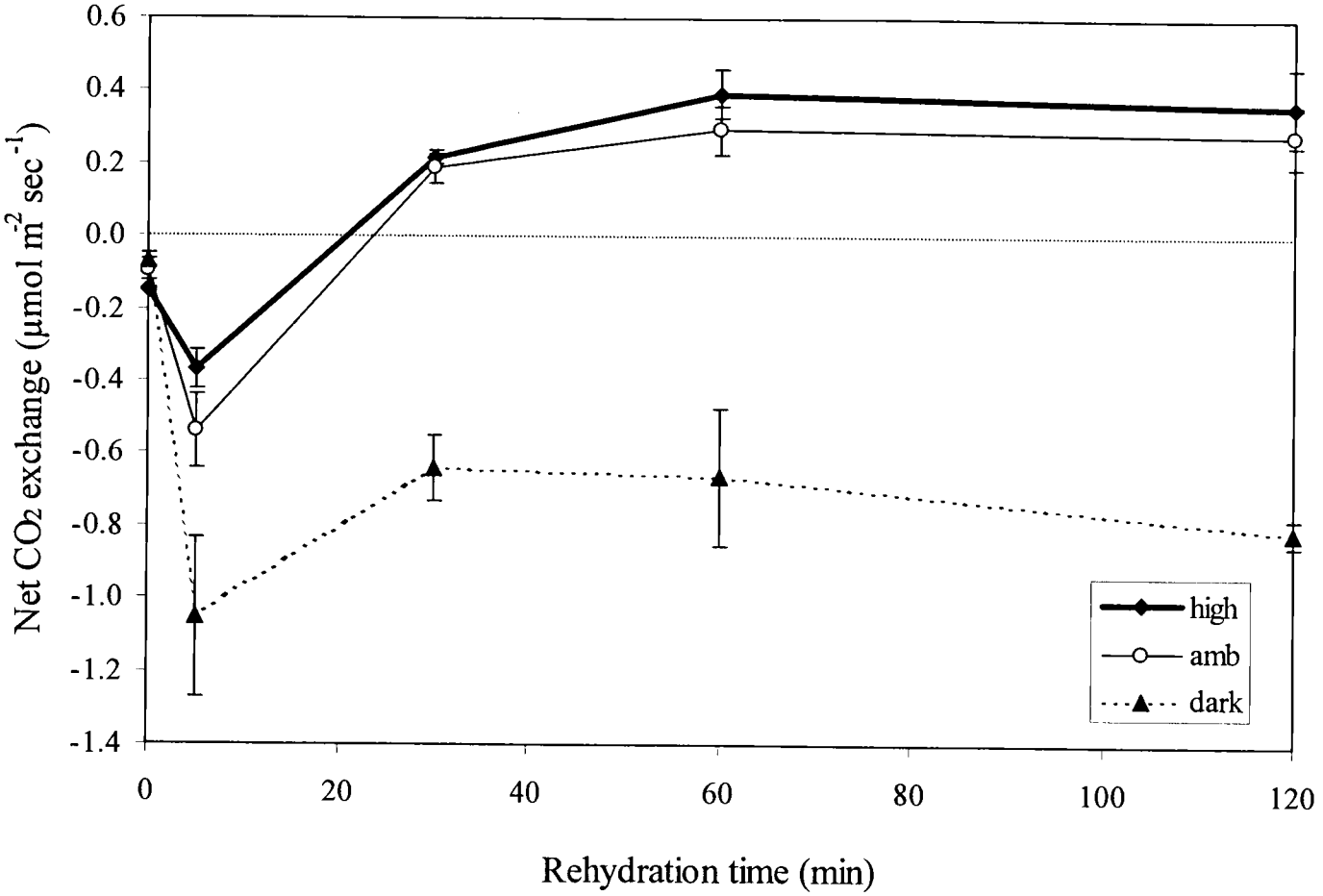

Figure 4.2. CO_2 exchange in *Lobaria oregana* with different rehydration times. Data are means of n=3 ± 1 SE.

