

--1 (11-73)

National Library of Canada

Bibliothèque nationale du Canada

CANADIAN THESES ON MICROFICHE

THÈSES CANADIENNES SUR WICROFICHE

TITLE OF THESIS/TITRE DE LA THÈSE Physiological Ecology and Stress Responses in the
medthe body and Stress Responses in the
Genus Peltigera
JNIVERSITY/UNIVERSITÉ MCMaster DEGREE FOR WHICH THESIS WAS PRESENTED/ GRADE POUR LEQUEL CETTE THESE FUT PRÉSENTÉE Ph.D. YEAR THIS DEGREE CONFERRED/ANNÉE D'OBTENTION DE CE DEGRÉ 1979
AME OF SUPERVISOR/NOM DU DIRECTEUR DE THÈSE Dr. K. Kershaw
CANADA to microfilm this thesis and to lend or sell copies of the film. The author reserves other publication rights, and neither the deprêter ou de vendre des examplaires du film. L'auteur se réserve les autres droits de publication: ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur. ATED/DATÉ Narch 26, 1979 SIGNED/SIGNÉ John D. Mochande. FAMANENT ADDRESS/RÉSIDENCE FIXÉ P. O. BOX 225 Way Day Signed S

PHYSIOLOGICAL ECOLOGY

AND

STRESS RESPONSES

IN THE GENUS

PELTIGERA

By

JOHN DAVID MACFARLANE, B.Sc.

A Thesis

Submitted to the School of Graduate Studies
In Partial Fulfilment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University
March 1979

PHYSIOLOGICAL ECOLOGY AND STRESS RESPONSES

DOCTOR OF PHILOSOPHY (1979)

McMASTER UNIVERSITY Hamilton, Ontario

TITLE: Physiological ecology and stress responses in the genus

Peltigera.

AUTHOR: John David MacFarlane, B.Sc. (McMaster)

SUPERVISOR: Professor K.A. Kershaw

NUMBER OF PAGES: xiii, 131

<u>ABSTRACT</u>

The multivariate examination of nitrogenase activity in two populations of the lichen Peltigera canina is described. Only a small increase in activity was observed with increasing radiant flux density and a marked but relatively constant temperature optimum was noted for both varieties that was independent of all other variables. However, very large rate changes were observed for all light levels and temperatures throughout the year. The influence of snowmelt on subsequent rates of nitrogenase activity is also described. While temperature was intimately involved in the velocity of the recovery of nitrogenase activity after snowmelt, the prime requirement appeared to be one of light.

Lichens are believed to be extremely resistant to high temperature stress when dessicated. A reexamination of this concept for several temperate and northern populations of lichens in the genera Peltigera and Stereocaulon indicated that some air-dry lichen thalli can be extremely sensitive to even moderate levels of heat stress whereas others exhibited a considerable degree of heat resistance. These differential levels of thermal resistance correlated exactly with the ecology of these populations. Thermal sensitivity was identifed as an important influence in the succession of plant species following forest fire and it is suggested that thermal stress may be one of the dominant parameters in the ecology of lichens.

Lichens are also believed to be extremely resistant to high light levels when in the air-dry state. A reexamination of this concept identified light stress as another ecological factor of considerable importance to some lichens.

A seasonal adjustment of net photosynthetic capacity is described for the woodland population of <u>Peltigera canina</u>. Changes in the maximal rates of net photosynthesis and respiration correlated strongly with concurrent changes in the leaf canopy. The seasonal adjustment of net photosynthetic rates is considered to be essential to the success of the organism which must adapt to a continually changing light environment.

ACKNOWLEDGEMENTS

I would like to express my very sincere thanks to my friend and supervisor Dr. K.A. Kershaw for his continued and unfailing interest, guidance and enthusiasm in every phase of this study. I also thank the members of the supervisory committee, Drs. W.R. Rouse and J.J. Miller for their advice throughout this study. Special thanks are offered to Ms. Margaret Webber for her expert assistance in the correction of successive drafts and to Ms. Sandra Farrell, Mr. Tom Morris, Mr. Brent Tegler and Mr. Mark Tysiaczny for stimulating and enlightening discussions. Finally, I wish to thank Miss Marsha Monster for her dedication in typing the final copy.

TABLE OF CONTENTS

				Page
				rage
	•			
Abstract	t	•		iii
Acknowle	edgm	ents	· · · · · · · · · · · · · · · · · · ·	v .
Table of	f Co	ntents		vi
List of	Fig	ures		x
List of	Tab.	les		xii:
Section	1.	Introdu	ection	1
Section	2.	Methods		6
	2.1	The Ni	trogenase Assay	6
	2.2	Season	al Changes in Nitrogenase Activity	8
		2.2.1	Seasonal Changes in Nitrogenase Activity of	8
	•	•	P. canina var. praetextata and P. canina var.	
	ٺ		rufescens	
		2.2.2	Light and Temperature Interactions with	11
			Nitrogenase Activity from P. canina var.	
			praetextata and P. canina var. rufescens	,
			Collected Under Snow	•

TABLE OF CONTENTS (continued)

 	<u> </u>		•
	2.3	Thermal Stress	712
	•	2.3.1 Thermal Stress in P. canina var. praetextata	12
	•	and P. canina var. rufescens 2.3.2 Microclimatic Factors	13
		2.3.3 Thermal Sensitivity in Northern Populations	16
· •		of <u>Peltigera</u> and <u>Stereocaulon</u> 2.3.4 Net Photosynthetic Responses to Thermal	18
	•	Stress in P. canina var. praetextata and	
	2.4	P. canina var. rufescens Light Stress and Light-Mediated Seasonal Adjust-	19
•		ments of Net Photosynthetic Capacity	ŧ
	•	2.4.1 Light Stress in <u>Peltigera aphthosa</u> . 2.4.2 Light-Mediated Seasonal Adjustments of	19
		Net Photosynthetic Capacity in P. canina	19
		var. praetextata	
Section	3.	Results	21
	3.1	Seasonal Changes in the Nitrogenase Activity of P. canina var. praetextata and P. canina var.	21
		rufescens	

TABLE OF CONTENTS (continued)

<u>.</u>			Pag
_			
	3.1.1	The Effect of Light Intensity on	21
		Nitrogenase Activity	
•	3.1.2	The Effect of Thallus Moisture on	23
		Nitrogenase Activity	
	3.1.3	The Effect of Temperature on Nitrogenase	23
		Activity	•
	3.1.4	Seasonal Variation in the Rate of	. 25
		Nitrogenase Activity	
	3.1.5	Light and Temperature Interactions with	27
		Nitrogenase Activity in P. canina var.	
	`	praetextata and P. canina var. rufescens	
		· Collected Under Snow	•
3.2	Therma	1 Stress	32
	3.2.1	Thermal Stress in P. canina var.	33
		praetextata and P. canina var. rufescens	
	3.2.2	Microclimatic Factors	35
¥	3.2.3	Thermal Sensitivity in Northern Populations	39
		of Peltigera and Stereocaulon	•

TABLE OF CONTENTS (continued)

• •				Pag
•				_
		3.2.4	Net Photosynthetic Responses to Thermal	46
	• ,	-	Stress in P. canina var. praetextata	•
		4	and P. canina var. rufescens	•
•	3.3	Light	Stress and Light-Mediated Seasonal	49
		Adjust	ments of Net Photosynthetic Capacity	•
	•	3.3.1	Light Stress in Peltigera aphthosa	49
		3.3.2	Light-Mediated Seasonal Adjustments of	52
•		•	Net Photosynthetic Capacity in Peltigera	
•		Ø.	canina var. praetextasa	
Section	4:	Discuss	ion	59
	4.1	The En	vironmental Control of Nitrogenase Activity	60
	4.2	Therma	l Stress as an Ecological Factor in Lichens	72
	4.3	Light	as an Ecological Factor in Lichens	84
Section	·5.^	Summary		93
Section	6.	Referen	ces	96
Section	7:	Appendi:	x "A"	111

