

AN ABSTRACT OF THE THESIS OF

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Title: VARIATION IN METABOLISM, THALLUS CHARACTERISTICS,  
AND WATER RELATIONS OF THREE POPULATIONS OF  
LOBARIA PULMONARIA FROM WESTERN OREGON

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Abstract approved: \_\_\_\_\_  
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A physio-ecological study of Lobaria pulmonaria (L.) Hoffm. was done to determine if geographic variation occurred within the species. Lobaria pulmonaria was collected from Table Mountain, Oak Creek, and Lost Prairie, located in the Coast Range, Willamette Valley, and Cascade Range respectively in Western Oregon.

During 1971 and 1972 experiments were conducted to determine physical and chemical characteristics, thallus water content-water potential relations, and metabolic oxygen exchange rates in relation to light intensity, temperature, relative water content (RWC), and water potential. No differences were observed in the morphology, thallus area-density, presence of stictic and norstictic acids, chlorophyll concentrations, or metabolic response to temperature among the three populations of L. pulmonaria. However, results of

other experiments indicated the possibility that L. pulmonaria has evolved intraspecific differences between the Oak Creek population and the montane groups.

The Oak Creek population, situated in the most xeric habitat, showed a slow decrease in water potential as the plant lost water (-0.9 bars /%RWC); a relatively high light compensation point (>50 ft-c); a high light saturation point (1000 ft-c); a high tolerance to high light intensities before photo-inhibition occurred ( $\pm$ 1900 ft-c); a low range of relative water content at maximum photosynthesis (53-75%); and an ability to maintain maximum net photosynthesis until water potential reached -12.5 bars. In contrast, the Table Mountain and Lost Prairie populations showed a rapid decrease in water potential as the plant lost water (-2.4 and -2.6 bars /%RWC respectively); a low light compensation point (<50 ft-c); a low light saturation point (440 and 300 ft-c respectively); a low tolerance to high light intensities indicated by photo-inhibition above 775 ft-c; and an inability to stay at maximum net photosynthesis once water potential dropped below -2.0 and -0.5 bars respectively. The Lost Prairie population was similar in its range of relative water content at maximum photosynthesis to that of the Oak Creek group (52-76%); however, the Table Mountain population had a higher range at 72 to 82%.

Variation in Metabolism, Thallus Characteristics, and  
Water Relations of Three Populations of Lobaria  
pulmonaria from Western Oregon

by

Garry Allen Neil

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VARIATION IN METABOLISM, THALLUS CHARACTERISTICS,  
AND WATER RELATIONS OF THREE POPULATIONS OF  
LOBARIA PULMONARIA FROM WESTERN OREGON

INTRODUCTION

Physio-ecological studies done on higher plants have shown evidence for the existence of ecologically adapted races within species (Björkman and Holmgren, 1963; Björkman, 1966; Hiesey et al., 1971). However, only recently have physiological studies been done on species of lichens growing in diverse habitats to determine the occurrence of intraspecific variation (Harris, 1971; Kershaw and Rouse, 1971; Kershaw, 1972; Rundel, 1972; Lechowicz and Adams, 1973).

Lobaria pulmonaria (L.) Hoffm. is a widely distributed foliose lichen, found growing in a variety of habitats in Europe, Africa, Asia, and in North America. Much work has been done on its chemistry, physiology, and ecology; however, no physio-ecological studies have been done to determine the possible occurrence of ecological races. Therefore, morphologically similar members of the lichen species L. pulmonaria, occupying dissimilar habitats in Western Oregon, were subjected to a variety of experiments to determine if geographic variation occurred within L. pulmonaria. These experiments included: physical and chemical analyses, thallus water content-water potential relations, and metabolic oxygen exchange rates in

relation to light intensity, temperature, and relative water content-water potential.

This thesis describes these experiments and discusses the variation encountered among three populations of L. pulmonaria collected from contrasting environments.

CHARACTERISTICS OF LOBARIA PULMONARIAMorphology

Species of the genus Lobaria are commonly studied organisms, mainly because of their size and availability. A monograph has been done on this genus for North America (Jordan, 1973); however, no worldwide monographic treatment exists (C.F. Culberson, 1969b). Lobaria pulmonaria is a foliose lichen belonging to the family Stictaceae. Its thallus, which is attached loosely to a substrate, is large, 5-25 cm broad and usually deeply lobed. The upper surface is light brownish green in color, turning dark green when wet. It is highly reticulated and is similar in appearance to lung tissue, thereby given the common name, lungwort. The reticulations and margins often contain soredia giving these ridges a grayish color. The lower surface is brownish to brown, covered with a close layer of tomentum. Apothecia are scattered, 1.0-4.0 mm in diameter, and usually a brownish-red color (Fink, 1935; Hale, 1969). Cephalodia are noticeable on the upper surface. They contain a secondary phycobiont, Nostoc, a member of Cyanophyta (Jordan, 1970). Coccomyxa, a chlorophyte, is the primary phycobiont of L. pulmonaria (Ahmadjian, 1967).

Another Lobaria species worth mentioning is Lobaria oregana (Tuck.) Müll. Arg. It grows in some of the same areas as L. pulmonaria. Its thallus is similar to L. pulmonaria; however, the

upper surface is light green to yellow and greenish-blue when wet, the lower surface is whitish-brown, and lobules are usually found along the margins of the lobes.

The three populations of L. pulmonaria studied are morphologically similar. Voucher specimens are on deposit in the Oregon State University Mycological Herbarium (OSC).

#### Distribution

Lobaria pulmonaria has a disjunct distribution through wide portions of Europe, Asia, Africa, and North America (Jordan, 1973). In Western and Central Europe it is confined to regions of more than 180 rainy days per year, whereas in Northern Europe it requires a lower number of rainy days (Barkman, 1958). In many areas of Western and Central Europe, Barkman (1958) reports that L. pulmonaria has become ". . . extinct or fragmentary. . ." because of air pollution, deforestation, and collecting for medicinal purposes. Lobaria pulmonaria resembles lung tissue, and during the middle ages it was used to treat lung diseases (Hale, 1967).

In the continental United States, L. pulmonaria is found along the West Coast from the montane regions of Northwest California to Western Oregon and large portions of Washington. Along the East Coast it extends from Maine west to Minnesota and south to North Carolina (Hale, 1969). In Canada, it has been reported in British

Columbia and from central Eastern Canada to the East Coast (Hale, 1967; Lambert and Maycock, 1968). Finally, in Alaska, it has been observed along the Southwest Coast (Jordan, 1973).

### Ecology

The majority of the populations of L. pulmonaria are corticolous but rarely it is saxicolous (growing on rocks). It has both a vertical and horizontal distribution on hardwoods and conifers. It begins its coverage from the base of a tree, thinning out as it moves up the canopy, and/or growing on the lower branches starting from the trunk of the tree. Lobaria pulmonaria grows mainly in old growth forest stands, and is rare on isolated trees, reforested areas, and in young trees (Hale, 1969).

The availability of water, as hinted by its distribution, is one of the major factors in the ecology of L. pulmonaria. Barkman (1958) reports finding L. pulmonaria only where either the precipitation is low and humidity is high, or the precipitation is high and humidity is low. For example, in areas of low humidity he found it growing only with 1000 to 1500 mm total rainfall whereas in high humidity areas it required only 500 to 700 mm to thrive. Lambert and Maycock (1968) observed that the range of L. pulmonaria in central Eastern Canada is from mesic to wet environments.

Lobaria pulmonaria's microhabitat is also strongly regulated

by water availability. The drier the climate the closer it grows to the base of trees and lower limbs. In some fairly dry areas it grows only on mossy boulders in forests (Barkman, 1958). It always occurs on the upper side of limbs and rocks. It needs direct water contact since absorption of water vapour is slow.

## THE STUDY AREAS

### General Description and Location

The study areas selected constitute an east-west transect from the Western Cascade Range to the Coast. The general description of the sample sites is given in Table I. Lost Prairie is in the Western Cascades, southwest of U. S. Highway 20, 12 km west of Santiam Junction. Hackleman Creek flows through this area. The Oak Creek sample site is located in the Willamette Valley, on a hillside 0.3 km northeast of Oak Creek Road, and 3 km northwest of Corvallis. The Table Mountain site is in the Coast Range, 1.4 km west on Forest Service Road 1246 from the junction of Forest Service Roads 1246 and 1354, and 16 km northeast of Waldport.

### Climatic Conditions

The proximity of the Pacific Ocean causes Western Oregon to have a maritime climate. The western slopes of the Coast Range are characterized by high precipitation, a long frost free period, and summer fog. The Coast Range, in turn, has a marked effect on the climate of the Willamette Valley. It serves as a partial barrier to the prevailing westerlies, causing less precipitation and warmer summers in the Valley. The Western Cascades provide the opposite effects by causing an uplifting and cooling of the air masses, thereby



Table I. Location, elevation, slope, and aspect of the three sample sites.

Site name	County	Latitude (N)	Longitude (W)	Elevation (m)	Distance from coast (km)	Aspect	Slope (%)
Lost Prairie	Linn	44°24'	122°03'	1009	163	-	0
Oak Creek	Benton	44°35'	123°20'	183	62	SW	23
Table Mountain	Lincoln	44°26'	123°52'	489	19	S	15

increasing precipitation in the Valley. The climate of the Western Cascades is mainly controlled by elevation, causing both precipitation and snowfall to increase and temperature to decrease (Franklin and Dyrness, 1969).

Climatic data were not obtained at the three sample sites. Instead, data were available from selected weather stations, reported by the U.S. Weather Service in Climatological Data from the years 1962 through 1971 (Tables II and III). Selection of these stations was based on similarity in elevation and terrain and on proximity to a sample site.

Since Lost Prairie has steep slopes on three sides and therefore receives considerable cold air drainage, the use of data from Marion Forks Fish Hatchery, which is similar in elevation, is inadequate. Instead, the climate of Lost Prairie is probably somewhere between that of Marion Forks Fish Hatchery and Santiam Pass.

The climographs of the four weather stations (Figure 1) and data from Table III demonstrate first, the high amount of year to year and month to month variation at the stations, and second, a considerable difference between the stations. Data for the Marion Forks Fish Hatchery and Santiam Pass weather stations indicate that Lost Prairie is characterized by very cold, wet winters. Snow in this region has started accumulating by the end of October and not subsided until at least the middle of April. Also, since this area is a frost pocket, its

Table II. Location and average annual precipitation, temperature, and frost-free period data for selected weather stations from 1962 through 1971.

Location	County	Sample site represented	Latitude (N)	Longitude (W)	Elevation (m)	Distance from site (km)	Direction from site	Average annual precipitation (mm)	Average annual temperature (°C)	Average frost-free period (days)
Marion Forks Fish Hatchery	Linn	Lost Prairie	44°36'	121°57'	757	27	NE	1764 ± 280 <sup>1</sup>	7.8 ± .6	115 ± 29
Santiam Pass	Linn	Lost Prairie	44°25'	121°52'	1452	20	E	2234 ± 357	4.9 ± .4	24 ± 16
Corvallis State College	Benton	Oak Creek	44°38'	123°12'	69	11	NE	1125 ± 228	10.9 ± .5	188 ± 26
Tidewater	Lincoln	Table Mountain	44°25'	123°54'	15	4.5	SW	2410 ± 325	11.6 ± .5	218 ± 38

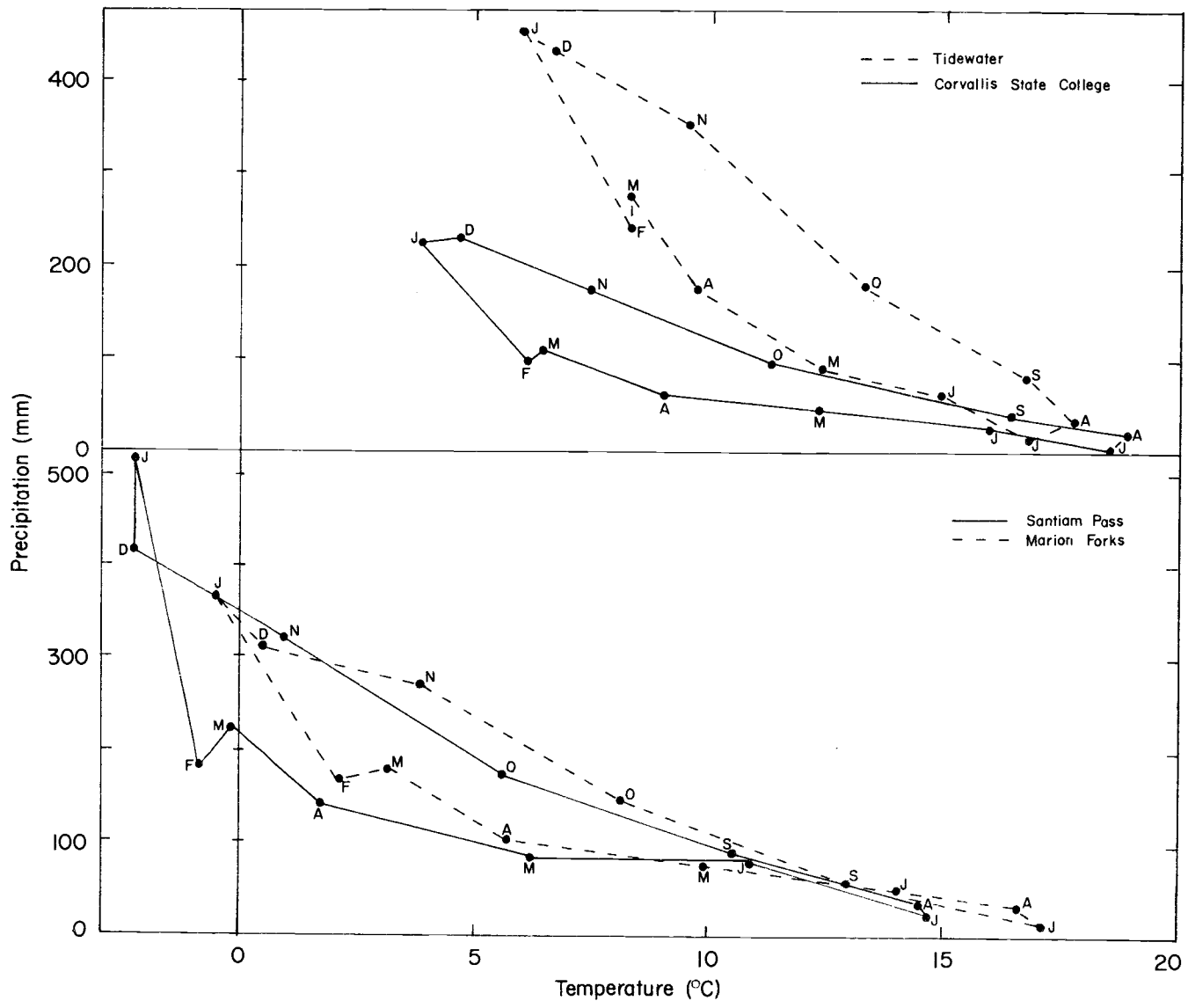
<sup>1</sup>The plus and minus values represent the standard deviation.