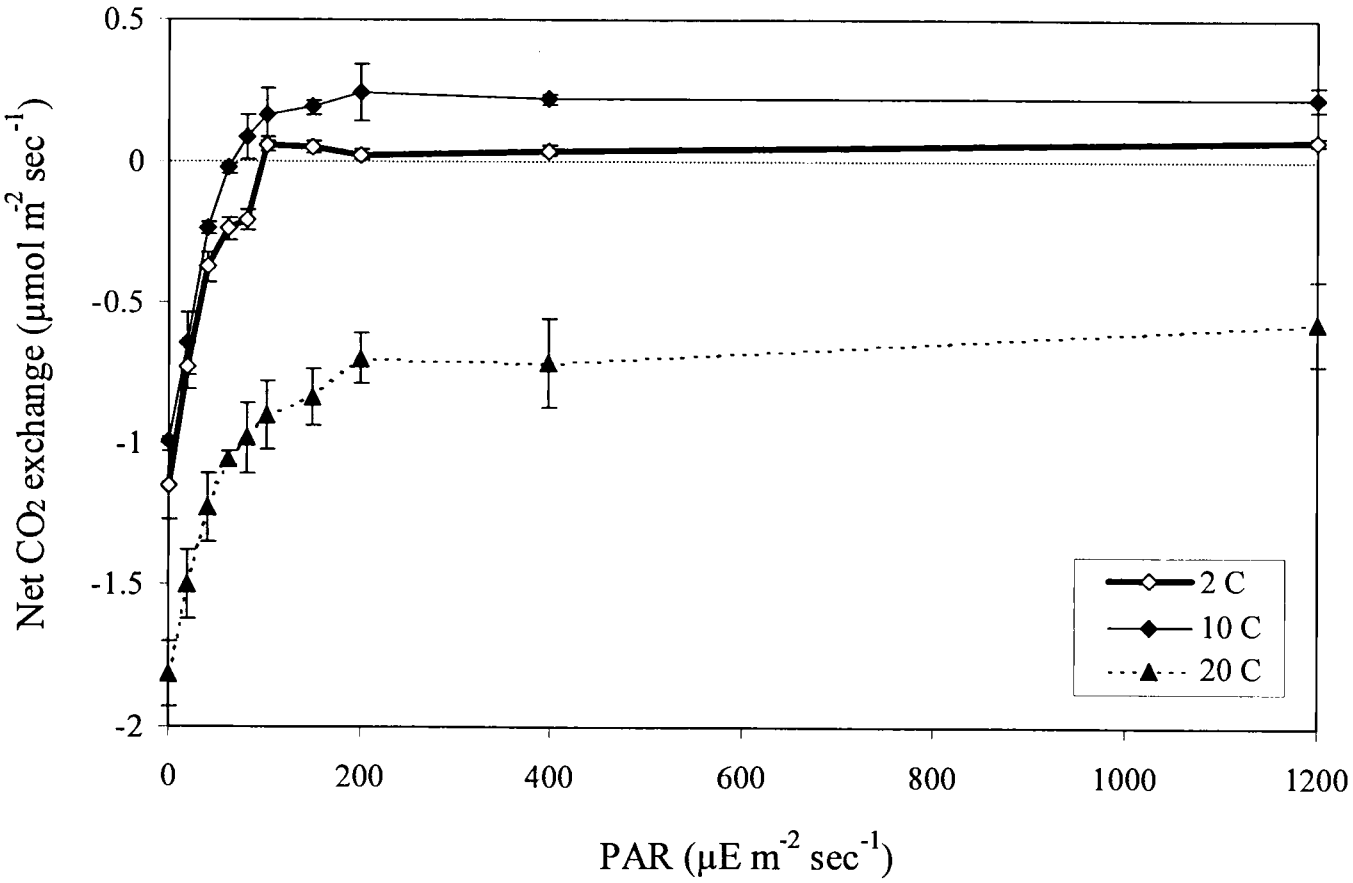

Figure 4.3. Light saturation curves for *Lobaria oregana* measured at 2°, 10°, and 20°C. Data are means of n=6 ± 1 SE.