LIST OF FIGURES

Figure		Page
. '		
1	Temperature and thermocouple emf	15
2.	Physiological data matrix for <u>Peltigera canina</u> var. <u>praetextata</u>	22
3.	Physiological data matrix for <u>Peltigera canina</u> var. <u>rufescens</u>	24
4.	Thermal inactivation of nitrogenase activity	26
5.	Snowmelt and nitrognesse activity for P. canina var. rufescens: provisional observations	29
6.	Snowmelt and nitrogenase activity for P. canina var. praetextata	30
7.	Snowmelt and nitrogenase activity for P. canina var. rufescens	31
8. a, b	Thermal stress and nitrogenase activity for P. canina var. praetextata and P. canina var. rufescens	34
9. a, b	Thermal stress and net photosynthesis and respiration for <u>P. canina</u> var. <u>praetextata</u> and <u>P. canina</u> var. <u>rufescens</u>	36
10. a, b	Thallus temperature and radiant flux density for June 22, 1977	37
ll. a, b	Thallus temperature and radiant flux density for June 26, 1977	38 `
12.	Thermal stress and nitrogenase activity for Peltigera scabrosa	41

LIST OF FIGURES (continued)

Figures		Page
13.	Thermal stress and nitrogenase activity for P. canina var. rufescens (Hawley Lake)	42
14.	Thermal stress and nitrogenase activity for Stereocaulon paschale	44
15.	Thermal stress and nitrogenase activity for Peltigera aphthosa	45
16.	Thermal stress and net photosynthesis and respiration for P. canina var. praetextata	47
17.	Thermal stress and net photosynthesis and respiration for P. canina var. rufescens	48
18.	Light Stress and nitrogenase activity for P. aphthosa	50
19.	Photograph depicting photooxidation	51
20.	Net photosynthetic adjustment for P. canina var. praetextata	54
21.	Inducement of net photosynthetic adjustment	58
22.	Microclimatic considerations in woodland and in open exposed areas.	76
23. a, b	Thallus temperature and radiant flux density for July 8, 1977	118
24. a, b	Thallus temperature and radiant flux density for July 9, 1977	120
25. а, ъ	Thallus temperature and radiant flux density for	. 122

LIST OF FIGURES (continued)

Figures				
		of.		
26. a, b	Thallus temperature and radiant flux density for July 11, 1977	124		
27. a, b	Thallus temperature and radiant flux density for July 17, 1977	126 -		
28. a, b	Thallus temperature and radiant flux density for July 22, 1977	128		
29. а, ъ	Thallus temperature and radiant flux density for August 3, 1977	130		

LIST OF TABLES

Table		Page
I.	Key to surface weather observations	111
II.	Surface weather observations for June 22, 1977	116
III.	Surface weather observations for June 26, 1977	117
IV.	Surface weather observations for July 8, 1977	119
v.	Surface weather observations for July 9, 1977	121
VI.	Surface weather observations for July 10, 1977	123
VII.	Surface weather observations for July 11, 1977	125
VIII.	Surface weather observations for July 17, 1977	127
IX.	Surface weather observations for July 22, 1977	129
х.	Surface weather observations for August 3, 1977	131

Section 1.

INTRODUCTION

The reports of nitrogen fixation in or upon lichen thalli date back more than 50 years. However, no definitive results were published until those of Bond and Scott (1955) from experiments on Collema granosum and Leptogium lichenoides using the heavy isotope 15N. This work was subsequently extended by Scott (1956) using Peltigera praetextata and Watanabe and Kiyohara (1963) with Peltigera virescens. These reports established that lichens with a blue-green algal phycobiont could reduce atmospheric nitrogen and it can now be assumed that any lichen with Nostoc, Calothrix or Scytonema as the phycobiont is capable of nitrogen fixation (Millbank, 1977). Following these earlier publications, a number of lichens were investigated by means of $^{15}\mathrm{N}_2$ and although the required techniques were cumbersome and the equipment expensive, force of necessity stimulated the development of procedures in which $^{15} exttt{N}_2$ studies could be carried out under field conditions (Stewart, 1967). Using such techniques, Fogg and Stewart (1968) described the gross effects of temperature on the fixation of elemental nitrogen by Collema pulposum and Stereocaulon sp. in the Antarctic. Simultaneously, a novel and revolutionary technique for the demonstration and assay of nitrogenase activity was being developed which involved the reduction of acetylene to ethylene by the nitrogenase enzyme complex. The versatility of the method has made it equally applicable in the field and in the laboratory and it has resulted in a surge of analytical activity unpredecedented in N_2 fixation research

(Burns and Hardy, 1975). Its sensitivity and other advantages have fostered laboratory and field experimentation which had previously been technically unfeasible and thus has been used to establish the nitrogen-fixing ability of a wide range of organisms including a variety of lichens (see Millbank, 1975). The extensive array of nitrogen-fixing organisms stimulated interest, in the environmental control of nitrogenase activity with a physiological-ecological perspective which was directly or indirectly focused on the nitrogen cycle within selected communities and ecosystems. The mapping of global nitrogen-fixing activity remains as the ultimate goal which is essential to understanding and evaluating the role of nitrogen fixation on a world scale.

Recent studies have examined the environmental control of nitrogenase activity in lichens with the experiments conducted either in the field or under more stringently controlled conditions in the laboratory. For example, the effect of temperature on the rate of nitrogenase activity has been surveyed in several lichen species. Hitch and Stewart (1973), Kallio et al., (1972), Kallio (1973), Kelly and Becker (1975), Maikawa and Kershaw (1975), Englund (1978), Kallio and Kallio (1978) etc. have all reported a range of temperature optima for nitrogenase activity. In a similar fashion, the intensity of light (Hitch and Stewart, 1973; Kallio et al., 1972; Kallio, 1973; Kelly and Becker, 1975; Englund, 1978), the presence or absence of light (MacFarlane et al., 1976; Kershaw et al., 1977; Huss-Danell, 1977a) and thallus moisture (Henriksson and Simu, 1971; Hitch and Stewart, 1973; Kershaw 1974; Kershaw and Dzikowski, 1977) have all been investigated in some detail. Unfortunately, the temporal and spatial limitations of the

experimental methods as employed have permitted only univariate or bivariate experimental designs. Even when more than two variables were examined, the experimental framework remained unaltered. For instance, Hitch and Stewart (1973) examined the response of nitrogenase activity in three lichens to thallus moisture content, thallus temperature and light intensity. In experiments examining the response to light intensity, the experimental replicates were held at maximum saturation whereas when thallus moisture content was varied, all experiments were performed at a constant single light intensity. Clearly these experiments do not show the interaction of variables. Thus, the attempt of some ecologists to seek correlations between single factors and single responses of organisms has sometimes led to erroneous conclusions and conflicting results. Many environmental characteristics are closely correlated and most ecological systems are so complex that multiple correlation is the rule rather than the exception. This situation presents considerable difficulties in the field where uncontrolled environmental fluctuations may become confounded with the experimental variates. The complexities of an almost unlimited number of variables and their interrelationships can, however, be more sharply defined in the laboratory. Controlled environmental facilities liberate the experimenter from the vagarious nature of the weather and enables the experiments to be replicated in time as well as in space. an adequate description of the environmental control of selected physiological processes and their ecological ramifications requires the basic data to be derived from factorially arranged experiments. Such experiments display the important secondary or tertiary interactions among the variables controlling the physiology of the organism.