Table III. Mean monthly and annual days of measurable precipitation ( $\geq 2.54$  mm) of the representative weather stations from 1962 through 1971.

Site:	Lost Prairie		Oak Creek	Table Mountain
Station:	Santiam Pass	Marion Forks Fish Hatchery	Corvallis State College	Tidewater
January	21.7 $\pm$ 2.9 <sup>1</sup>	17.8 $\pm$ 6.7	15.1 $\pm$ 5.5	19.1 $\pm$ 4.9
February	10.7 $\pm$ 1.7	10.8 $\pm$ 2.0	8.2 $\pm$ 3.2	13.9 $\pm$ 2.2
March	11.8 $\pm$ 5.2	11.7 $\pm$ 5.4	10.6 $\pm$ 5.0	14.2 $\pm$ 5.6
April	12.9 $\pm$ 3.6	11.1 $\pm$ 3.9	7.6 $\pm$ 3.5	13.7 $\pm$ 3.1
May	7.3 $\pm$ 1.6	8.3 $\pm$ 2.8	4.9 $\pm$ 2.3	8.3 $\pm$ 2.6
June	5.7 $\pm$ 3.1	6.3 $\pm$ 3.1	3.1 $\pm$ 1.3	5.0 $\pm$ 2.1
July	3.4 $\pm$ 2.7	3.8 $\pm$ 2.3	1.2 $\pm$ 1.1	1.9 $\pm$ 1.6
August	3.6 $\pm$ 4.7	3.5 $\pm$ 4.3	2.1 $\pm$ 3.0	2.7 $\pm$ 3.6
September	5.1 $\pm$ 1.6	5.0 $\pm$ 2.3	3.6 $\pm$ 2.3	5.9 $\pm$ 2.6
October	10.6 $\pm$ 2.8	9.6 $\pm$ 3.3	8.0 $\pm$ 2.3	11.0 $\pm$ 2.4
November	13.4 $\pm$ 5.5	14.3 $\pm$ 4.3	12.7 $\pm$ 4.4	18.3 $\pm$ 4.7
December	<u>17.9 <math>\pm</math> 4.7</u>	<u>16.4 <math>\pm</math> 5.7</u>	<u>15.8 <math>\pm</math> 5.2</u>	<u>19.7 <math>\pm</math> 5.6</u>
Total	124.1	118.6	92.9	133.7

<sup>1</sup>The plus and minus values represent the standard deviation.

Figure 1. Climographs for the Tidewater, Corvallis State College, Santiam Pass, and Marion Forks Fish Hatchery weather stations. The data were averaged from U. S. Weather Bureau Climatological Data, 1962 through 1971. Letters indicate month of the year.



frost free period is probably similar to the figures reported for Santiam Pass (Table II). Lost Prairie is homologous to the other areas in two ways. Its summers are dry and warm, being the same as that of the Oak Creek area, and its monthly rainfall and number of days of rain per month follow closely those of the Table Mountain area.

Winter in the Oak Creek region is cool and wet. It receives only 50% of the amount of precipitation that Lost Prairie and Table Mountain do. Some snow is common to the area but it does not stay more than a few days. Its summers are warm and dry with some rain in June and September.

The Table Mountain region is characterized by very wet, cool winters. Like the Oak Creek area, some snow is common at the elevation of the sample site but it does not usually last more than a week. Its summers are usually not extremely dry or warm except the month of July and part of August. The summer fog brought in from the ocean by the movement of air masses usually keeps the summers cool and moist.

The data of Table III combined with the yearly total precipitation reported in Table II indicate that Table Mountain is the most mesic location, whereas the Oak Creek site is the most xeric.

### Vegetation

The three sample sites are placed in vegetation zones based on

the paper by Franklin and Dyrness (1969): the Table Mountain site is located within the Tsuga heterophylla Zone; the Oak Creek site is within the Pinus-Quercus-Pseudotsuga Zone; and the Lost Prairie site is part of the Abies amabilis Zone.

The Table Mountain location is dominated by Pseudotsuga menziesii with a few Tsuga heterophylla and no hardwoods. Lobaria pulmonaria was found only on the limbs of P. menziesii up to approximately 40% of total tree height. The average height of the trees sampled was  $49 \pm 1$  m and their average diameter at breast height (DBH) equalled  $119 \pm 4$  cm.

The Oak Creek site is dominated by Quercus garryana with a small number of young P. menziesii growing within the location. At this site L. pulmonaria grows on both the trunk and branches of Q. garryana to a height ranging from 30 to 50% of total tree height. No L. pulmonaria was observed on the P. menziesii. The average height of the trees sampled was  $5.4 \pm 1.9$  m and their average DBH equalled  $9.0 \pm 1.1$  cm.

Picea engelmannii is the dominant tree at the Lost Prairie site. Lobaria pulmonaria occurs only on the limbs of P. engelmannii that are at least partly open grown. It was not found inside the stand perimeter except where there were openings in the P. engelmannii stand. Lobaria pulmonaria was found on the limbs to approximately 40 to 60% of total tree height. The average height of the trees



sampled was  $49.5 \pm 0.7$  m and the average DBH equalled  $118.5 \pm 3.5$  cm.

The epiphytic vegetation associated with L. pulmonaria was determined by collecting from only the branches of the trees at all sites. Branches ranging in height from one to five meters and facing in all directions were selected. The epiphytes were determined by Dr. Lawrence H. Pike, Oregon State University. As indicated by Table IV, there is more similarity in the epiphytic vegetation for Table Mountain and Lost Prairie than for Oak Creek. Pseudocyphelaria anomala was the only epiphyte found at all three sites, whereas only four other lichens are associated with L. pulmonaria at Lost Prairie and Table Mountain. This gives further evidence that the habitat of L. pulmonaria in the three areas is different.

Table IV. Systematic checklist of epiphytes found with Lobaria pulmonaria.<sup>1</sup>

	Table Mountain	Oak Creek	Lost Prairie
<u>Lichens</u> <sup>2</sup>			
<u>Alectoria</u> sp. ( <u>jubata</u> group)			X
<u>Alectoria sarmentosa</u> (Ach.) Ach.			X
<u>Caloplaca oregona</u> Magn.		X	
<u>Caloplaca vicaria</u> Magn.		X	
<u>Cetraria chlorophylla</u> (Willd.) Vain.			X
<u>Cladonia fimbriata</u> (L.) Fr.			X
<u>Evernia prunastri</u> (L.) Ach.		X	
<u>Hypogymnia duplicata</u> (Sm. ex Ach.) Rass.	X		
<u>Hypogymnia enteromorpha</u> (Ach.) Nyl.	X		
<u>Hypogymnia tubulosa</u> (Schaer.) Hav.			X
<u>Lecanora</u> sp. ( <u>varia</u> group)		X	
<u>Lecanora confusa</u> Almb.		X	
<u>Lecanora pacifica</u> Tuck.		X	
<u>Lepraria membranacea</u> (Dicks.) Vain.	X	X	
<u>Lobaria oregana</u> (Tuck.) Mull. Arg.	X		
<u>Lobaria scrobiculata</u> (Scop.) DC.		X	
<u>Nephroma bellum</u> (Spreng.) Tuck.	X		
<u>Nephroma helveticum</u> var. <u>sipeanum</u> (Gyeln.) Wetm.		X	
<u>Nephroma laevigatum</u> Ach.	X	X	
<u>Nephroma parile</u> (Ach.) Ach.			X
<u>Nephroma resupinatum</u> (L.) Ach.		X	
<u>Ochrolechia farinacea</u> Howard		X	
<u>Parmelia subaurifera</u> Nyl.		X	
<u>Parmelia sulcata</u> Tayl.		X	
<u>Parmeliella saubinetii</u> Zahlbr.	X		
<u>Pertusaria amara</u> (Ach.) Nyl.		X	
<u>Pertusaria ambigens</u> (Nyl.) Tuck.	X		
<u>Platismatia glauca</u> (L.) W. Culb. & C. Culb.	X		X
<u>Platismatia herrei</u> (Imsh.) W. Culb. & C. Culb.	X		X

(Continued on next page)

Table IV. (Continued)

	Table Mountain	Oak Creek	Lost Prairie
<u>Lichens</u> (continued)			
<u>Pseudocyphellaria anomala</u> Magn.	X	X	X
<u>Pseudocyphellaria anthraspis</u> (Ach. ) Magn.	X		X
<u>Pseudocyphellaria crocata</u> (L. ) Magn.	X		X
<u>Ramalina farinacea</u> (L. ) Ach.		X	
<u>Ramalina inflata</u> Hook. f. & Tayl.		X	
<u>Rinodina archaea</u> (Ach. ) Arn.		X	
<u>Rinodina marysvillensis</u> Magn.		X	
<u>Sphaerophorus globosus</u> (Huds. ) Vain.	X		
<u>Sticta fuliginosa</u> (Dicks. ) Ach.		X	
<u>Sticta limbata</u> (Sm. ) Ach.		X	
<u>Usnea subfloridana</u> Stirt.	X		
<u>Mosses</u> <sup>3</sup>			
<u>Antitrichia curtipendula</u> (Hedw. ) Brid.	X		
<u>Dicranum fuscescens</u> Turn.			X
<u>Hypnum circinale</u> Hook.			X
<u>Isothecium spiculiferum</u> (Mitt. ) Ren. & Card.	X		
<u>Neckera douglasii</u> Hook.	X		
<u>Orthotrichum</u> sp.	X		
<u>Orthotrichum consimile</u> Mitt.			X
<u>Orthotrichum lyellii</u> Hook. & Tayl.		X	
<u>Liverworts</u> <sup>4</sup>			
<u>Frullania nisquallensis</u> Sull.	X		
<u>Porella navicularis</u> (Lehm. & Lindenb. ) Lindb.	X		

<sup>1</sup> Voucher specimens are on deposit at the OSU Mycological Herbarium (OSC).

<sup>2</sup> Nomenclature follows Hale and Culberson (1970).

<sup>3</sup> Nomenclature follows Lawton (1971).

<sup>4</sup> Nomenclature follows Conard (1956).

## PHYSICAL-CHEMICAL STUDIES

Thallus Area-DensityMethods

In June, 1971, thalli of L. pulmonaria were obtained from the three sample sites from branches at one to five meters above the ground, and were allowed to dry at room temperature for 24 hours. The material was then stored in the dark at room temperature up to one week before use. It was noticed with earlier samples that if the lichen material was left exposed to high intensity light the thalli would become slightly bleached after one to two weeks. One to two lobes were then removed from the outer edge of each thallus from a total of 30 plants for each location. The lobes were selected according to their health and physical condition as noted by color and absence of damage. The material used represented the youngest growth and the whole population.

Following the procedures of Harley and Smith (1956), the lobes were washed in tap water to remove any dirt. Discs 11 mm in diameter were cut with a brass cork borer and soaked in Petri dishes of distilled water at room temperature for 12 to 20 hours, allowing complete saturation. After soaking, the discs were blotted twice with Kimwipes to remove any surface water and weighed ( $\pm 0.1$  mg) to

determine their saturated weight (results are reported in thallus water content-water potential section). For dry weight determination, the material came to a constant weight when kept at 80°C for 24 hours. This temperature was adopted to avoid charring. The area-density values were calculated for each disc using the following formula:

$$\text{Area-Density (mg/cm}^2\text{)} = \frac{\text{dry weight}}{\text{disc area}}$$

An F-test ( $\alpha = .05$ ) was calculated on the null hypothesis that the means for area-density are similar at the three locations.