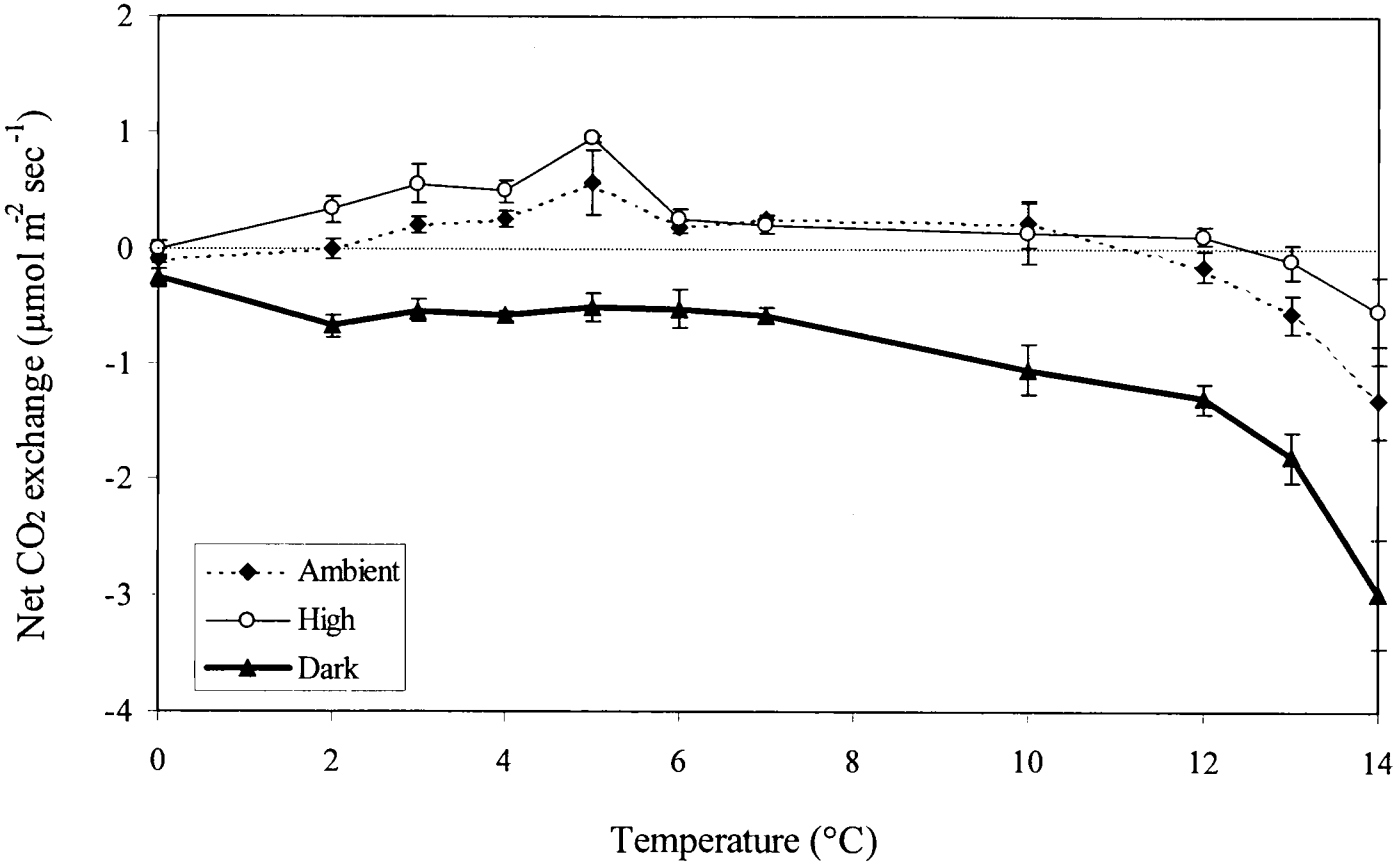

Figure 4.4. CO_2 exchange in *Lobaria oregana* measured in the field at different temperatures. Data are means of n=3-8 ± 1 SE.

photosynthetic rate under ambient light peaked at 0.58 μmol CO_2 m^{-2} sec^{-1} at a temperature of 5°C. The temperature compensation point under high light conditions (1200 μE m^{-2} sec^{-1}) was slightly below 1°C, and net photosynthesis was positive between 1 and 12°C. The highest average photosynthesis rate under high light was 0.95 μmol CO_2 m^{-2} sec^{-1}, again occurring at 5°C.

Respiration remained low (i.e., < 0.6 μmol CO_2 m^{-2} sec^{-1}) until around 6 °C and then increased rapidly as temperature increased (Figure 4.4). Respiration rates doubled between 7 and 10°C and again between 10 and 12°C. Maximum average respiration rates occurred at 14 °C, the warmest temperature under which field measurements were made. Respiration rates continue to increase with increasing temperature (Kershaw 1985). Therefore, these data suggest that net carbon gain in *L. oregana* is only physiologically possible between about 1 and 12°C.