This thesis concerns itself with examining the relationship between the distribution of plants and the physiological adaptations linking them to measurable physical characteristics of the surfaces on which they grow. More specifically, the study involved the multivariate examination of nitrogenase activity in two populations of the lichen Peltigera canina collected from deciduous woodland in southern Ontario and from an open and exposed xeric roadside in the Muskoka region of central Ontario. The responses of nitrogenase activity for both species were examined as a function of thallus moisture, thallus temperature, radiant flux density, the time of the year and the influences of snowcover with the experiments arranged factorially to produce a physiological data matrix for each collection. A secondary interaction between nitrogenase activity and high temperature pointed to the potential importance of high temperatures and the contrasting microclimates of the two respective habitats. These results lead ultimately to a reexamination of the concept of thermal stress as an ecological factor in lichens of fundamental importance which has been severely neglected. The examination of thermal stress was consequently extended to northern populations of Peltigera and Stereocaulon from equally diverse habitats. Thermal sensitivity was identified as a dominant influence governing the succession of lichen species following forest fire and was strongly correlated with the gross microclimatic changes accompanying this natural process. The multivariate theme was developed further with the examination of light as another neglected ecological

parameter of substantial impact. Specifically, light stress was recognized as an important factor in the ecology of some lichens in the dessicated state. In addition, the adjustment of net photosynthetic capacity in response to a seasonally changing light environment was documented for the woodland population of <u>Peltigera canina</u> in some detail.

Section 2.

METHODS

(2.1) The Nitrogenase Assay

methods were employed to examine nitrogen fixation (see Burris, 1974). Fundamentally, the most satisfactory method was, and still is, the measurement of the uptake of ¹⁵N-enriched N₂. This constitutes an absolute method of comparatively high sensitivity, but it requires the use of relatively expensive ¹⁵N₂, the subsequent analysis requires the use of a mass spectrometer and the sensitivity of this method is less than that of acetylene reduction. An alternative approach, namely the substitution of acetylene as the substrate for nitrogenase, was first reported by Schollhorn and Burris (1966) and Dilworth (1966). The product ethylene, a hydrocarbon which is readily separated by gas chromatography, can be measured in low concentration with a hydrogen flame ionization detector (Purnell, 1962). Shortly after this initial discovery, Sloger and Silver (1967), Stewart et al. (1967) and Hardy et al. (1968) reported the use of the method for measuring nitrogenase activity.

Because of the extreme flexibility of this method, it has been possible to make numerous measurements either under a range of environmentally-controlled conditions in the laboratory or in the field, whereas earlier methods limited experimentation to a few samples. The method has permitted field studies on both higher and lower plants that were impractical previously. It has also been employed extensively in studies

of biological N_2 fixation in lakes, streams and the ocean. The method is simple, sensitive and inexpensive and hence has been widely adopted as a measure of N_2 fixation; hundreds of publications in which it has been used already have appeared in the literature. Despite the widespread appeal, it should be emphasized that it constitutes an indirect measurement rather than a direct measurement of N_2 fixation.

In detail, the method used in this investigation was as follows: nitrogenase activity of lichen thalli of both the genus Peltigera and the genus Stereocaulon was estimated by acetylene reduction. A Pye-Unicam series 104 gas chromatograph fitted with two hydrogen flame ionization detectors and two 1.6m glass columns packed with Poropak R, 50-80 mesh, was used throughout. The columns were run at 50° C with argon as carrier flowing at 75ml/min and with hydrogen and air for the detector flame flowing at 75ml/min and 600ml/min respectively. The attentuation was set at 20×10^2 and signals were amplified and then recorded on a twin pen Rikadenki recorder. Recorder deflections appeared as sharp peaks from the base line; peak height was calibrated against known standards and was accurate to about 2ppm. The calibration was linear for the flow rates and attenuation settings noted for the gas chromatograph and for all attenuation settings on the recorder. Results were expressed as nanomoles of ethylene produced per gram dry weight of lichen material (80°C, 12 hrs) per hour. Acetylene reduction has not been expressed in terms of potential biological N_2 fixation because a proper conversion factor was not determined (see Discussion).

(2.2) Seasonal Changes in Nitrogenase Activity

(2.2.1) Seasonal Changes in Nitrogenase Activity of P. canina var. praetextata and P. canina var. rufescens

P. canina var. praetextata was collected from a wooded area north of Waterdown, Ontario while P. canina var. rufescens was collected in the Muskoka region of central Ontario from a very exposed and xeric habitat adjacent to a highway. P. canina var. praetextata was collected in July, September and December 1975 and in March, May and July of 1976 while P. canina var. rufescens was collected in July, September and December, 1975 and in May and July of 1976. It should be emphasized that material was never collected from under snow for this series. Experimental lichen material was collected dry in the field and stored in a Conviron growth chamber at 50 microEinsteins m⁻² sec⁻¹ (μ E m⁻²s⁻¹) and a 12/12 h day/night cycle. Storage temperatures were maintained at 10°C night and 15°C day for all collections. This set of conditions in the growth chamber represented the standard pretreatment of all experimental material for this particular series, and was an essential experimental control. Prior to an experiment, individual lobes of material were dissected from the outside margin of the lichen mats and soaked overnight for 12 hours in distilled water at 20°C and a radiant flux density of $450\mu\text{E}~\text{m}^{-2}\text{s}^{-1}$. This pretreatment also served to avoid a hydration response characteristic of some species in this genus (MacFarlane et al., 1976). Soaking temperature controls at 10°C, 20°C and 30°C showed an identical pattern of response to subsequent experimental conditions and accordingly 20°C was used throughout the remainder of the experimental series. In a

further series of controls it was established that there was no significant loss of nitrogenase activity as a result of the length and conditions of storage. Material was not kept longer than three weeks but in fact retains its viability under the described conditions for up to six weeks. Dead lichen thalli were used as controls and showed no gas exchange. Similarly, live thalli were tested for natural ethylene production in the absence of acetylene and none was detected.

The experimental system necessary for this study required the measurement of nitrogenase activity in each species as a function of temperature, thallus moisture content, light intensity, time of year and contrasting habitats to form a physiological data matrix. The matrix of nitrogenase activity was established for both species with light intensities of 75, 150, 300 and $450\mu \text{E} \text{ m}^{-2} \text{ s}^{-1}$ and with temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35°C at all levels of thallus moisture. The light regime was representative of conditions found on the forest floor ranging from full summer canopy conditions to dulkand rainy days in the spring and fall. Fluorescent light tubes were used to provide the required radiant flux densities as measured using a Lambda quantum sensor (400-700nm) at the thallus surface. A spectroradiometer was used to check the spectrum of the fluorescent tubes and showed only a small contribution in the infra-red with a maximum at 600nm. Experimental temperature control was obtained using a Conviron model E7 growth chamber equipped with specially designed cooling equipment which provided additional temperature buffering at the colder temperatures. Instead of using 20ml serum vials of standard design, 16ml gas exchange cells were used in order to examine the moisture interactions with nitrogenase

žú.

activity. These were used with ground glass covers sealed with Dow-Corning high vacuum grease and with small syringe ports for removal of gas samples (see Larson and Kershaw, 1975a for details). Tests were conducted to show that the grease is impervious to acetylene or ethylene and unless otherwise stated, these gas exchange cells were used throughout all experiments. Thallus temperature was measured with embedded microthermocouples (see section 2.3.2 for details) and throughout each experiment, thallus temperature was controlled to within 1°C. This temperature control was achieved by control of the air temperature around the gas exchange cells. Through the use and correct positioning of two blower-type fans inside the growth chamber, thallus temperatures were typically ±1°C of the chamber air temperature for all light intensities used.