### Results and Discussion

Area-densities for the three populations were similar (Table V). This was further confirmed by the F-test which failed to reject the hypothesis. The L. pulmonaria populations seem to have no obvious physical differences, with the external morphology and area-density being the same. However, the area-density gives no clue as to the compactness of the thalli at each locality. It does not indicate the amount of material, by volume, in the upper cortex, medulla, and lower cortex.

Smith (1961), working with Peltigera polydactyla, found that seasonal variation occurred in the dry weight of sample discs, with a higher dry weight in late winter and early spring and a decrease in

Table V. Mean thallus area-density of three populations of Lobaria pulmonaria.

Sample site	No. of samples (n)	Mean (mg/cm <sup>2</sup> )	Standard deviation (mg/cm <sup>2</sup> )
Table Mountain	30	9.0	0.9
Oak Creek	47	10.6	1.0
Lost Prairie	36	9.0	0.6

the summer. He concluded that higher assimilation activity in the winter and spring may account for this observation. Therefore, the area-density data reported here for L. pulmonaria should not be considered as necessarily constant throughout the year.

### Chemical Tests

#### Methods

One lobe from five individual thalli from each location was removed from material collected in June, 1972, to determine the presence of the chemical constituents, stictic and norstictic acids. The molecular structure of these acids is described by C. F. Culberson (1969a). Following the procedures of Hale (1967), each lobe was soaked in distilled water for one minute to allow the thallus to become soft and pliable. Each lobe was then divided in half and placed on a porcelain spot plate. One drop of 55% potassium hydroxide (K) in aqueous solution was placed on the medulla and upper cortex of one

set of divided lobes and one drop of 5% p-phenylenediamine (PD) in ethyl alcohol on the other set. Five minutes were allowed for any color reactions. If stictic and norstictic acids are present, a positive reaction will occur with K giving a yellow coloration and PD a red color.

### Results and Discussion

For all samples, the results were positive for both K and PD, indicating the presence of stictic and norstictic acid. This provides evidence that there are no chemical variants in any of the three populations as far as these two chemical constituents; however, it does not give any indication of possible variation with any other compounds.

Other studies of the chemistry of L. pulmonaria have shown several distinct varieties and strains. These strains and varieties have been recognized by combinations of the following products: stictic acid, norstictic acid, constictic acid, and triepside gyrophoric acid (C.F. Culberson, 1969b). Of importance to this study, some of her populations were found to be chemical varieties or strains because of the absence of either stictic or norstictic acid.

It is not uncommon to find chemical variants of morphologically uniform lichens exhibiting distinct geographical distributions (Hale, 1967). For example, in the Ramalina siliquosa group, there were four chemical races, each at a different locality in Europe (W. L. Culberson,

1967). In fact, upon further study, where the ranges of these races overlapped, the races selected different ecological habitats (Culberson and Culberson, 1967). Other lichen species that demonstrate the same phenomenon are the Cladonia chlorophaea group (Evans, 1944), the Parmelia dubia group (Culberson and Culberson, 1956), the Usnea strigosa group (Hale, 1962), and the Thamnolia vermicularis group (Sato, 1965).

### Chlorophyll Concentration

#### Methods

In March, 1972, it was noticed that there seemed to be a difference in the intensity of the greenish color in the thalli at the three sample areas, indicating a possible difference in chlorophyll concentrations. Therefore, one lobe from 30 thalli at each location was collected in order to determine if this was the case.

The lobes were immediately air dried over calcium sulfate in a sealed glass jar for 24 hours, since oven drying would cause decomposition of the chlorophyll. Then the 30 lobe samples were placed in liquid nitrogen causing the thalli to become extremely brittle, and allowing them to be ground into a fine powder with a mortar and pestle. It was found through earlier trials that chlorophyll would not be entirely extracted from air dried thalli placed directly into a glass to



glass homogenizer with the organic solvent. The finely powdered thalli were next separated into four to five samples per location, each weighing 30 to 50 mg.

The procedure for chlorophyll extraction followed that of Hill and Woolhouse (1966). Each sample was placed in a glass homogenizer tube and 0.4 ml of 80% (v/v) pyridine added. The pyridine served to swell and soften the lichen tissue, thereby allowing rapid release of chlorophyll, and it reduced phaeophytin formation by keeping the pH above 7.0 as other organic solvents were added. This mixture was allowed to stand for 15 minutes. One and six-tenths (1.6) milliliters of methanol was then added and the mixture was hand homogenized for 10 minutes. This was usually enough time for complete extraction of the chlorophyll. Ten milliliters of 80% acetone was added to the homogenized mixture, which was then centrifuged at 6,000 x g at 0°C for 10 minutes. The supernatant was decanted and the volume measured. The optical density of the supernatant was recorded at 645 and 663 m $\mu$  in a Beckman DB spectrophotometer, using 1 cm cells.

Chlorophyll concentrations were calculated from the following equations (Maclachlan and Zalik, 1963):

$$C_a = \frac{(12.3 D_{663} - 0.86 D_{645}) V}{d \times 1000 \times W}$$

$$C_b = \frac{(19.3 D_{645} - 3.6 D_{663}) V}{d \times 1000 \times W}$$

where C = concentration in mg/g dry weight; a = chlorophyll a;  
b = chlorophyll b; D = optical density at wavelength indicated; V =  
volume of extract in ml; d = length of light path in cm; W = dry  
weight of thalli in g.

Test extractions were also made using only 80% acetone to prove that additions of methanol and pyridine would not effect the position of absorption maxima at 645 and 663 m $\mu$ , since Maclachlan and Zalik's equation is primarily used for 80% acetone extracts.

Chlorophyll concentrations of thalli were determined in June, 1972, using the above procedure, to determine if there were any seasonal variation.

A Least Significance Difference Test (LSD) at 5% level was done to determine which populations were significantly different in mean chlorophyll concentration for March and June.

### Results and Discussion

The mean total chlorophyll content of samples collected in March was over twice those collected in June. There was no significant difference between locations in June and only a slight difference between the population at Table Mountain and the other two locations for March (Table VI). This indicates that the visual color difference observed was not due to chlorophyll concentration, and the algal layer of the lobes contains approximately the same amount of chlorophyll

Table VI. Mean chlorophyll content, standard deviation, and LSD results for populations of Lobaria pulmonaria.

Sample site	Month collected	Mean chlorophyll concentration <sup>1</sup> (mg/g dry wt)	Standard deviation (mg/g dry wt)	LSD results <sup>2</sup>
Table Mountain	March	2.21	0.19	a
Oak Creek	March	1.86	0.16	b
Lost Prairie	March	1.66	0.14	b
Table Mountain	June	0.92	0.09	c
Oak Creek	June	0.91	0.07	c
Lost Prairie	June	0.77	0.07	c

<sup>1</sup> Each mean value represents the replicate analysis of four to five separate samples from each habitat.

<sup>2</sup> Like letters indicate no significant difference (5% level); unlike letters indicate significant difference.

with the possible exception of the Table Mountain population. The results also show that seasonal variation occurs in chlorophyll concentration of the thalli.

Before conducting this experiment it was thought that the Oak Creek population might have the lowest chlorophyll content. Lobaria pulmonaria grows on a deciduous tree at this location and is therefore in a more exposed habitat than the populations at the other localities. Rundel (1972), working with sun and shade races of Cladonia sub-tenuis, found the mean total chlorophyll content of shade populations was over two times that of sun populations. Hill and Woolhouse (1966), working with Xanthoria parietina, found a 40% decrease in chlorophyll content in exposed thalli compared to shaded ones. This observed decrease in chlorophyll content was attributed to a decrease in number of algal cells per unit surface area and a decrease in chlorophyll content per algal cell (Rundel, 1972). Higher plants show similar variations between shade and sun species and races (Björkman and Holmgren, 1963; Björkman, 1970).

Seasonal variation in chlorophyll content in lichens was first reported by Wilhelmsen (1959). Working with Peltigera canina, Parmelia physodes and Xanthoria parietina, it was reported that in the summer these lichens contained one-half to two-thirds of their winter chlorophyll content. Plummer and Gray (1972) demonstrated that the density of algal cells in the thalli of three species of Cladonia

was about equal for winter and spring; however, no measurement was made in the summer. As with shade and sun species and races of lichens, seasonal variation might be caused by decrease in chlorophyll content per algal cell and decrease in algal cell number. However, Plummer and Gray's (1972) results raise doubts in the idea of algal cell decrease. Wilhelmsen (1959) stated a third possibility, that summer drought may be partly responsible for low levels of chlorophyll during the summer.

Finally, it should be noted that the summer chlorophyll content of L. pulmonaria on a dry weight basis is only 25% of the content in the leaves of higher plants as reported by Wilhelmsen (1959) and Björkman (1970).

## THALLUS WATER CONTENT-WATER POTENTIAL RELATIONS

Methods

Many lichen species are highly drought resistant; consequently, a number of studies have been conducted on water relations with respect to distribution and metabolism. However, only one investigation has been done on the relationship of thallus water content to water potential (Klepper, 1968).

Since the study sites varied considerably in precipitation received, it was expected that their water relations might also vary. To examine this possibility, thalli of L. pulmonaria were collected, from branches one to five meters in height at the three sites, in June and July of 1971 and 1972, to determine variation between populations in the water holding capacity of the thalli and in the water potential at different relative water contents (RWC). The thalli were stored up to one month in an air dried condition at room temperature and in the dark. No difference was observed in comparing water potential of thalli used immediately after collection to those a month old.

Data were obtained on thallus saturation weight from the study done on thallus area-density. Water holding capacity at saturation is reported in the results as percent of oven dry weight and was determined by the equation:

$$\text{Water Holding Capacity (\%ODW)} = \frac{\text{SW} - \text{ODW}}{\text{ODW}} \times 100$$

where, %ODW = percent oven dry weight; SW = saturated weight; ODW = oven dry weight. An F-test ( $\alpha = .05$ ) and an LSD test were done on the means for the three locations.

The water potential of thalli at different RWC's was determined by use of the Schardakow dye method (Knipling, 1967a). In this method, preweighed samples of five 11-mm discs of a known RWC were immersed in test tubes (10 x 75 mm), sealed with Parafilm, containing 1.0 ml of a graduated series of sucrose solutions (test solutions) of known water potentials. Five disc samples were used rather than one to reduce individual variation and to provide sufficient tissue for the method to be used. The sucrose used was of a commercial type (C&H Sugar), and the solutions of known water potential were prepared according to the table in Knipling (1967b). Fresh solutions were made every fourth day to avoid the possibility of contamination. Two series of test solutions were used for each RWC, with the first series being at five bar increments to determine the approximate water potential, and the second series being at one bar increments to refine the estimate. The immersion time for the discs was tested. They came to equilibrium with the test solutions in 30 minutes. Knipling and Kramer (1967) reported that it takes from two to eight hours for broadleaf samples and up to 24 hours for conifer needles to reach equilibrium.

The precision of this method depends on three factors: 1) lack of contamination on the discs (Knipling and Kramer, 1967), 2) no escape of free water from the cut cells of the discs (Brix, 1966; Hellmuth and Grieve, 1969), and 3) a constant RWC within each series of discs. The first two factors were accounted for by treating the lichen thalli in the same manner as described in the methods section of thallus area-density, with the addition that the thalli were washed in distilled water as well as tap water to reduce contamination. Since diffusion of water from freshly cut cells of the discs was a source of error, the discs were soaked for 12 to 20 hours in Petri dishes filled with distilled water to allow for this diffusion.

Discs used for water potential studies could not be used to measure the RWC, since the sucrose would enter into the intercellular spaces as well as the cells and would therefore affect the ODW. Consequently, control samples, of five discs each, were set up for RWC calculations. In determining RWC, two facts about lichens had to be controlled: 1) the greater the dry weight of the disc, the lower the rate of water loss, and 2) humidity affects the rate of water loss (Smyth, 1934; Scofield and Yarman, 1943; Heatwole, 1966; Mutch and Gastineau, 1970).

Saturated weight (SW) was determined by weighing water saturated disc samples, with all samples being within 10.0 mg of each other. This was accomplished by taking soaked discs, blotting them



dry of surface water with Kimwipes, and weighing them, in sets of five, rapidly on a Mettler Balance ( $\pm 0.1$  mg). The five disc samples were then placed into "humidity jars" of a screw cap type, 5.75 cm in height, 5.25 cm in diameter, and 115 ml in volume. The jars contained 10 ml of a saturated salt solution and a number 8 rubber stopper which served as a platform for the discs. The saturated salt solutions controlled the relative humidity inside the jar. Winston and Bates (1960) reported that saturated salt solutions maintain constant relative humidity in the atmosphere over them because each solution has a definite water vapor pressure at a given temperature. Two salts with the least variability caused by temperature were selected, since the jars were left in an open lab with variable temperature during disc desiccation. The solutions selected were KCl with a relative humidity of 85% at 20°C and  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  with a relative humidity of 55% at 20°C. The discs were placed in the jars of one or the other salt solutions depending on whether a high or low RWC was desired.

The five disc samples in the jars were allowed to lose water for a set period of time, again depending on the RWC to be measured for water potential. The samples were then removed and fresh weight (FW) determined. They were oven dried at 80°C for 24 hours, and finally weighed again to obtain their ODW. The RWC for the samples of five discs was calculated using the formula (Barrs, 1968):

$$\text{RWC} = \frac{\text{FW} - \text{ODW}}{\text{SW} - \text{ODW}} \times 100$$

Confidence intervals (95%) were determined for every RWC.