DISCUSSION

The environmental parameters (i.e., water, light, and temperature) that control lichen CO_2 exchange are, of course, interdependent. However, manipulating each of these parameters separately is a necessary first step towards understanding a lichen's carbon metabolism. In addition, it is useful to consider some of *L. oregana*'s ecological characteristics in light of the physiological constraints within which it must perform.

Thallus water content

Lichens are poikilohydric organisms that undergo repeated cycles of desiccation and rehydration. Analyses of photosynthetic responses to thallus water contents in many different lichen species have repeatedly produced triphasic (i.e., low, optimal, and high) response curves (Green et al. 1994). CO_2 exchange in *L. oregana* follows the general pattern seen in other lichens at low and optimal water contents. At low water contents, reactivation of physiological activity does not

occur until a minimum hydration level of around 50% is reached. Optimal water content in *L. oregana* is around 250%, and gas exchange rates peak and then plateau beyond this point.

Depression of photosynthetic rates is often observed at high thallus water contents (Lange et al. 1996, Lange et al 1993a). The depression is attributed to increased resistance to CO_2 diffusion into a "saturated" thallus (Green et al. 1994). This hypothesis is supported by the fact that increased CO_2 concentrations decrease the magnitude of the depression (Cowan et al. 1992). There is only a very slight decrease in *L. oregana*'s net photosynthesis at the highest water contents attainable in the laboratory (325%), suggesting that this hydration level is insufficient to cause appreciable thallus CO_2 resistance. The highest water contents of *L. oregana* observed in the field are between 200-300%. Therefore, it seems unlikely that thallus diffusion resistance is important in limiting photosynthetic CO_2 exchange in this epiphytic species.

The primary photobiont in *L. oregana* is green algal, suggesting that this lichen may be able to obtain positive net photosynthesis through equilibration with high air humidity. This phenomenon has been observed for *L. pulmonaria* (Lange et al. 1986). Re-activation of physiological activity in cyanobacteria requires liquid water (Lange et al. 1993b), and cyanobionts have a physiological advantage over green algal bionts at very high thallus water contents (Green et al. 1993). The fact that *L. oregana* has both types of photobionts may allow physiological activity over a wider range of meteorological conditions than would be possible for lichens containing only one type of photobiont.

Light

Photosynthetic rates in *L. oregana* reach light saturation at PAR levels of 100-200 $\mu E\ m^{-2}\ sec^{-1}$, which is consistent with the range of 100-400 $\mu E\ m^{-2}\ sec^{-1}$ typical of other lichen species (Demmig-Adams et al. 1990). These low saturation values indicate that *L. oregana* is not a light-demanding species, and high light levels may even result in decreased photosynthetic efficiency. Irreversible damage

to the photosynthetic apparatus of air-dried *L. pulmonaria* occurred after thalli were exposed to PAR levels of 1000 $\mu E\ m^{-2}\ sec^{-1}$ (Gauslaa & Solhaug 1999). In contrast, *L. pulmonaria* thalli transplanted into the high-light environment of a clear-cut grew at least as well as in old-growth or young forests (Sillett et al. 2000). It is likely that these different responses to high light by lichens in field transplant experiments are due to the pre-experimental conditions to which the thalli were acclimated (Sillett 1994). The increased temperatures typically accompanying high PAR values are probably more detrimental to *L. oregana*'s physiology than are high light levels alone (Gauslaa & Solhaug 1999).

Temperature

The light saturation experiment demonstrates that thalli are unable to reach compensation points at temperatures of 20°C. In other words, net photosynthesis remains negative even at the highest light levels. Fungal tissue accounts for 90-95% of the biomass of lichen thalli, and at higher temperatures the respiratory demand of the mycobiont exceeds the photosynthetic capabilities of the photobiont(s) (Kershaw 1985).