Prior to each experiment, excess surface moisture was shaken off each thallus replicate and the initial level of saturation was thus typical of wet field conditions. Saturated lichen thalli were then placed in the gas exchange cells under given experimental conditions. A 1.6ml gas sample was removed and 1.6ml of purified acetylene (Matheson Co.) was replaced to effect a 10% acetylene-air mixture. An atmosphere of 10-20% acetylene gave consistent results and removed the requirement of a nitrogen-free atmosphere necessary at lower acetylene concentrations (Millbank, 1972; Kershaw, 1974). This was confirmed by experiment and a 10% by volume acetylene-air atmosphere was used throughout all experimental series. The plants were sealed in the gas exchange cells for one hour. At the end of the incubation period, a

analysis. The lichen material was then removed, weighed on an analytical balance and the process repeated until the thallus was airdry. Thus each replicate sample showed the response of nitrogenase activity to thallus water content from the saturated to the airdry state. All experiments used five replicates at each light and temperature level. Means were calculated within 50% thallus moisture classes and the results were expressed as a mean level of activity for each experiment in nanomoles of ethylene produced per gram oven dry weight of experimental material (80°C, 12 hrs) per hour.

(2.2.2) Light and Temperature Interactions with Nitrogenase Activity from P. canina var. praetextata and P. canina var. rufescens Collected Under Snow

Peltigera canina var. rufescens was collected in the Muskoka region of central Ontario in January, 1978 from under 50cm of snow. Lichen material was kept dark and cold after collection and was returned to the laboratory immediately where it was stored in growth chambers at 2°C and in continuous darkness. Upon collection, P. canina var. rufescens was saturated but not frozen and as a necessary control was maintained in this condition by frequent spraying with a mist of distilled water. Subsequently, nitrogenase activity was assayed at temperatures of 5°, 15°, 25°, 35°C. Handling and pretreatment of experimental material was as described in section 2.2.1. Four replicate thalli and 20ml vials were used for each experimental temperature. Thalli were kept saturated during each experiment and the use of 20ml vials allowed the comparison of results with earlier studies (i.e. Kershaw, 1974; Maikawa

and Kershaw, 1975; MacFarlane et al., 1976; Kershaw et al., 1977). After the examination of nitrogenase activity in material kept under dark cold storage conditions, the experimental material was placed in a Conviron growth chamber set at a 15°/10°C day/night temperature regime with a 12 hr photoperiod (300µE m⁻²s⁻¹) and kept moist with frequent misting of distilled water for a further two weeks. Nitrogenase activity was then reexamined as before.

The confounding and interacting effects of light and temperature on the recovery of hitrogenase activity after a simulated snow melt could not be separated within the confines of the present experimental. design. Accordingly, P. canina var. praetextata and P. canina var. rufescens were collected from under the snow in their respective habitats during March, 1978 and pretreated as before. Experimental material was subsequently stored in Conviron growth chambers under the following 12 hr temperature and light regimes: $2^{\circ}/0^{\circ}$ C day/night thermoperiod with either $450\mu\text{E}$ m⁻²s⁻¹ or $0\mu\text{E}$ m⁻²s⁻¹ daylight illumination. Twenty ml vials were used throughout, four replicate thalli were kept saturated for all experiments and nitrogenase activity was assayed at 25° C and $300\mu\text{E}$ m⁻²s⁻¹ every second day for an eight day period.

(2.3) Thermal Stress

(2.3.1) Thermal Stress in P. canina var. praetextata and P. canina var. rufescens

Replicate thalli of P. canina var. praetextata were collected

in the air-dry state from deciduous woodland in Waterdown, Ontario and P. canina var. rufescens from an open xeric roadside habitat in Muskoka, Ontario. The material was returned within hours of collection and stored at 15°C with a 12 hr.photoperiod of $300\mu\text{E m}^{-2}\text{s}^{-1}$ for two days to allow further equilibration with the humidity of the laboratory. Subsequently, the material was stored air-dry (10% oven dry weight) in Conviron growth chambers at 25°, 35°, and 45°C with a 12 hr photoperiod of $300\mu E\ m^{-2}s^{-1}$ for varying experimental periods up to 40 days. Nitrogenase activity was examined to assess the level of damage to each population over time. Experiments were run at $25^{\circ}C$ and $300\mu E$ m $^{-2}$ and four replicates were used for each treatment temperature. Further evidence of thermal stress was obtained by examining the rates of net photosynthesis and respiration using infra-red gas analysis on both populations. Gas exchange was measured using a Beckman 865 infra-red gas analyzer and the closed experimental system described by Larson and Kershaw (1975a). Incubation dishes (100ml) each with an injection side arm closed by a rubber septum were used throughout the experimental series. Three-millilitre gas samples were injected into a carrier gas of 200ppm $\rm CO_2$ in $\rm N_2$ (see Larson and Kershaw, 1975a for further details). Three replicates from 25° and 45°C storage were examined after 27 days of storage treatment at these temperatures.

(2.3.2) Microclimatic Factors

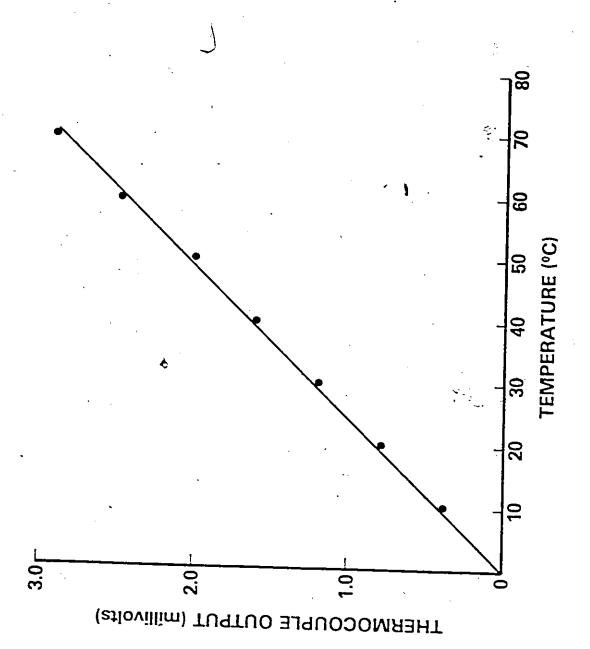
The measurement of temperature differences in the two contrasting habitats was achieved using thermocouples with their characteristic high sensitivity, accuracy and low radiation errors, (Tanner, 1963; Pieters

and Schurer, 1973). The thermocouples used in this study were constructed of 42 (0.064mm) guage copper-constantan wire to maximize the above characteristics. Probes were built with five sensing junctions and an ice reference temperature of 0°C was used and maintained with an Omega electronic ice reference cell. Calibration from millivolts to degrees Celsius is approximately linear from 0-70°C (Figure 1) and accurate to within 12 of the temperature difference between the measuring and reference junction (Schimmelpfennig, 1975).

It was essential to find woodland and roadside locations which were in juxtaposition to one another so that thallus temperatures could be measured simultaneously. None were found in Ontario but a suitable site was located near Pellston in northern Michigan. Thallus temperature probes were embedded into three replicate whole lichen clones in situ and temperatures were measured simultaneously from the woodland and the adjacent roadside. The resultant signals were stepped through a multichannel automatic stepping switch every three minutes and were recorded on a Rikadenki strip-chart recorder housed in a tent some 30m distant (see Field et al., 1974 for details). Radiant flux density was also measured every 15 min with a Lambda quantum sensor held horizontally level at the surface of the three lichen clones in their respective habitats. An average of three figures was taken and recorded. This entire procedure was repeated for a second site which was located about 0.6km from the first. Temperature data were recorded on June 22, 26; July 8, 9, 10 and 11, 1977 for Site 1 and on July 17, 22 and August 3, 1977 for Site 2. Surface weather observations recorded at the Pellston airport 2.5km away were obtained for the above-noted days from the U.S. National Climatic Data Centre in

Figure i

The relation between thermoelectric voltage in absolute millivolts and temperature in °C for copper-constantan thermocouples. (After Schimmelpfennig, 1975).