The five disc samples used for the water potential determination were preweighed to within 10 mg of their corresponding samples used for RWC and then placed in the humidity jars with the same saturated salt solution for the same time period. At the end of that period they were assumed to be at the same RWC as the control samples.

Since a large number of discs were needed for the above methods, more than 50 thalli were collected from each location.

### Results and Discussion

The mean water holding capacity at saturation of samples collected in June, 1971, ranged from 164 to 182% of ODW, with the Lost Prairie population being significantly higher than those of Oak Creek and Table Mountain (Table VII). From these results, all three populations of L. pulmonaria can be characterized as hygrophytic. Barkman (1958) reported that hygrophytic lichen species, which make up the majority of the lichens, have a water capacity at saturation that ranges from 50 to 400% of ODW, whereas xerophytic species have a range from 400% to as high as 3900% in a species of Collema. This hygrophytic characteristic is further confirmed for

Table VII. Water content at saturation as percent oven dry weight (% ODW) of discs of three populations of Lobaria pulmonaria collected in June, 1971.

Sample site	No. of samples (n)	Mean (% ODW)	Standard deviation (%)	LSD results <sup>1</sup>
Lost Prairie	36	182	21	a
Oak Creek	47	164	9	b
Table Mountain	30	164	18	b

<sup>1</sup> Like letters indicate no significant difference (5% level); unlike letters indicate significant difference.

L. pulmonaria by the observation that it takes six to nine days for L. pulmonaria to reach equilibrium when placed in an environment of 100% relative humidity (Stocker, 1927). Lange et al. (1970), working on lichens of the Negev Desert, Israel, found that xeric species reach equilibrium in only two or three hours.

One might infer from Table VII that the Lost Prairie population is in a drier habitat than the others since its water capacity is higher. On the other hand, the data in Tables II and III and Figure 1 in the climate section, and the results obtained on the relative water content-water potential experiments (Figure 2) give strong evidence that the Oak Creek population is in the more xeric environment.

The results of the relative water content-water potential experiments are given in Figure 2. The Oak Creek population gradually decreases in water potential as the RWC decreases below

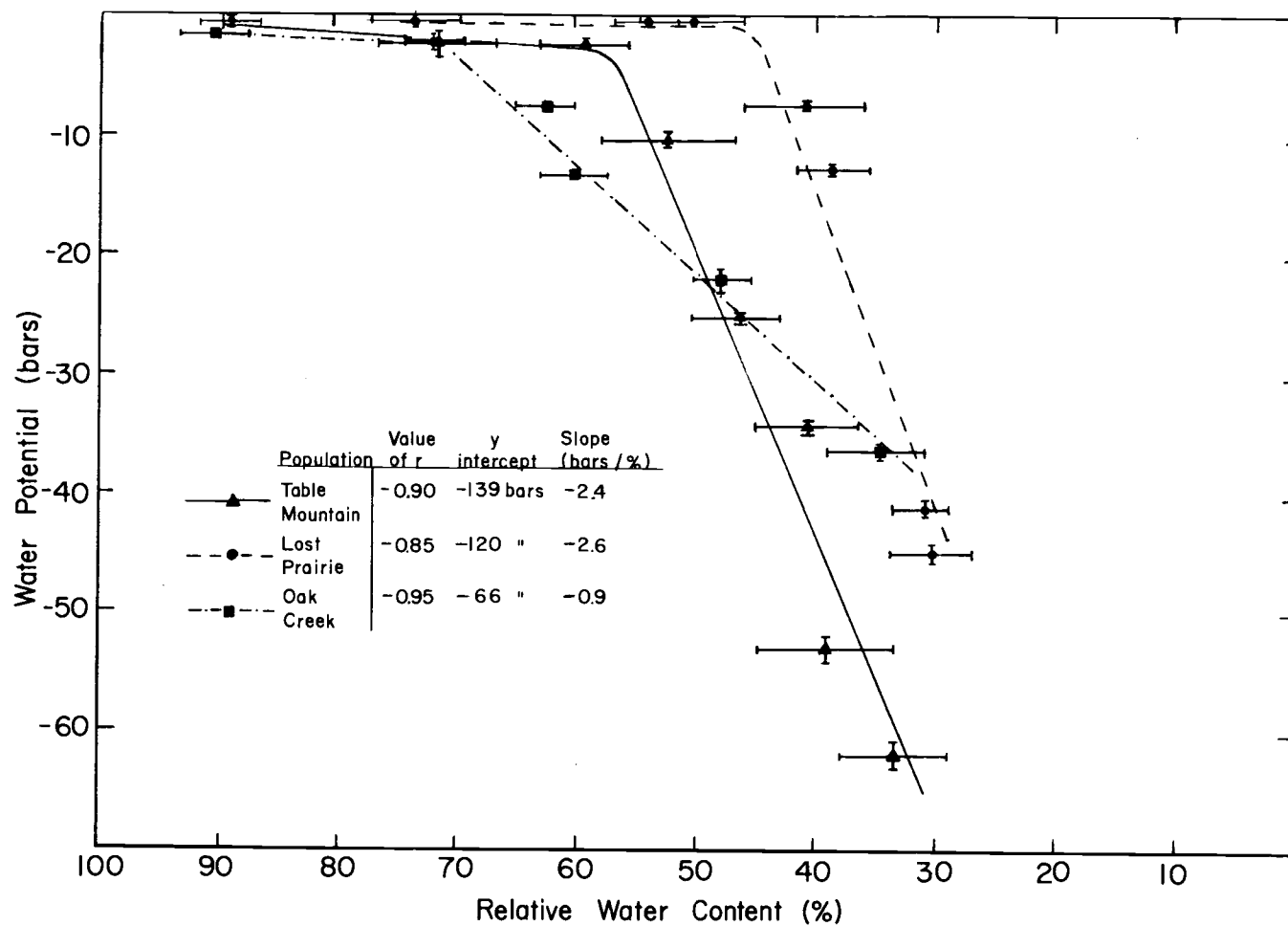


Figure 2. Relationship of thallus water content to water potential for three populations of Lobaria pulmonaria collected in June, 1971 and 1972. The horizontal lines at each point represent the confidence interval (95%) for that particular RWC. The value of r represents the correlation coefficient. The value of r, y intercept, and slope refer to the non-asymptotic part of the curves.

70%, whereas the Table Mountain and Lost Prairie groups decrease rapidly in water potential below 58 and 45% RWC respectively.

A plant that is distributed in a dry area might theoretically be expected to decline to a lower moisture content before undergoing stress. One would also look for its water potential to decrease gradually as RWC decreases in comparison to other members of the species growing in more mesic environments (Iljin, 1957; Slatyer, 1958). As can be seen by the results, only one of those expectations turned out to be the case for the Oak Creek population. The populations at the more mesic sites of Lost Prairie and Table Mountain did decrease rapidly in water potential in comparison to L. pulmonaria at Oak Creek; however, the Oak Creek population began to undergo stress at higher RWC than either of the other groups. This unexpected difference in RWC at which water potential starts to decline can be clarified by the studies done on the water holding capacity of living and dead lichen thalli. The amount of water absorbed by dead thalli, depending on the species, ranges from 50 to 75% of ODW of corresponding living thalli (Cuthbert, 1934; Smyth, 1934; Heatwole, 1966). This would seem to indicate that much of the water absorbed by lichens is held in the intercellular spaces. This is substantiated by experiments on Peltigera canina by Smyth (1934) in which it was observed that the hyphae swell less in dead thalli than living thalli. These findings led Smith (1962) to caution that measurements of

osmotic pressure must take into consideration intercellular water as a possible error.

Stocker (1927) reported that water in L. pulmonaria is mainly held outside the hyphal and algal cells in the intercellular spaces. Therefore, water must first be removed from the intercellular spaces, mainly located in the medulla region (Smith, 1960), before cellular water loss necessary to increase stress can occur. The amount of water that L. pulmonaria at Oak Creek holds in its intercellular spaces may be less than the other two populations; thereby stress begins at a higher RWC than the other populations. Consequently, the RWC below which water potential declines may have no relation to the types of environment the three populations inhabit, but instead to internal morphological differences unaccounted for in this study. However, comparison of the slopes of the non-asymptotic part of the curves shows that the Oak Creek population undergoes less moisture stress than both the Table Mountain and Lost Prairie groups as they become desiccated. This makes the Oak Creek population best suited for its habitat, since it is more exposed to desiccating conditions than the others.

## THALLUS METABOLISM

Relationship of Photosynthesis  
to Light IntensityMethods

Since Lobaria pulmonaria grows as an epiphyte on a deciduous tree at Oak Creek and on an evergreen tree at both Lost Prairie and Table Mountain, there is the possibility that the maximum photosynthetic rate of the population at Oak Creek occurs at a higher light intensity than the other two groups. Thalli were collected, in the manner described earlier, from the Oak Creek site on June 15, 1972, and ending with the Table Mountain site on June 17, 1972. Experiments were done on the day of collection.

One 13-mm diameter disc was excised from the youngest growth of one lobe of five or six separate individuals. The discs were washed in distilled water and placed in separate Petri dishes containing 10 ml of 10 mM Tris-Cl buffer and 10 mM NaHCO<sub>3</sub> at a pH of 7.0 for 30 minutes. During soaking, the discs were illuminated at room temperature using a 100 watt incandescent light source placed 1.0 m from the discs.

The rates of net photosynthesis and dark respiration were monitored using a Clark Oxygen Electrode (Baddeley et al., 1971) which was connected to a Gilson mini-polygraph (GME Model #M5P).

The cuvette in which the assimilation rates of each disc were measured contained 2.0 ml of the above buffer, with  $\text{NaHCO}_3$  serving as a  $\text{CO}_2$  source. Prior to placing the buffer into the cuvette,  $\text{CO}_2$ -free air (air bubbled through 0.1 M KOH), was bubbled into a buffer stock. At air saturation, the buffer contains  $0.304 \mu\text{M O}_2/\text{ml}$ .

The temperature of the buffer solution and disc in the cuvette was controlled by a continuous pump circulating distilled water from a constant-temperature refrigerated water bath to a glass water jacket enclosing the cuvette. The flow rate was 1300 ml/minute and the temperature was constant at  $20 \pm .5^\circ\text{C}$ . To absorb heat from the light source, a flat culture flask, 2.0 cm thick, containing distilled water, was placed 20 cm in front of the cuvette in the direction of the light source. Three sides of the water jacket were covered with tin foil. This allowed the light to reflect into the cuvette from all sides since it was impossible to keep the disc from moving in the cuvette. Two layers of red cellophane, which absorbed all wavelengths below  $620 \text{ m}\mu$ , were placed on the front of the water jacket. The use of red cellophane was recommended by Dr. Robert Gee (personal communication) so that only wavelengths utilized by chlorophyll a and b would pass to the discs. The light intensity readings that were measured, therefore, were only of those wavelengths that were actually used by the photosynthetic machinery. The light source used was a Sylvania tungsten halogen lamp ( $3400^\circ\text{K}$ ,



650 watts, 120 volts). The light intensity was controlled by varying the distance from the lamp to the cuvette and was measured in foot-candles (ft-c) using a Weston Model 756 Illumination Meter.

Each disc was placed in the cuvette containing buffer solution, after its 30 minutes soaking time, and allowed to equilibrate in the dark at 20°C for 15 minutes. To allow for equal circulation of the 2.0 ml buffer solution around the disc and the Clark Oxygen Electrode, a 5.0 mm Teflon stirring rod was placed in the cuvette, and was activated by a Micro 5 magnetic stirrer.

Dark respiration was measured before and after the photosynthetic measurements. Net photosynthesis was measured starting with the lowest light intensity. This precaution was necessary since it has been observed that using high intensities on higher plants and algae growing in shade habitats can damage the light-absorbing part of the photosynthetic mechanism (Kok, 1956; Hiesey et al., 1971). After the measurements on photosynthesis and dark respiration, the ODW for each disc was determined as described earlier.

Net photosynthesis and dark respiration are expressed as a percentage of maximum net assimilation rate (NAR) and as the absolute rate,  $\mu\text{M O}_2/\text{min/g dry weight}$ . An F-test ( $\alpha = .05$ ) was calculated on the mean absolute rates.