The field-based temperature response curve for gas exchange indicates that positive net photosynthesis in *L. oregana* is not physiologically possible above approximately 12°C. Net carbon gain must occur during relatively cool temperatures when fungal respiration is inhibited. *Lobaria oregana* is a nitrogen-fixing lichen, and nitrogenase activity increases linearly with temperature (Chapter 3). At any given time, high rates of nitrogen fixation and positive net photosynthesis may be mutually exclusive. The temporal separation of net carbon and nitrogen fixation suggests a mechanism for storing fixed carbon until warmer temperatures allow nitrogen fixation and the capacity for rapid fungal respiration and growth.

The temperature constraints on net carbon gain may explain some of the distribution patterns of *L. oregana*. This species is often sparse or absent in humid, low-elevation forests (Sillett, unpublished data), where winters are mild and the

summer drought is ameliorated by persistent coastal fog. Perhaps conditions are too warm and wet to allow sufficient net photosynthesis for survival. In other words, *L. oregana* might simply respire itself to death in these forests. A similar phenomenon has been observed in tropical rain forests, where bryophyte abundance increases with altitude. Bryophyte species that thrive in tropical montane forests are absent or sparse at lower elevations because of an inability to sustain positive net photosynthesis in warm, wet lowland forests (Frahm 1990). The critical experiments to test this hypothesis in temperate rainforests have yet to be conducted.

Lobaria oregana's specificity to humid, mid-elevation forests may be based upon physiological constraints. At elevations above its distribution range, cold temperatures may prohibit sufficient nitrogen fixation, while at low elevations warm temperatures may not allow adequate positive net photosynthesis. These hypotheses can be tested by transplanting *L. oregana* thalli into humid forests above and below its natural elevation range along with meteorological data-loggers that track temperature and precipitation. Subsequent analyses of growth rates and/or mortality rates combined with the meteorological data may help explain the landscape distribution patterns of *L. oregana*.

Modeling carbon fixation by L. oregana

Another useful application of *L. oregana*'s CO_2 exchange physiology would be the development of a model of annual carbon gain (e.g., Sundberg et al. 1997), which would allow re-evaluation of ecosystem-level productivity estimates for this species. A model of annual nitrogen fixation by *L. oregana* is readily developed because the parameters controlling nitrogenase activity are relatively simple. In hydrated thalli, nitrogen fixation is controlled primarily by temperature and is not light-limited (Chapter 3). The parameters controlling carbon fixation are more complex. For example, the interactions among light levels, temperature, and CO_2 exchange rates at different canopy heights would have to be monitored. Furthermore, high atmospheric humidity and actual precipitation events would both

activate CO_2 exchange, but thalli of different size classes would have hydration and desiccation rates (Gauslaa & Solhaug 1998). A more sophisticated characterization of stand-level CO_2 exchange dynamics would be necessary to accurately model carbon gain by *L. oregana.*

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Chapter 5. Conclusion

This thesis contributes to a better understanding of the ecological role of *L. oregana* in Douglas-fir forests of the Pacific Northwest. *Lobaria oregana*'s N-fixing capabilities are defined by local patterns of temperature and precipitation. The magnitude of its ecosystem contribution depends on stand-level biomass and is therefore site-specific. Temperature is more important than light level, host tree species, or canopy position in controlling nitrogenase activity in hydrated lichens. At the WRCC, where winters are cold and cyanolichen biomass is relatively low, annual N fixation by *L. oregana* is lower than past estimates would have predicted. In warmer sites with more abundant cyanolichen biomass (e.g., HJA), *L. oregana*'s contribution to the forest N cycle may be higher than previously recognized.

It is possible to estimate how much N *L. oregana* fixes based solely on litterfall data, canopy biomass data, and growth rates. However, a model based upon the physiology of *L. oregana*'s nitrogenase activity is a more powerful tool because it can be applied to any site where meteorological data and canopy biomass estimates exist.

This research also expands what is known about the physiological constraints on carbon metabolism in *L. oregana*. Its photosynthetic response to thallus water content is typical of other lichens, and like other lichens it becomes light-saturated at low PAR levels. The effect of temperature on both carbon metabolism and N fixation may provide clues to some of *L. oregana*'s landscape distribution patterns. The fact that N fixation is inhibited by low temperatures may explain why *L. oregana* drops out of epiphyte assemblages at higher elevations. The narrow temperature range within which net carbon gain occurs may preclude *L. oregana*'s ability to grow and survive in warmer low-elevation forests. These hypotheses are compelling, but the research to test their validity has yet to be conducted.

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