Asheville, N.C. (Appendix A).

(2.3.3) Thermal Sensitivity in Northern Populations of Peltigera and Stereocaulon

Northern populations of Peltigera scabrosa from spruce-lichen woodland and P. canina var. rufescens from a 25 yr old burn were collected at-Hawley Lake in the Hudson Bay lowlands of northern Ontario (54°20'N, 84°20'W), during late September, 1977. Lichen material was collected and allowed to dry indoors for 24 hours at ambient temperatures $(8^{\circ}-10^{\circ}\text{C}$ day, 0° C night), then packed in plastic bags and shipped south in two days. Experimental material was then stored at 15°C for two further days to allow moisture levels to equilibrate with those of the laboratory (10 $^{\circ}$ % of the ovendry weight). Daylight illumination for <u>P. canina</u> var rufescens was 300 μ E m⁻²s⁻¹ while <u>P. scabrosa</u> was exposed to 25 μ E m⁻²s⁻¹. Following this pretreatment, thermal stress conditions were established on the air-dry material at temperatures of 25°, 30°, 35°, 40°, and 45°C for P. scabrosa and 25°, 35°, and 45°C for P. canina var. rufescens. These 12 hr day temperatures were coupled with a 12 hour night at 15°C. Daylight illumination remained unchanged from the pretreatment regime. The storage temperature modifications were incorporated into the experimental design based on previously collected field data (section 2.3.2) and essentially represented much more realistic day/night temperature regimes than the continuous storage temperature treatments using in previous experimental designs. Storage light intensities were also modified in keeping with the light levels encountered in the

natural habitats. Nitrogenase activity was assayed at 25° C and $300\mu E$ m⁻²s⁻¹ for five replicates from each storage condition for both species up to periods of 30 days.

Stereocaulon paschale was collected from spruce lichen woodland in the Abitau-Dunvegan Lakes region of the Northwest Territories $(60^{\circ}21^{\circ}\text{N},\ 106^{\circ}54^{\circ}\text{W})$ during November, 1977. Mats of <u>S. paschale</u> were collected in dry conditions and packed over linen bags of silica gel in plastic boxes for transportation to the laboratory. Transit time was limited to a maximum of two days. The lichen mats were subsequently stored in Conviron growth chambers at 15°C and a 12 hr photoperiod of $300\mu\text{E m}^{-2}\text{s}^{-1}$ for two days to allow moisture equilibration. Lichen material was then stored air-dry in growth chambers with 12 hr thermoperiods of $25^{\circ}/15^{\circ}$, $35^{\circ}/15^{\circ}$, and $45^{\circ}/15^{\circ}\text{C}$ and $300\mu\text{E m}^{-2}\text{s}^{-1}$ for 21 days. Nitrogenase activity was assayed at 25°C and $300\mu\text{E m}^{-2}\text{s}^{-1}$ for five replicates at weekly intervals.

In June, 1978, <u>Peltigera aphthosa</u> was collected from dense spruce-lichen woodland at Hawley Lake, northern Ontario. Experimental material was dried, and shipped south as described for other <u>Peltigera</u> species collected in this area. Upon its arrival, <u>P. aphthosa</u> was stored at 10°C and a 12 hour photoperiod of 25µE m⁻²s⁻¹ for a minimum of two further days. Subsequently, the experimental material was stored in growth chambers with 12 hr day/night periods of 15°/15°, 25°/15°, 35°/15°, and 45°/15°C at 25µE m⁻²s⁻¹ for periods up to 24 days. The 15°C storage treatment was included as a necessary control in the experimental design because some preliminary results suggested that the 25°/15°C storage treatment could be stressful over long periods of time

to some of the more sensitive lichen species. Nitrogenase activity was examined at 20° C, 300μ E m $^{-2}$ and four replicates were used throughout.

(2.3.4) Net Photosynthetic Responses to Thermal Stress in P. canina var. praetextata and P. canina var. rufescens

P. canina var. praetextata and P. canina var. rufescens were collected during the first week of September, 1978 in Waterdown, Ontario and Muskoka, Ontario respectively. After being collected dry in the field, experimental material was returned to the laboratory within hours and stored in growth chambers at 15°C for a minimum of two days. P. canina var. praetextata collected from the woodland beneath a full canopy was stored under a 12 h period of 25 µE m s while P. canina var. rufescens was maintained with a 12 hour daylength of $300\mu E$ m⁻²s⁻¹. After the experimental material had equilibrated with ambient humidity, thermal stress experiments were initiated by storing the lichens in growth chambers with a 12 hr thermoperiod of 15°/15°, 25°/15°, 35°/15°, and 45°/15°C; storage light intensities for both species remained unchanged. Six replicates from each storage temperature (three each for photosynthesis and respiration) were dissected from whole clones and soaked overnight at 15°C prior to the experiment. The 15°/15°C storage treatment served as a necessary control in light of previous results and material from this temperature was run at the beginning and the end of the experimental series. Net photosynthesis and respiration were monitored at 25°C and $150\mu E m^{-2}s^{-1}$.

(2.4) Light Stress and Light-Mediated Seasonal Adjustments of Net Photosynthetic Capacity

(2.4.1) Light Stress in Peltigera aphthosa

Peltigera aphthosa was collected from dense spruce-lichen woodland at Hawley Lake during October, 1978. Collection, pretreatment and shipping details were as described in section 2.3.3. Upon its arrival, P. aphthosa was stored in growth chambers at 15° C and with a 12 hr photoperiod of $25\mu \text{E m}^{-2} \text{s}^{-1}$ for a minimum of two days. One half of the experimental material was then stored at 15° C and a 12 h photoperiod of $450-600\mu \text{E m}^{-2} \text{s}^{-1}$ for periods up to 30 days. Nitrogenase activity was assayed at 25° C and $300\mu \text{E m}^{-2} \text{s}^{-1}$ for five replicates following an overnight soaking at 15° C.

(2.4.2) Light-Mediated Seasonal Adjustments of Net Photosynthetic Capacity in P. canina var. praetextata

One clone of <u>P. canina</u> var. <u>praetextata</u> was collected in Waterdown and returned to the laboratory within minutes. The experimental material was placed in a growth chamber at 15° C and a 12 h photoperiod of $25\mu\text{E m}^{-2}\text{s}^{-1}$. Lichen material was usually damp when collected in the late afternoon and was kept saturated with distilled water until its use the following day. Net photosynthesis was examined on the next day for three replicates at each light level of 450, 150 and $0\mu\text{E m}^{-2}\text{s}^{-1}$ and 25° C. This light intensity series was amplified by the addition of one further light level at $75\mu\text{E m}^{-2}\text{s}^{-1}$. After all the leaves had fallen, lichen material was collected and stored as before but at $300\mu\text{E m}^{-2}\text{s}^{-1}$ rather than at $25\mu\text{E m}^{-2}\text{s}^{-1}$. Experimental material was collected on

September 4, October 1, and October 19, 1978. Weekly collections were made on October 27, November 3, and November 11, 1978 to accommodate the rapid changes in the canopy that were taking place. Based on the work of Kershaw (1977b, c), extra material was collected on October 27, when the canopy was completely devoid of leaves and Peltigera clones growing on the forest floor were exposed to naturally high levels of incident radiation (> 1000µE m⁻²s⁻¹). After examining net photosynthesis and respiration (see 2.3.1) experimental material was stored at 15°C and a 12 hour photoperiod of 25µE m⁻²s⁻¹ for two further weeks. Thalli were kept moist with frequent misting of distilled water. Subsequently, net photosynthesis was examined as before at 450, 150, 75 and 0µE m⁻²s⁻¹ and 25°C

Section 3.