## Results and Discussion

The results indicate some physiological variation in relation to distribution of L. pulmonaria in Western Oregon (Figures 3, 4, and 5). There was a clear difference between the material collected in the deciduous forest of Oak Creek, having a maximum NAR between 775 and 1900 ft-c, and individuals in the evergreen forests, having a maximum NAR between 215 and 775 ft-c. This difference was also evident in the rate of NAR increase in relation to light intensity. The Oak Creek group increased gradually (Figure 3) in comparison to both the Table Mountain and Lost Prairie groups (Figures 4 and 5). Also, when the lines were extrapolated to the dark respiration rates, it was noted that the compensation points were lower for the two mountain populations than for that of the valley. Finally, differences were found in the light intensity at which photo-inhibition occurred. NAR of individuals from Table Mountain started to decline sometime above 775 ft-c. This was the same for the Lost Prairie group, except for one individual which started to decline after 325 ft-c. However, individuals from Oak Creek gave an indication that they were just starting to be inhibited by the light intensity at 1900 ft-c. It should be noted, because of experimental error caused by the discs moving in the cuvette, that after a disc reached 100% maximum NAR, it was considered to stay at 100% until after it dropped below approximately

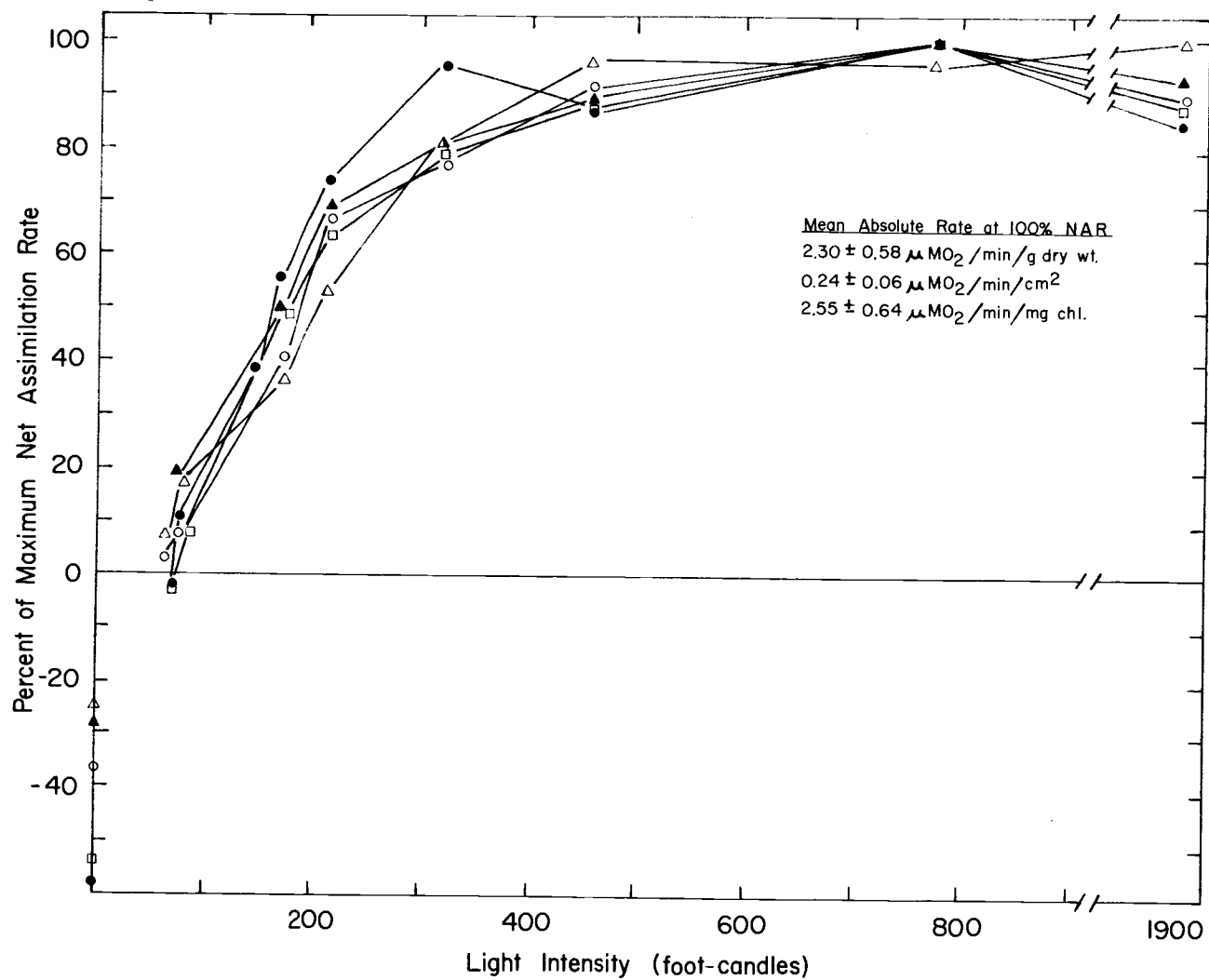


Figure 3. Relationship between net assimilation rate (NAR) and light intensity at 100% RWC for five replicates of *Lobaria pulmonaria* collected at Oak Creek. Absolute rates were converted using data from the area-density and chlorophyll sections.

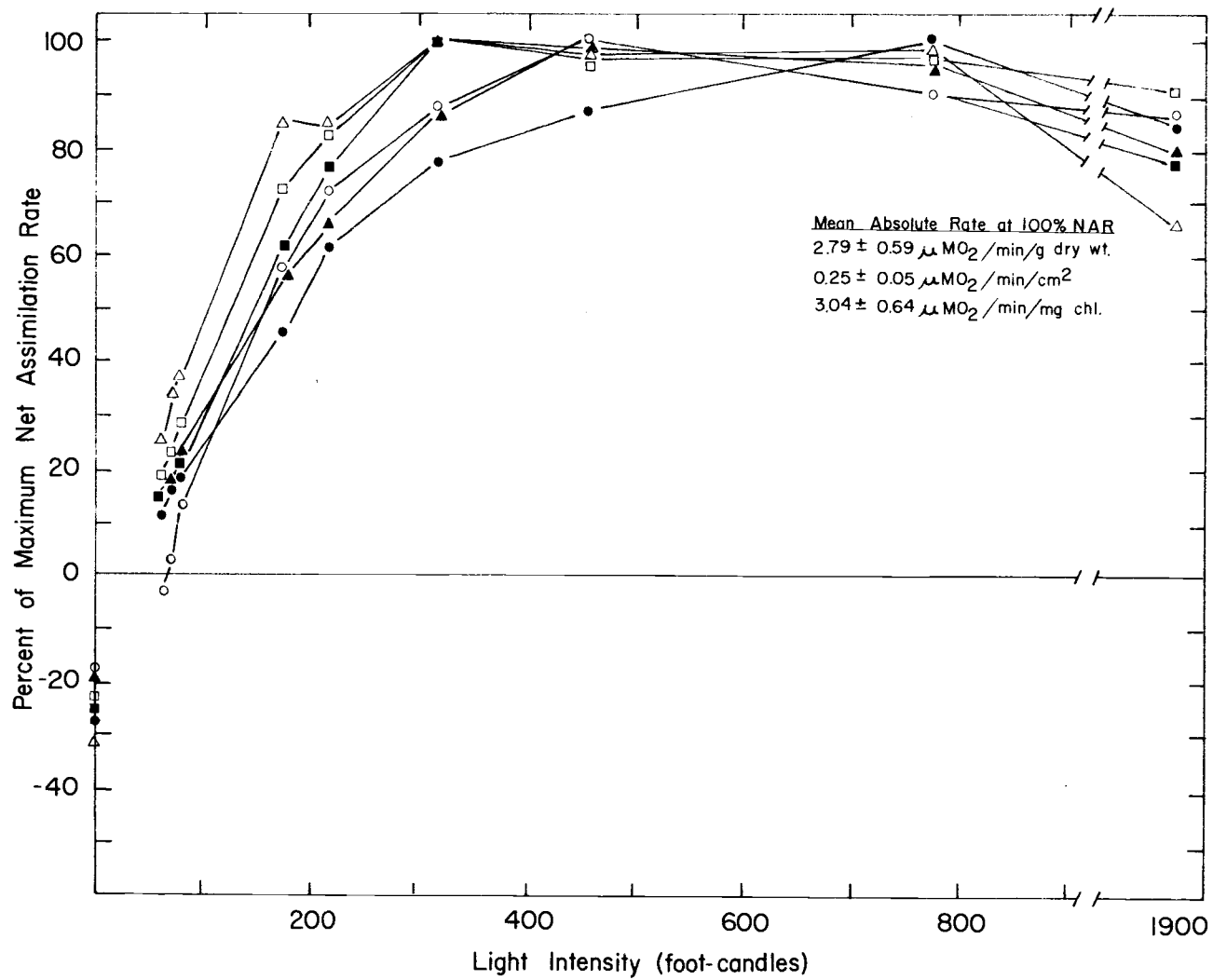


Figure 4. Relationship between net assimilation rate (NAR) and light intensity at 100% RWC for six replicates of *Lobaria pulmonaria* collected at Table Mountain. Absolute rates were converted using data from the area-density and chlorophyll sections.

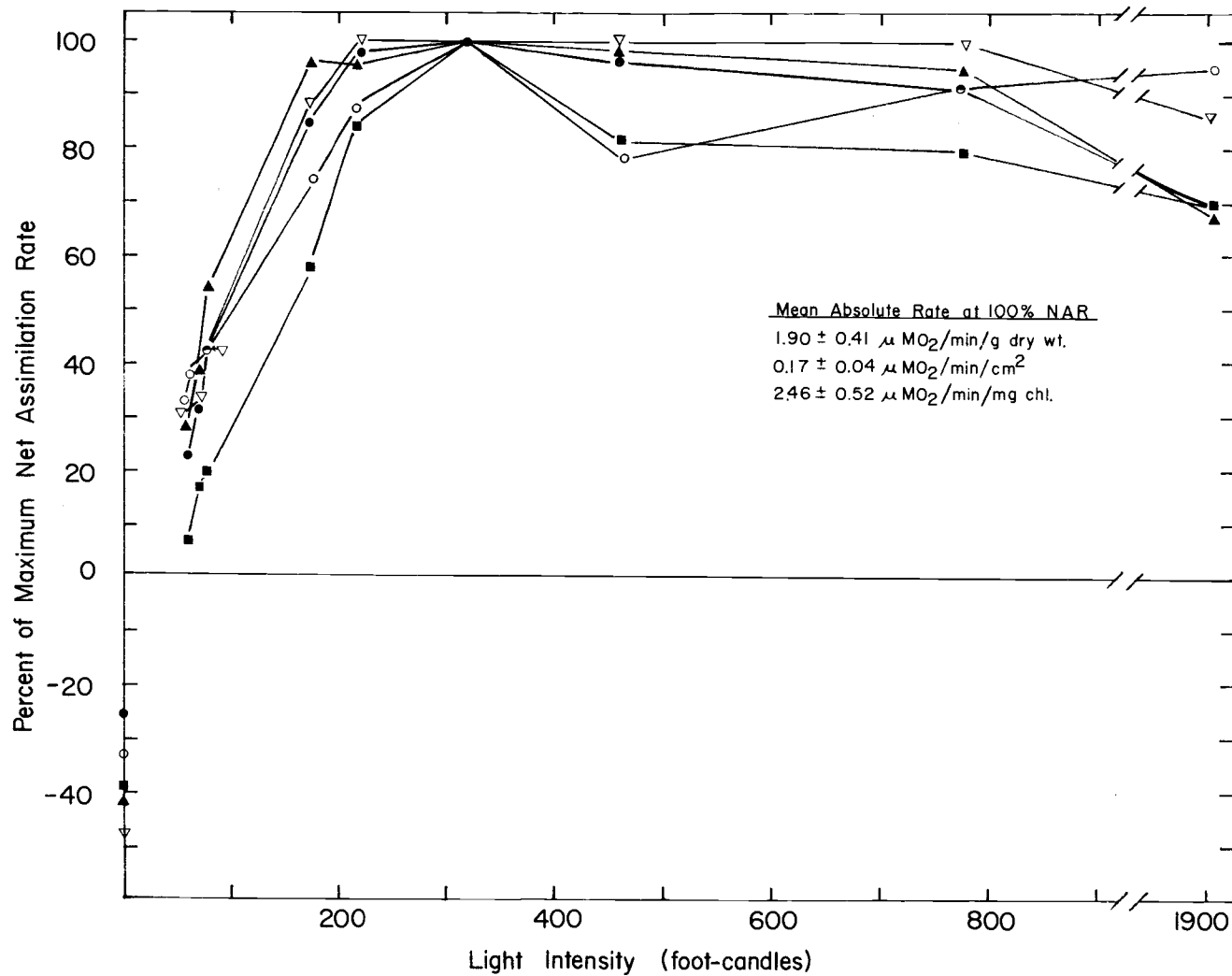


Figure 5. Relationship between net assimilation rate (NAR) and light intensity at 100% RWC for five replicates of *Lobaria pulmonaria* collected at Lost Prairie. Absolute rates were converted using data from the area-density and chlorophyll sections.

90% NAR. An F-test done on the mean absolute rates at 100% NAR for the three populations indicated no significant difference. No statistical analysis was done on the dark respiration rates; however, the Oak Creek individuals seem to have a higher rate than both those of Table Mountain and Lost Prairie (Figures 3, 4, and 5).

Each of the three populations of L. pulmonaria has characteristics similar to either sun or shade species and races found in a variety of higher and lower plants as indicated by differences in their light compensation points, rate of NAR increase in relation to light intensity, light saturation, and photo-inhibition.

Harris (1971) found that the NAR of Parmelia caperata growing at the top of an oak forest canopy started to level off at 1600 ft-c, whereas the same species, growing at the bottom of the tree, leveled off at 800 ft-c. In relating this observation to vertical light profiles in the forest, it was found that there was an increase of 25 to 50%, winter and summer respectively, in light intensity from ground level to the top of the canopy (Harris, 1972). Similar studies done by Hosokawa et al. (1964) found that lichens growing at the base of a tree had an optimal intensity at about 10,000 lux, and species growing at the top of the canopy were at optimum above 20,000 lux. Light saturation has also been found to be higher in sun species and races of higher plants as reported by Lundegardh (1921) and Björkman (1966, 1970).

Differences in the rate of increase of NAR in relation to light intensity increase have been observed in sun and shade races of Parmelia caperata (Harris, 1971) and Cladonia mitis (Lechowicz and Adams, 1973). In both studies, the sun races increased at a slower rate than did their corresponding shade races. Again, this is the case for higher plants (Björkman, 1966, 1970). Work done by Hosokawa and Odani (1957) and Harris (1971) also points out the fact that the compensation points for shade species of lichens are lower than those of related sun species. Similar observations have been made on the sun and shade races of the higher plant Solidago virgaurea (Björkman, 1966). No comparisons of photo-inhibition have been made between shade and sun species or races in lichens; however, photo-inhibition in lichens has been reported (Bliss and Hadley, 1964). In algae and flowering plants, photo-inhibition has been observed to occur in shade plants, whereas at the same light intensities, sun species and races show no inhibition (Kok, 1956; Björkman, 1966).

Higher rates of photosynthesis at light saturation have been observed for the sun races of Parmelia caperata than in its shade races (Harris, 1971). Björkman (1970) found that sun races and species of higher plants have a much higher rate than do their corresponding shade groups. Dark respiration rates determined on lichens by other researchers conflict with the apparent differences

noted in the L. pulmonaria results, in that Harris (1971), Rundel (1972), and Lechowicz and Adams (1973) observed no difference in dark respiration between shade and sun races.

It should be noted that Rundel (1972) and Lechowicz and Adams (1973) could find no significant difference in light saturation intensities between their sun and shade races of Cladonia subtenuis and Cladonia mitis respectively. However, with the observations made on L. pulmonaria and the data reported by Harris (1971), it can be concluded that sun-shade variation of the type found in higher plants does occur in some foliose, corticolous lichens. Consequently, in comparing the results of the three populations of L. pulmonaria with the results reported on sun and shade species and races of algae, other lichens, and higher plants, the Oak Creek population can be considered to be a more sun tolerant group than both the Table Mountain and Lost Prairie populations.

#### Relationship of Photosynthesis and Dark Respiration to Temperature

##### Methods

Experiments were conducted on the effect of temperature on dark respiration and photosynthesis at the end of June, 1972, using the same thalli previously collected for the light saturation experiment. One 13-mm diameter disc was excised, and treated as before,



from one lobe of six separate thalli from each location. Three replicates were used for measurement at  $5^{\circ}$  and  $10 \pm .5^{\circ}\text{C}$ , while the other three were used at  $10^{\circ}$  and  $20 \pm .5^{\circ}\text{C}$ . This was necessary because the discs were unduly injured if used more than twice. The excised discs were washed in distilled water and placed into test tubes containing the same buffer previously described. The test tubes were placed in a water bath for 30 minutes at the temperature of measurement. The discs were illuminated during this time with a General Electric reflector lamp at a light intensity of approximately 600 ft-c.

The rate of oxygen uptake and release was measured polarographically at 800 ft-c, as described in the previous experiment. All discs were assumed to be at light saturation at this light intensity (Figures 3, 4, and 5). At the end of the experiment, the ODW of all discs was determined.

Net photosynthesis and dark respiration are expressed as both absolute rates,  $\mu\text{M O}_2/\text{min/g}$  dry weight, and as relative rates, percent of photosynthesis and respiration relative to the average of all rates at  $10^{\circ}\text{C}$ . Statistical analyses were made of the absolute values at each temperature.

### Results and Discussion

The photosynthetic rate of the Lost Prairie population was lower

than both the Oak Creek and Table Mountain groups at all three temperatures (Table VIII). However, no significant difference between the dark respiration rates of the three populations was observed. Figure 6 seems to indicate that the three groups have a similar metabolic response to temperature up to 20°C.

Lange and Kappen (1972) observed that arctic lichen species have a higher rate of dark respiration at higher temperatures than do desert and tropical species. Billings and Mooney (1968) report the same type of response with higher plants, with higher dark respiration rates in species of colder regions than those of warmer areas. Physiological adaptation of species to warm and cold habitats was also found in photosynthetic rates. Peet and Adams (1972), comparing the temperature optima for net photosynthesis of Cladonia subtenuis, a southern species, to the temperature optima of Cladonia rangiferina, a more northern species, reported that C. rangiferina reached maximum photosynthesis at 15°C whereas the optimum for C. subtenuis was between 20°C and 25°C. This ecophysiological adaptation was further observed by Eickmeier and Adams (1973), with the alpine lichen species Cladonia ecmocyna having a lower temperature optimum than a related subalpine species, Cladonia rangiferina. One might have expected the Lost Prairie population, from the coldest habitat, to have a lower temperature optimum than the others.

Table VIII. Mean absolute rates of net photosynthesis and dark respiration in relation to temperature at 100% RWC for *Lobaria pulmonaria* collected in June, 1972. The abbreviations TM, OC, and LP represent the sample sites Table Mountain, Oak Creek, and Lost Prairie respectively.

Temperature (°C)	Rate: Population:	$\mu\text{M O}_2/\text{min/g dry wt.}$			$\mu\text{M O}_2/\text{min/cm}^2$			$\mu\text{M O}_2/\text{min/mg chl.}$		
		TM	OC	LP	TM	OC	LP	TM	OC	LP
<u>Net Photosynthesis</u>										
5°	Mean rate <sup>1</sup>	0.414	0.347	0.172	0.0037	0.0036	0.0015	0.45	0.38	0.22
	Std. dev.	0.098	0.150	0.096	0.0009	0.0016	0.0009	0.11	0.16	0.12
	LSD result <sup>2</sup>	a	a, b	b						
10°	Mean rate	0.720	0.955	0.395	0.0064	0.0101	0.0035	0.78	1.10	0.51
	Std. dev.	0.116	0.333	0.101	0.0015	0.0035	0.0009	0.19	0.37	0.13
	LSD result	a	a	b						
20°	Mean rate	2.41	2.88	1.33	0.021	0.030	0.012	2.62	3.18	1.72
	Std. dev.	0.38	0.15	0.11	0.0035	0.0017	0.0010	0.41	0.16	0.14
	LSD result	a	a	b						
<u>Dark Respiration</u>										
5°	Mean rate	0.130	0.261	0.162	0.0011	0.0027	0.0015			
	Std. dev.	0.047	0.048	0.068	0.0004	0.0006	0.0005			
	LSD result	a	a	a						
10°	Mean rate	0.267	0.332	0.249	0.0024	0.0035	0.0022			
	Std. dev.	0.095	0.061	0.087	0.0008	0.0007	0.0008			
	LSD result	a	a	a						
20°	Mean rate	1.35	1.68	1.37	0.012	0.018	0.012			
	Std. dev.	0.13	0.29	0.47	0.001	0.003	0.004			
	LSD result	a	a	a						

<sup>1</sup>The rates  $\mu\text{M O}_2/\text{min/cm}^2$  and  $\mu\text{M O}_2/\text{min/mg chl.}$  were calculated using the conversion data from the area-density and chlorophyll sections.

<sup>2</sup>Like letters indicate no significant difference (5% level!); unlike letters indicate significant difference.

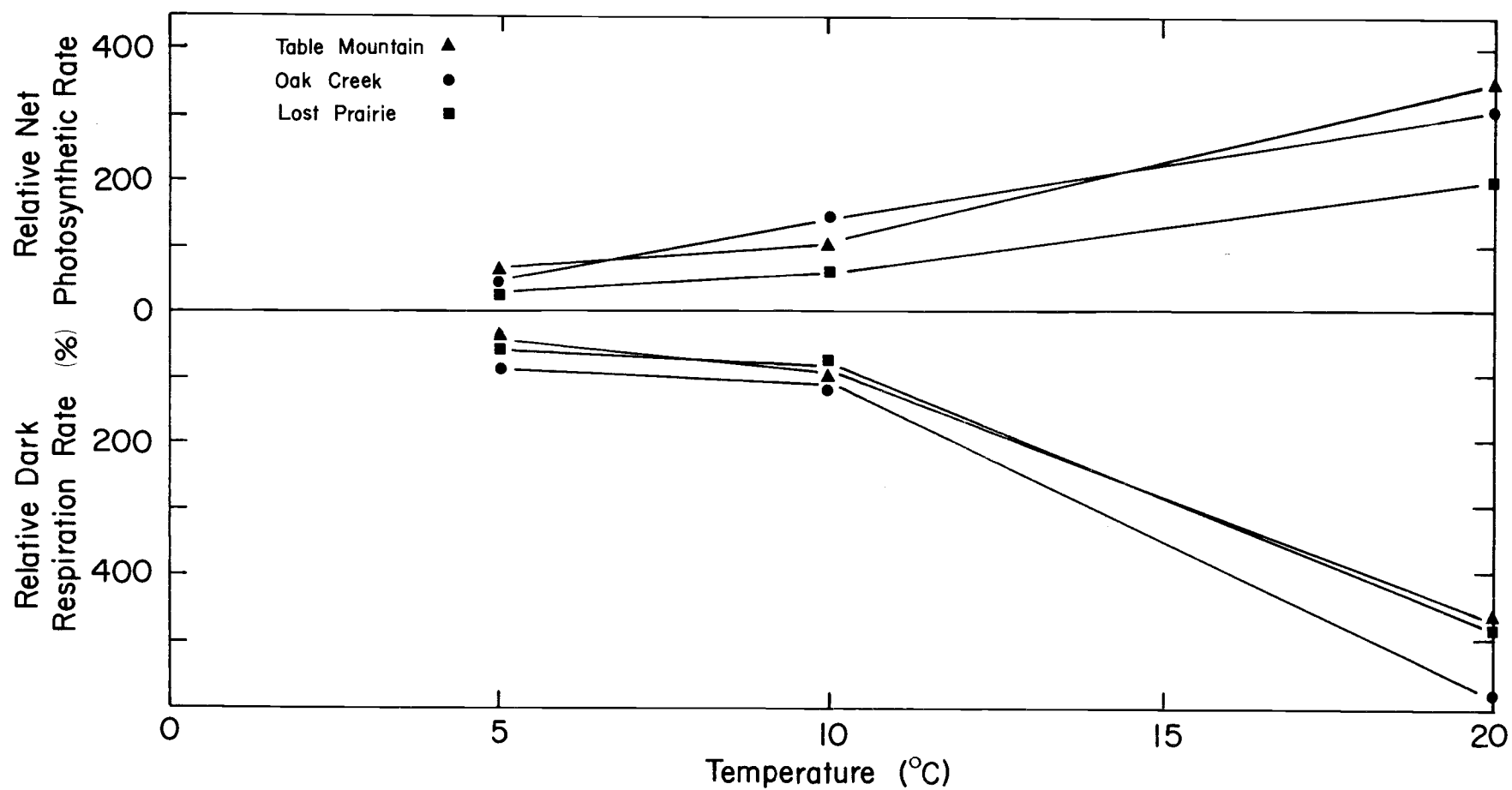


Figure 6. Relative rates of dark respiration and net photosynthesis in relation to temperature at 100% RWC for *Lobaria pulmonaria* collected in June. One hundred percent is equal to the average respiration rate and average photosynthetic rate for all discs at 10°C. The relative change at 5°C and 20°C was calculated for each set of three discs in relation to the 10°C values.

One of four possible situations could cause the lack of difference with temperature: 1) variability within the populations measured; 2) small temperature difference between the sample sites; 3) lack of enough temperatures tested to determine the optimum for maximum net photosynthesis for any of the three populations; or 4) the occurrence of acclimatization after collection. Scholander et al. (1952), studying the effect of temperature on respiration of arctic and tropical lichens, could arrive at no general conclusion because of the variability within the species which was as high as 100% in their metabolic rate. As was seen in Table VIII, the values of the standard deviation of the mean rates for respiration and photosynthesis in many cases were very high, and therefore the variability it indicates could have affected the overall results.

The temperature difference for any one month does not diverge by more than 4° to 5°C between any of the three sites (Figure 1). Therefore, no physiological differentiation may need to exist in photosynthesis or dark respiration in relation to temperature.

In order to determine the optimum temperature for maximum net photosynthesis, temperatures between 10° and 20°C and above 20°C needed to be tested. Maximum net photosynthesis for any one of the populations could have been within or above the temperatures measured.

Finally, the results may indicate that the discs used became

acclimatized. Chabot and Billings (1972), working on higher plants of the Sierran alpine, found acclimation of dark respiration to a change in temperature to occur as rapidly as within eight to ten hours. Acclimation of photosynthesis also occurred, but it needed a longer time period. Acclimation of photosynthesis has also been observed in the orchid, Aplectrum hyemale (Adams, 1970), and in the shrub, Encelia californica (Mooney and Shropshire, 1967). However, no study has been done on the ability of lichens to acclimatize.

Relationship of Photosynthesis and Dark  
Respiration to Relative Water  
Content and Water Potential

Methods

Experiments were conducted on thalli collected on July 1, 1972, to determine if variation exists between any of the three populations of Lobaria pulmonaria in relation to the effects RWC and water potential have on photosynthesis and dark respiration. The air dried thalli were stored in the dark at room temperature until used. The metabolic measurements were conducted during July 3-11, 1972. On the day of measurement, two lobes from each of two separate thalli from each location were removed and cut to approximately 2.0 cm by 1.5 cm. The lobes were then washed in distilled water and soaked in Petri dishes of distilled water at room temperature for two hours,

more than sufficient time for the thalli to become saturated. During soaking the cut lobes were illuminated as described for the light intensity experiment.