RESULTS

(3.1) Seasonal Changes in the Nitrogenase Activity of P. canina var. praetextata and P. canina var. rufescens

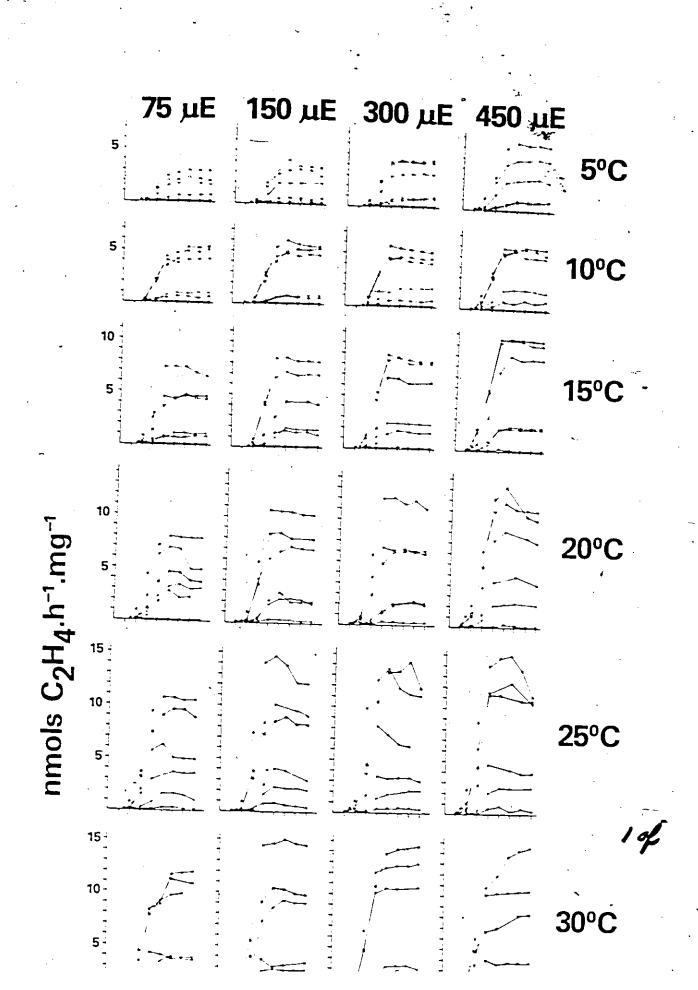
In previous studies of N₂ fixation in lichens, the effects of various environmental parameters such as light, temperature and moisture have been investigated but only in a univariate or bivariate manner. This has resulted in a tendency to obscure any seasonal variation in the rate of nitrogenase activity and to mask any non-linear responses which might have otherwise been recognized. Recent work by Kershaw (1977b, c) has shown substantial seasonal variations of net photosynthetic activity in the genus <u>Peltigera</u> and it was therefore essential to examine nitrogenase activity for seasonal fluctuations.

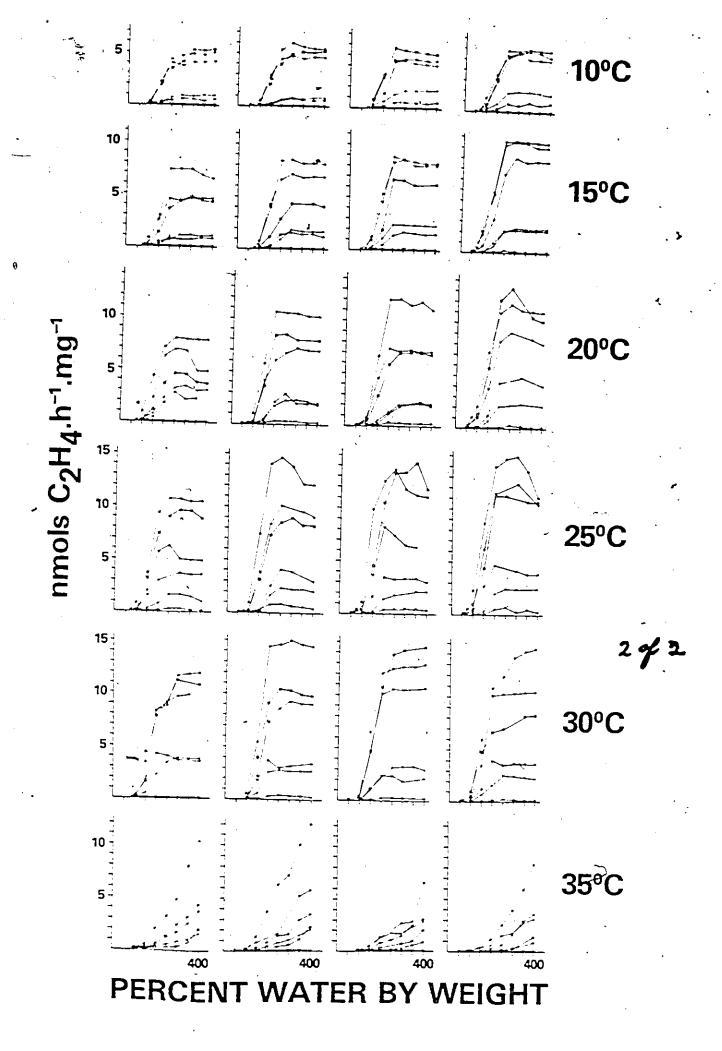
<u>Peltigera canina</u> var. <u>praetextata</u> and <u>P. canina</u> var. <u>rufescens</u> were selected for detailed examination because of their relative abundance, their proximity to the laboratory and their contrasting habitats.

(3.1.1) The Effect of Light Intensity on Nitrogenase Activity

The physiological data matrices for <u>Peltigera canina</u> var. <u>praetextata</u> and <u>P. canina</u> var. <u>rufescens</u> are given in Figures 2 and 3 respectively. There is no significant increase in nitrogenase activity with radiant flux density although, typically, the rate is greater at $450\mu E$ m⁻²s⁻¹ than at $75\mu E$ m⁻²s⁻¹. This pattern of response is evident at all experimental temperatures and throughout the seasons.

The nitrogenase activity (nmols $C_2^{H_4} \cdot h^{-1} \cdot ms^{-1}$) in <u>Peltigera canina</u> var. <u>praetextata</u> at all levels of thallus moisture, under 75, 150, 300 and 450 μ E m⁻² s⁻¹ illumination, and at 5, 10, 15, 20, 25, 30 and 35°C. The data matrix was established for July 1975 (o); October 1975 (\blacktriangle), December 1975 (\bullet), March 1976 (\square), May 1976 (Δ), and July 1976 (\blacksquare).





(3-1.2) The Effect of Thallus Moisture on Nitrogenase Activity

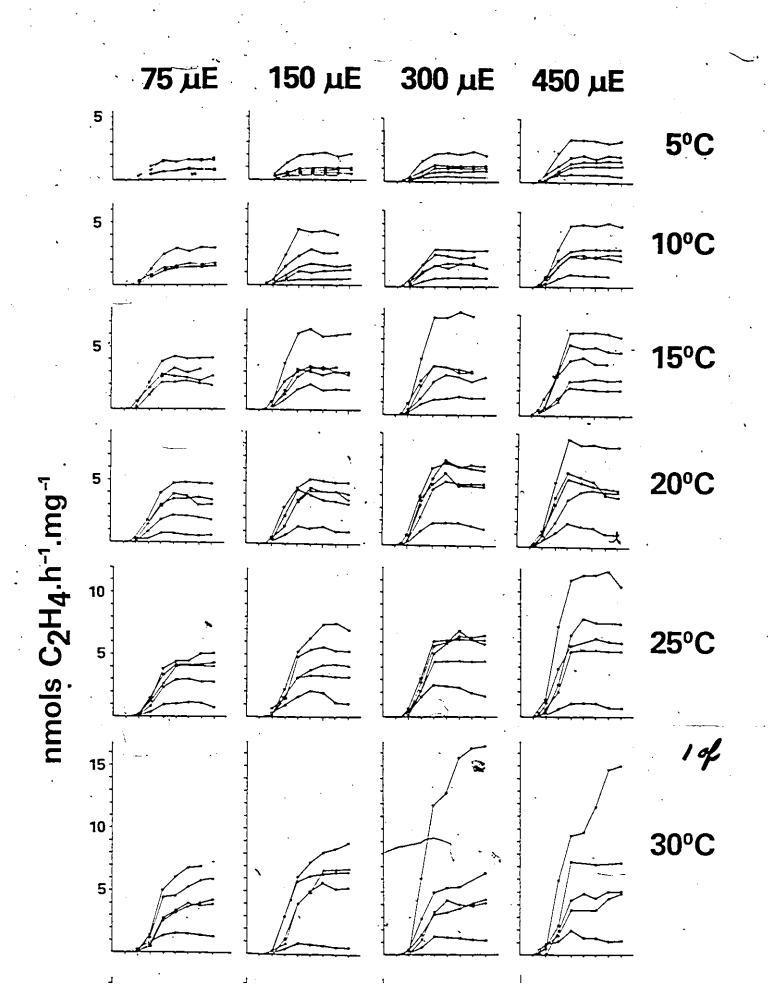
For all cells of both matrices with the exception of 35°C, (see section 3.1.3), P. canina var. praetextata and P. canina var.

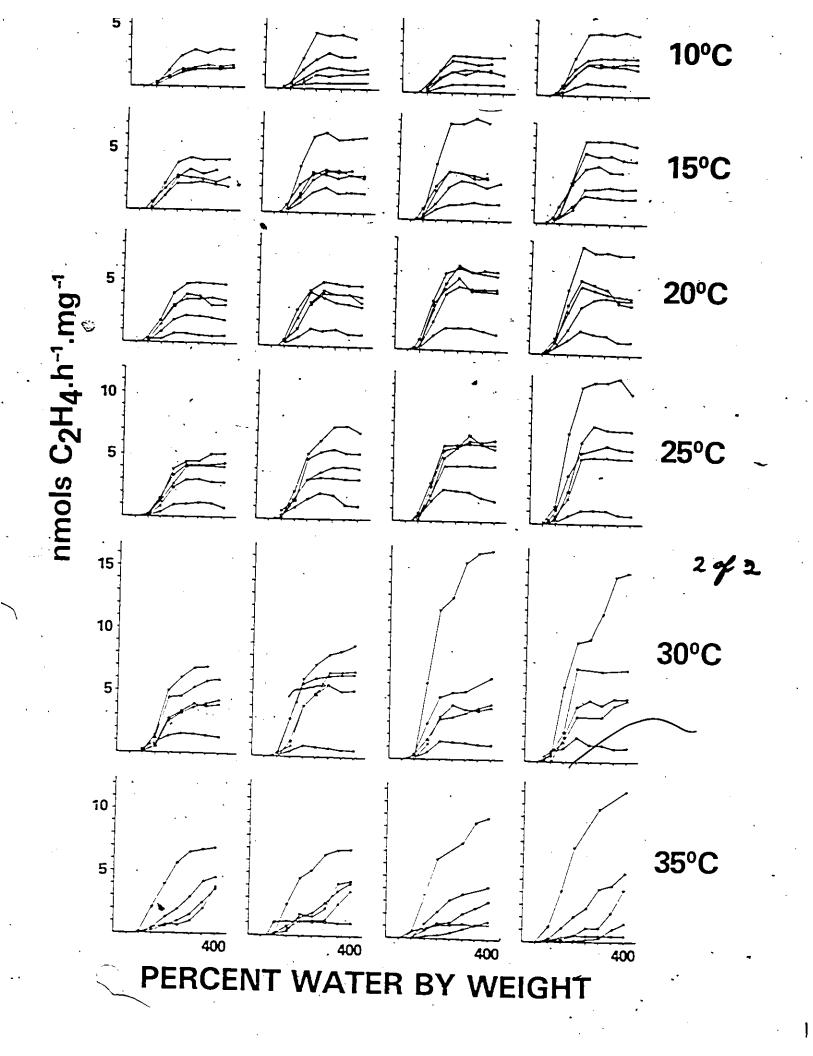
rufescens exhibit a maximum and relatively stable rate of nitrogenase activity between 200% and 400% relative water content. Below 200% saturation, the rate of acetylene reduction declines sharply and nitrogenase activity ceases completely in a range from 50 to 100% relative moisture content. It is also evident that both species possess very similar water holding capacities.

(3.1.3) The Effect of Temperature on Nitrogenase Activity

Very pronounced response of nitrogenase activity to temperature at all light levels throughout the year (Figures 2 and 3). Typically, minimum levels of nitrogenase activity were obtained at 5°C while maximum fixation was observed in the temperature range 20°-30°C. At 35°C however, nitrogenase activity declined extremely rapidly after the first incubation and apparently reflected the sensitivity of the nitrogenase enzyme system to high temperatures. This response was largely independent of the season and was particularly pronounced in P. canina var. praetextata (Figure 2). This phenomenon was reexamined in a further series of experiments which followed the time course of nitrogenase activity in saturated thalli of P. canina var. praetextata maintained at 35°C (Figure 4). The pattern of response under constant saturation levels was very similar to the drying response (Figure 2) and apparently the rapidly declining activity is thus a function of high temperature rather than

The nitrogenase activity (nmols C_2H_4 h $^{-1}$ mg $^{-1}$) in <u>Peltigera canina</u> var. <u>rufescens</u> at all levels of thallus moisture, under 75, 150, 300 and 450 μ E m $^{-2}$ s $^{-1}$ illumination, and at 5, 10, 15, 20, 25, 30 and 35°C. The data matrix was established for August 1975 (o), October 1975 (Δ), December 1975 (\bullet), May 1976 (Δ), August 1976 (\blacksquare).





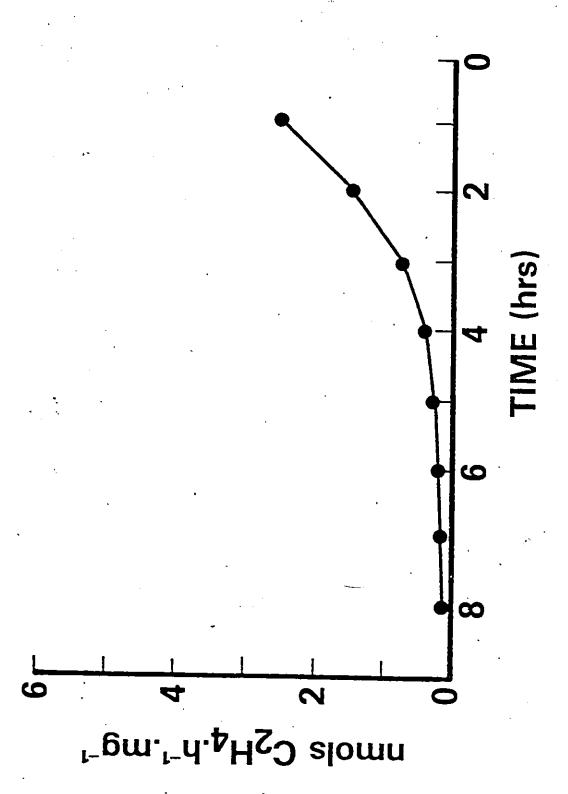
the loss of thallus moisture. Subsequently, nitrogenase activity was restarted in the experimental 35°C replicates by soaking at 20°C for two hours, confirming the interpretation that heat inactivation of the nitrogenase system occurs at 35°C.