Photosynthesis and respiration were estimated by measuring uptake and evolution of oxygen with a Gilson Differential Respirometer. Carbon dioxide concentration in the flask atmosphere was maintained at 1.0% to insure that the  $\text{CO}_2$  concentration would not be rate limiting. This level was controlled by a  $\text{CO}_2$  buffering mixture containing 6.0 ml diethanolamine, 15 mg thiourea, 3.0 g  $\text{KHCO}_3$ , 2.2 ml 6N HCl, and 6.8 ml  $\text{H}_2\text{O}$  (Pardee, 1949) and prepared 24 hours before its use according to the method of Umbreit et al. (1964). In all experiments, 0.6 ml of the buffer was divided between the two side arms and center-well in each of six 20-ml reaction flasks.

Once the reaction flasks were prepared with the  $\text{CO}_2$  buffer, the cut lobes were blotted dry twice to remove surface water and weighed ( $\pm 0.1$  mg). The saturated lobes were then placed upside down in the bottom dried portion of each flask, which was connected to the respirometer and lowered in the water bath, kept at  $20 \pm .5^\circ\text{C}$ . An opaque, black plastic sheet was placed over the water bath and the thalli were allowed to equilibrate for 15 minutes. After 15 minutes, dark respiration was measured for 10 minutes. The lobes were then illuminated with six General Electric reflector lamps (30 watts, 115 volts) situated below the water bath. The light intensity at thallus

level was approximately 800 ft-c as measured with a Weston Model 756 Illumination Meter. This light intensity was assumed to be light saturating from the data in Figures 3, 4, and 5. Beginning five minutes after the lights were turned on, photosynthesis was measured for ten minutes. Then the lights were turned off and dark respiration was again measured for ten minutes after a five minute waiting period. The thalli were removed from the flasks, weighed immediately to allow calculation of their RWC, placed back in the flasks, and photosynthesis and respiration measured again. This was repeated five to eight times for each lobe. At the end of a complete run the lobes were oven dried and weighed.

The RWC for each trial was assumed to be the average value of RWC before and after each set of measurements. The net photosynthetic rate used was from the one measurement for each trial, and dark respiration was assumed to be the average of the two measurements at each trial. Variability using this procedure was high and therefore thalli from all three locations were always included at the same time on the respirometer.

Net photosynthesis and dark respiration are expressed in the results as both absolute rates and relative rates as described in the temperature experiment. Since the thalli used in this section were collected during the same season as the thalli used in the thallus water content-water potential section, it is assumed that the water



potential values can be calculated from RWC. Therefore, graphs indicating the relationship between relative net photosynthesis and dark respiration and RWC also have the calculated water potential values listed. The conversion of RWC to water potential is from Figure 2. Statistical analyses were made on the absolute photosynthetic rates at the optimum level of thallus saturation.

### Results and Discussion

Maximum dark respiration occurs from 70 to 90% RWC for the Lost Prairie group, 67 to 95% RWC for the population at Table Mountain, and 61 to 90% for the replicates at Oak Creek (Figure 7). The majority of all replicates begin to decline in dark respiration at RWC above their optimum level; however, because of variability that can be attributed to error in experimental procedure and the normal variability found in the metabolic rates of lichens (Scholander *et al.*, 1952), it could probably be assumed that respiration stays maximal up to 100% RWC. The rate of decrease in respiration below optimum RWC is similar for all three populations. In relation to water potential, the populations' dark respiration rates start to decline at very high water potentials (-0.5 to -2.0 bars) with the exception of one Oak Creek replicate which does not decline until it reaches approximately -12.5 bars (Figure 7). The values for the absolute rates of dark respiration given in Table IX indicate no significant difference between groups.

Figure 7. Relationship of relative net respiration to water potential-  
relative water content for six, seven, and five replicates  
of Lobaria pulmonaria collected at Oak Creek, Lost  
Prairie, and Table Mountain respectively. The water  
potential values for each population are given on the  
x-axis of each graph.

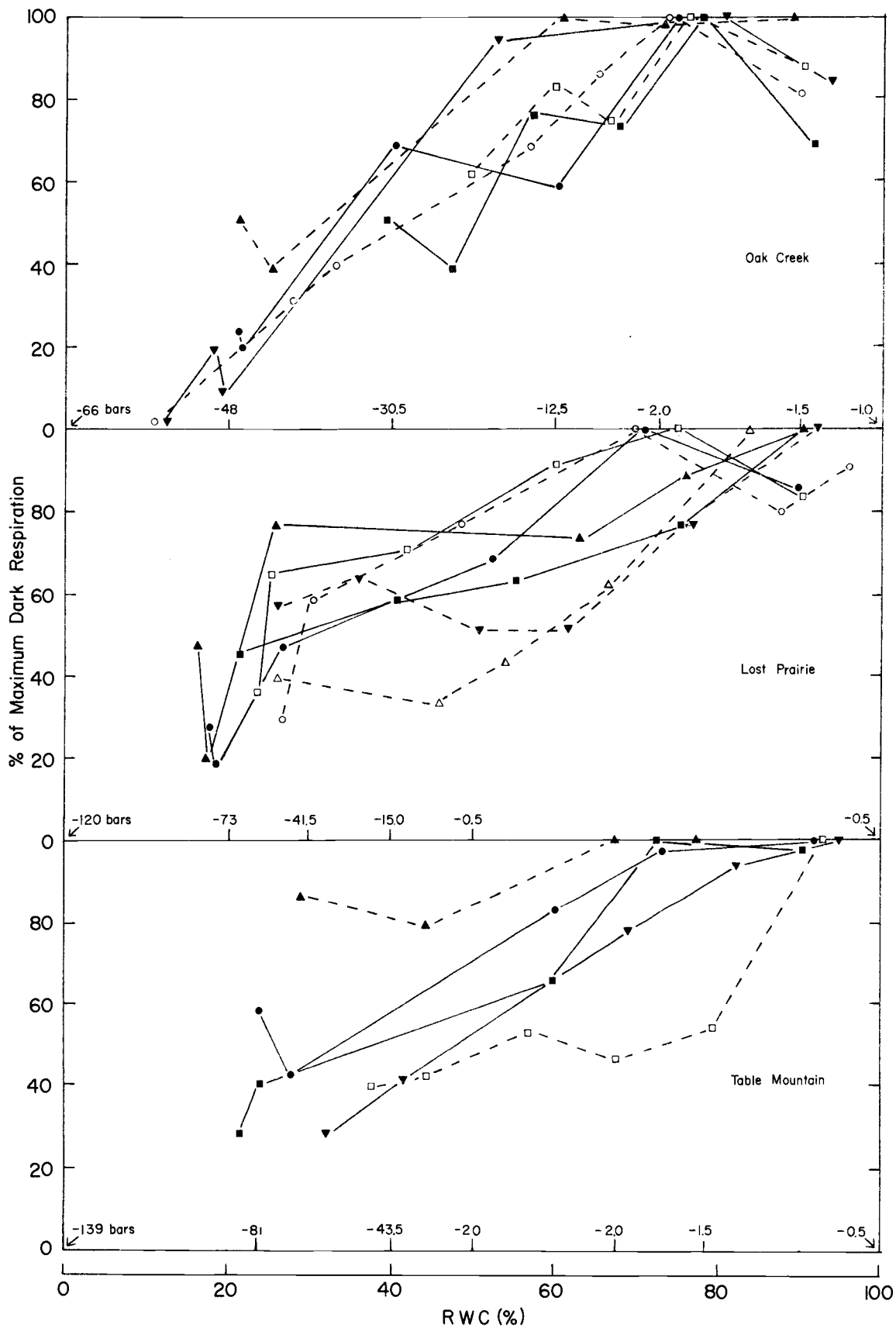


Table IX. Mean values and standard deviations for absolute net photosynthesis and dark respiration at the optimum level of thallus saturation for three populations of Lobaria pulmonaria.

Sample site	Net photosynthesis			Dark respiration	
	$\mu\text{M O}_2/\text{min/g}$ dry wt	$\mu\text{M O}_2/\text{min}/$ $\text{cm}^2$ <sup>1</sup>	$\mu\text{M O}_2/\text{min}/$ mg chl. <sup>1</sup>	$\mu\text{M O}_2/\text{min/g}$ dry wt	$\mu\text{M O}_2/\text{min}/$ $\text{cm}^2$ <sup>1</sup>
Lost Prairie	$2.18 \pm 0.60$ a <sup>2</sup>	$0.019 \pm 0.005$	$2.83 \pm 0.78$	$1.10 \pm 0.30$ a	$0.011 \pm 0.005$
Table Mountain	$3.17 \pm 1.14$ a,b	$0.028 \pm 0.010$	$3.45 \pm 1.24$	$1.15 \pm 0.27$ a	$0.010 \pm 0.002$
Oak Creek	$3.23 \pm 0.18$ b	$0.034 \pm 0.002$	$3.57 \pm 0.20$	$1.18 \pm 0.24$ a	$0.013 \pm 0.003$

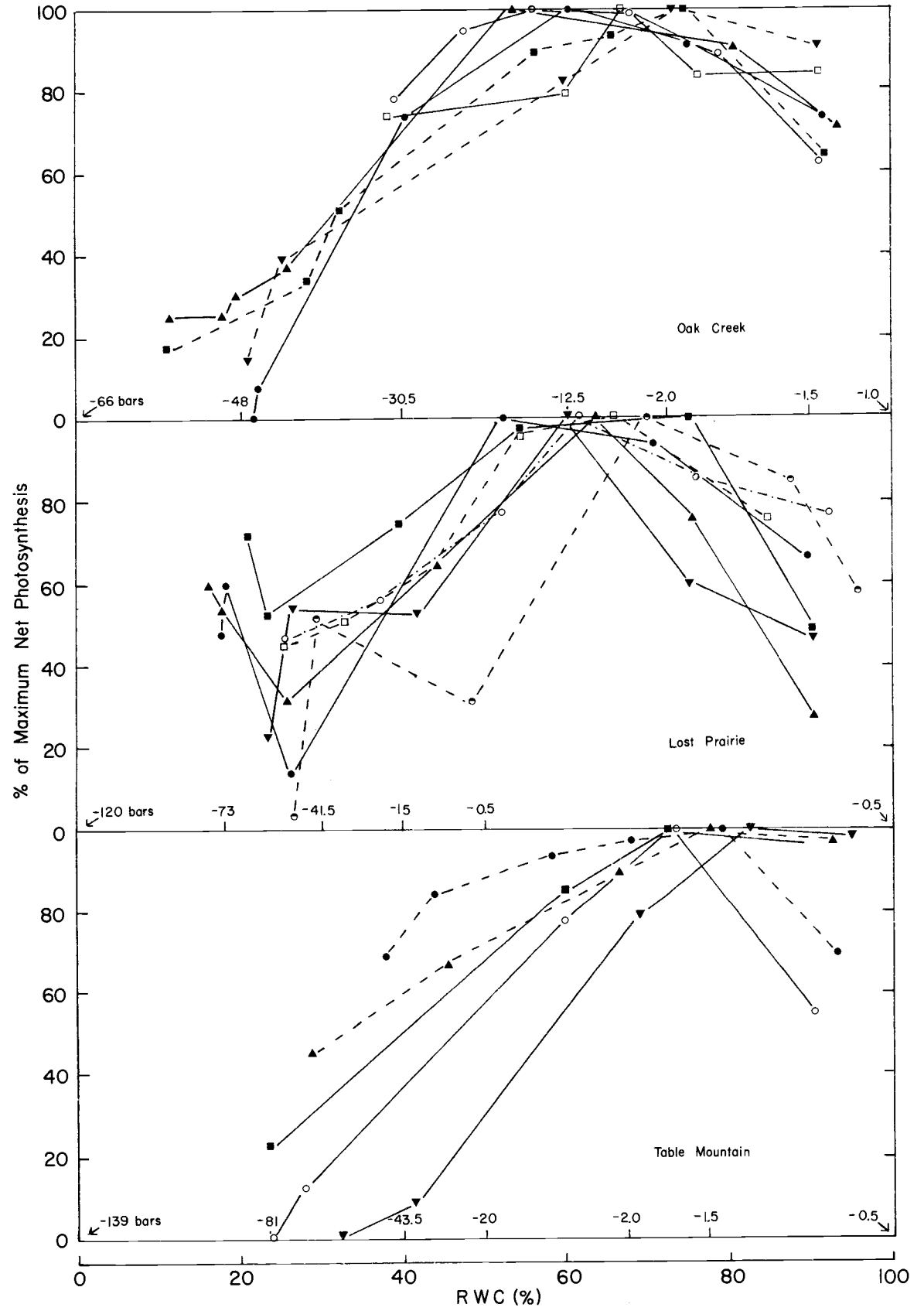
<sup>1</sup>The rates  $\mu\text{M O}_2/\text{min}/\text{cm}^2$  and  $\mu\text{M O}_2/\text{min}/\text{mg chl.}$  were calculated using the conversion data from the area-density and chlorophyll sections.

<sup>2</sup>Like letters indicate no significant difference (5% level); unlike letters indicate significant difference.

These results therefore indicate a lack of difference in respiration in relation to RWC and water potential. This has also been observed for RWC in other comparative studies of lichens (Harris, 1971; Peet and Adams, 1972; Eickmeier and Adams, 1973).

Photosynthesis for Lost Prairie and Oak Creek samples is optimum at similar RWC, from 52 to 76% saturation. The Table Mountain replicates, however, do not reach maximum photosynthesis until 72 to 82% RWC (Figure 8). Above optimum RWC, the decline of photosynthesis of the Oak Creek group is greater than three of the five Table Mountain replicates but less than, on the average, the Lost Prairie samples. As the thalli dry below their optimum RWC, the decline in photosynthesis is similar for both the Table Mountain and Lost Prairie replicates; however, the Oak Creek population declines more gradually. With respect to hydration compensation, the Oak Creek replicates do not reach it until 22% RWC or lower, whereas two of the Table Mountain replicates reach hydration compensation at 24 and 32% RWC. The hydration compensation points for the Lost Prairie group cannot be determined. In relation to water potential, net photosynthesis for both Table Mountain and Lost Prairie populations begin to decline at high water potentials (-1.5 and -0.5 bars respectively), whereas the Oak Creek group does not decrease, on the average, until the plant is under stress at approximately -12 bars. The average net photosynthetic rate at optimum RWC for the Oak

Figure 8. Relationship of relative net photosynthesis to water potential-relative water content for six, seven, and five replicates of Lobaria pulmonaria collected at Oak Creek, Lost Prairie, and Table Mountain respectively. The water potential values for each population are given on the x-axis of each graph.



Creek replicates was significantly higher than that of Lost Prairie (Table IX).

The Oak Creek site is composed of a deciduous forest and has a very dry summer, and therefore it should allow survival only of the more drought resistant individuals. At the other two sites, with a high annual precipitation and cooler summer, selection of individuals will probably be based on other ecological factors (Zavitkovski and Ferrell, 1970). The results obtained in this experiment, with the results of other investigators, would seem to back up this expectation.

From the data, it was noted that the Table Mountain and Lost Prairie groups do not maintain their maximal photosynthetic rates once water stress is below -1.5 and -0.5 bars respectively, whereas the Oak Creek group stayed at maximum until -12.5 bars. This makes the latter population more fit for its habitat, since L. pulmonaria at Oak Creek is exposed to more desiccating conditions than the other groups. Also, since this population is in a drier site, it will lose water at a faster rate after a rain than those exposed to more mesic conditions. Lechowicz and Adams (1973) have observed that, after a rain, sun populations of Cladonia mitis dropped to 12% RWC within one hour, whereas two hours after the rain the shade population had only decreased to 32% RWC. Therefore, in order for the Oak Creek population to be efficient in carbohydrate metabolism, it must maintain photosynthesis at low RWC even if under moisture stress.



The Oak Creek population, on the average, seems to have a lower hydration compensation point than that of the Table Mountain group. Lower hydration compensation points have been reported for populations from xeric environments than for populations of the same species growing in more mesic habitats. Harris (1971) observed that Parmelia caperata collected from the top of an oak forest canopy had an average hydration compensation point at 10% RWC, whereas for the same species collected at the base of the tree, it was at 25% RWC. Kershaw and Rouse (1971) observed the same type of correlation with Cladonia alpestris. In measuring net assimilation rates of two populations, one growing under a spruce canopy and the other from an open area, it was noted that the hydration compensation point averaged 10% higher for the one growing under the canopy. Even more dramatic differences were reported by Peet and Adams (1972). Investigating the differences between Cladonia rangiferina from a mesic site and C. subtenuis from a more xeric area, they found that the compensation point was 25% higher for C. rangiferina. Finally, Kershaw (1972), working with a variety of lichen species in southern Ontario, found in all cases a lower hydration compensation point in those species from a more xeric environment.

Differences ranging from 10 to 60% RWC between optima for photosynthesis for sun and shade species have been reported by many of the investigators mentioned above. In all cases the optimum rates

were at a lower RWC for those species in more xeric environments (Harris, 1971; Kershaw and Rouse, 1971; Kershaw, 1972). This was also the case in the results mentioned in this section. The average RWC at maximum photosynthesis was approximately 12% higher for the more mesic environment of Table Mountain in comparison to the average optimum RWC for the Oak Creek and Lost Prairie populations. Therefore, the results and the above investigations show a possible correlation to the lichen habitat preference. Since the Table Mountain site is near the coast, it receives considerable fog during the year. Stocker (1927) reported that L. pulmonaria will remain saturated for longer periods of time on foggy days. Also, since during the winter season much of the precipitation is snow at Lost Prairie, it probably does not aid in thallus saturation as would rain. Consequently, even though both Lost Prairie and Table Mountain are considered mesic sites, the chances of L. pulmonaria at Lost Prairie staying at saturation for long periods of time are probably low, and therefore it would be important for the Lost Prairie population to reach maximum photosynthesis at a lower RWC than the Table Mountain group.

The occurrence of maximal assimilation below 100% saturation is commonly observed in lichens (Stocker, 1927; Cuthbert, 1934; Lange, 1969; and others), as well as in the results of this experiment (Figure 8). The reason for this is that at saturation, water held in the

intercellular spaces blocks gas diffusion into the algal cells (Stocker, 1927; Smyth, 1934; Quispel, 1959). Valley and coast groups showed, on the average, only a slight decline as water content increased after maximum photosynthesis; however, Lost Prairie showed a very rapid decline. This is a further indication that the Cascade group is probably not at full saturation for the same length of time as the coast group.

In both the light saturation and temperature experiments, photosynthesis and respiration were measured in an aqueous solution, the thalli thereby being at 100% saturation. Baddeley et al. (1971), the first to use a Clark oxygen electrode in the measurement of lichen metabolism, did not do any photosynthetic measurements because they thought photosynthesis would be below maximum as described above. However, since the oxygen molecules that are being released or taken into the thallus are constantly in a water medium, and are also measured in water, the water in the intercellular spaces should not serve to block oxygen diffusion. The decrease in maximum photosynthesis above lichen optimum RWC reported in this section and by others is because the gas molecules had to move from a liquid phase (intercellular water) to a gas phase (air in a cuvette), or vice versa, to be measured. Therefore, the rates of photosynthesis at 100% saturation in both previous sections are assumed to be at maximum.

The valley group had the highest absolute net photosynthetic rate (Table IX); however, the only statistically significant difference is between Oak Creek and Lost Prairie. It has been reported that drought resistant lichen species have a higher maximal photosynthetic rate than those in mesic environments (Harris, 1971; Kershaw and Harris, 1971; and others). This is important since the amount of time that thalli in a dry habitat are able to photosynthesize at maximum is less than those of more mesic environments (Lechowicz and Adams, 1973). It should also be noted that the absolute maximal rates reported in Table IX were expected to be, and were, higher than those previously reported in the light saturation (Figures 3, 4, and 5) and temperature (Table VIII) studies. However, differences in the rates were not statistically significant. These visually higher rates are attributed to three possible sources: 1) use of white light rather than red, as in the other experiments; 2) experimental error; or 3) concentration of CO<sub>2</sub> being different from previous experiments. Use of white light instead of just red wavelengths would cause a higher rate of photosynthesis due to the presence of blue wavelengths which are also active in photosynthesis. The amount of variability, as indicated by the standard deviation, could have resulted in the averages being higher. Finally, since the concentration of carbon dioxide may have been higher in this experiment, a higher rate in photosynthesis could have occurred (Salisbury and Ross, 1969).

Clearly, there is considerable correlation of the physiological response of L. pulmonaria to thallus saturation with its distribution in Western Oregon. The Oak Creek population is more drought adapted than the Table Mountain population, with the Lost Prairie group being somewhat intermediate.

## GENERAL DISCUSSION AND CONCLUSION

There were no differences observed in the morphology, thallus area-density, presence of the chemical constituents stictic and norstictic acids, chlorophyll concentrations, or metabolic response to temperature among the three populations of Lobaria pulmonaria studied in Western Oregon during the summer months of 1971 and 1972. However, intraspecific variation in physiological responses in relation to light and water content have been observed and are to some extent correlated with the regional environment the populations occupy.

The Table Mountain population is located in a mesic environment, characterized by high yearly rainfall, a large number of days of measurable rain, and summer fog. The temperature throughout the year is mild to cool. The amount of direct sunlight that L. pulmonaria receives in a day is low, as it occupies the branches of the lower half of a canopy of Pseudotsuga menziesii. The physiology of the population at this site is characterized by 1) a rapid increase in water stress as the plant loses water, 2) a low light compensation point, 3) a low light saturation point, 4) the occurrence of photo-inhibition at high light intensity, 5) a high RWC necessary for maximum net photosynthesis, and 6) an inability to stay at maximum net photosynthesis once water potential decreases below -2.0 bars

(Table X). All of these characteristics indicate that the Table Mountain population is a shade tolerant plant with a low capability of living in a dry habitat.

The Oak Creek population is in an oak forest in the Willamette Valley. The climate at this location fluctuates with the seasons in having a cool, wet winter and a warm, dry summer. Lobaria pulmonaria grows in the lower half of the canopy of Quercus garryana, a deciduous tree, and therefore the amount of direct sunlight that L. pulmonaria receives changes with the seasons, being relatively high in the fall and winter and decreasing in the spring and summer. The physiology of this population in comparison to the others is characteristic of sun-tolerant groups with the capability of withstanding low water availability. These physiological properties include 1) a slow increase in moisture stress as the thallus becomes dehydrated, 2) a high light compensation point, 3) a high light saturation point, 4) reduced photo-inhibition at high light intensities, 5) a low RWC necessary for maximal net photosynthesis, and 6) an ability to stay at maximum net photosynthesis once it sustains moisture stress (-12.5 bars) (Table X).

The Lost Prairie population is situated in a more extreme temperature environment. The area has a very short frost-free period indicating harsh, cold winters, and summer temperatures which are similar to the Oak Creek location. Much of its winter

Table X. Summary of the differences found in the physiology of the three populations of Lobaria pulmonaria. The abbreviations TM, OC, and LP represent Table Mountain, Oak Creek, and Lost Prairie respectively.

Physiological characteristics	Population		
	TM	OC	LP
Decline of water potential in relation to thallus dehydration (bars /%RWC)	-2.4	-0.9	-2.6
Light compensation point (ft-c)	< 50	> 50	< 50
Average light saturation point (ft-c)	440	1000	300
Range of light saturation points (ft-c)	320-775	775-1900	215-320
Light intensity for photo-inhibition (ft-c)	> 775 <sup>1</sup>	±1900	> 775
Range of RWC at maximum photosynthesis (%)	72-82	53-75	52-76
Water potential at which photosynthesis decreases from maximum (bars)	-2.0	-12.5	-0.5

<sup>1</sup>One individual showed photo-inhibition above 320 ft-c.



precipitation is snow. Water availability seems to be higher in the summer than at Oak Creek, as it averages approximately the same number of days of measurable precipitation as Table Mountain in June, July, and August. The amount of light that Lobaria pulmonaria receives is low in comparison to the Oak Creek group, as it is found at the bases of branches in the lower half of the canopy of Picea engelmannii. The population's physiological characteristics indicate that it is as shade-tolerant as the Table Mountain group; however, there is a slight indication that the plant has some drought resistant characteristics. Its physiological properties include 1) a rapid increase in moisture stress as the plant becomes dehydrated, 2) a low light compensation point, 3) a low light saturation point, 4) an occurrence of photo-inhibition at high light intensities, 5) a low RWC necessary for maximal net photosynthesis, and 6) an inability to stay at optimum net photosynthesis once it sustains moisture stress (-0.5 bars) (Table X).

The results of the various experiments conducted in this investigation, therefore, indicate that morphologically and chemically similar members of the lichen species, Lobaria pulmonaria, have physiological differences that probably underlie and delimit their ecological tolerance. Consequently, the results suggest that L. pulmonaria may have evolved intraspecific differences between the Oak Creek population and the montane groups, similar to those

encountered in shade and sun races of herbaceous plants (Björkman and Holmgren, 1963) and in mesic and xeric races of Douglas-fir (Zavitkovski and Ferrell, 1970). All physiological measurements were conducted in the same types of artificial environment. Therefore, if responses of L. pulmonaria to its environment were strictly short-term acclimatization, the results would have shown similarities, not differences.

To confirm this conclusion other studies need to be done, for instance, into the possibilities of morphological differences not observed here, especially the compactness of the thalli. Plummer and Gray (1972) and Hill and Woolhouse (1966) found that shade species of lichens have a higher population density of algal cells in their thalli than those growing in exposed areas. Wilhelmsen (1959) reported that one of the reasons that photosynthesis of lichens decreases during dehydration is a decrease in cell wall transparency. This could be different between lichen races. In relation to this, field work should be done on the rate of water loss after the thalli are saturated by rain. This would further indicate the importance of the water content-water potential experiments reported in this paper. Also, with the growing knowledge about how to culture whole lichen thalli (Pearson, 1970; Dibben, 1971), the three populations of L. pulmonaria could be inoculated in a controlled environment and then subsequently studied in order to determine if their physiological

differences are still present when thalli develop in the same environment. Finally, further work should be done on the microclimate in the sample areas. The climatic differences reported in this paper can only represent differences between habitats in a very general way. Microenvironmental characteristics may compensate for, or may increase, the differences in operational environment for the lichen thalli extrapolated from the Weather Bureau data.

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