(3.1.4). Seasonal Variation in the Rate of Nitrogenase Activity

Both P. canina var. praetextata and P. canina var. rufescens show very substantial seasonal rate changes in nitrogenase activity for all combinations of light intensities and temperatures. Extremely low levels of fixation were recorded throughout the matrix for P. canina var. praetextata in the summer of 1975. However, by the end of September of that year, nitrogenase activity had increased to an average of 2.5-3.0 nmols $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ for the $20^{\circ} \sqrt{30^{\circ}C}$ cells of the matrix. By December 1975, very large increases in the rate of acetylene reduction were observed for all cells in the matrix with a maximum rate of 14-15 nmols $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ at $25^{\circ} - 30^{\circ}C$ over all light intensities. These very high rates of activity declined by March and then remained more or less constant until May, 1976. In mid July, 1976, the potential to fix nitrogen had declined to the September 1975, level.

The seasonal pattern of acetylene reduction by <u>P. canina</u> var. <u>rufescens</u> was basically similar. In July and August of 1975, nitrogenase activity on average was low and ranged from 1.5 nmols ${}^{\text{C}}_{2}{}^{\text{H}}_{4} \cdot {}^{\text{h}}^{-1} \cdot {}^{\text{mg}}^{-1}$ to a maximum of 2.5 nmols ${}^{\text{C}}_{2}{}^{\text{H}}_{4} \cdot {}^{\text{h}}^{-1} \cdot {}^{\text{mg}}^{-1}$ at temperatures of 15°C and 30°C respectively. By October 1975, nitrogenase activity had increased markedly above the summer rates with maximum levels of activity at temperatures of 25°-30°C. By December of that year, rates of acetylene reduction had increased still further to an

The time course of nitrogenase activity in <u>Peltigera canina</u> var. <u>praetextata</u> in water saturated thalli at 35° C and under $300\mu E$ m⁻² s⁻¹ illumination.



average of 6.5-7.0 nmols C₂H₄·h⁻¹·mg⁻¹ at 30°C. By summer 1976, the rate of nitrogenase activity had declined considerably to approximately the 1975 fall and winter rates. Thus in both species, seasonal changes in nitrogenase activity were large but there was no appreciable difference in temperature optima or response to increasing radiant flux densities or moisture content.

(3.1.5) Light and Temperature Interactions with Nitrogenase Activity in P. canina var. praetextata and P. canina var. rufescens Collected Under Snow

Some previous research studies in the genus <u>Peltigera</u> have dealt separately with the light and temperature interactions with nitrogenase activity (e.g. MacFarlane <u>et al.</u>, 1976; Kershaw <u>et al.</u>, 1977). It was of interest to extend the investigation of light and temperature interrelations to the recovery of nitrogenase activity following a naturally imposed extensive cold and dark period. It became apparent that previously reported rates of acetylene reduction for <u>Peltigera canina</u> differed considerably from those observed during the course of this work (see Maikawa & Kershaw, 1975). Since portions of the experimental material that were used in earlier studies were collected from under the snow, the potential effect of snow cover on subsequent rates of nitrogenase activity needed clarification.

The level of nitrogenase activity for thallus replicates of P. canina var. rufescens collected from under lm of snow is given in Figure 5. Very low rates of acetylene reduction are evident at all temperatures for both $300\mu E$ m⁻²s⁻¹ and $0\mu E$ m⁻²s⁻¹ illumination. After

two weeks of storage at a 12 hr photoperiod of 300 µE m⁻²s⁻¹ and 15°/10°C, the rates of nitrogenase activity increased almost two fold for all temperatures and light levels (Figure 5). Clearly, these results establish that both light and temperature are significantly involved in the recovery of nitrogenase activity after snowmelt but the assessment of the relative contributions of each required further experimentation.

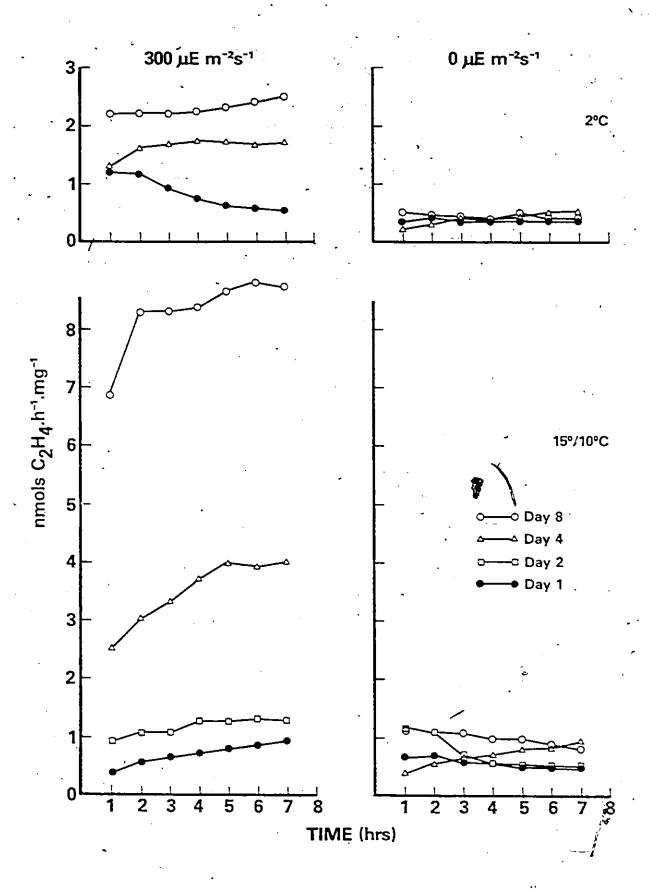
P. canina var. praetextata and P. canina var. rufescens collected from under the snow in March, 1978 show a similar pattern of nitrogenase activity (Figure 6 and 7). Replicate thalli of P. canina var. praetextata stored at a 12 h thermoperiod of $15^{\circ}/10^{\circ}$ C day/night temperature and a 12 h photoperiod of $300\mu E \text{ m}^{-2} \text{ show a remarkable recovery in their rates of }$ nitrogenase activity after eight days under these storage conditions (Figure 6). Nitrogenase activity was approximately 0.5-0.7 nmols $C_2H_A \cdot h^{-1} \cdot mg^{-1}$ after the first day and had slowly increased by day two to 1.2 nmols $C_2^{\rm H}_4 \cdot h^{-1} \cdot mg^{-1}$. By day 4, the rates had more than doubled and by day 8, rates of acetylene reduction reached a maximum of 8.8 nmols $C_{9}H_{\Delta} \cdot h^{-1} \cdot mg^{-1}$. The response of nitrogenase activity in replicates stored at $300\mu E$ m $^{-2}$ but at a constant temperature of only 2°C showed a similar but less rapid resurgence of activity. After eight days at these storagé conditions, rates of acetylene reduction which initially were c. 0.75 nmols $C_2H_4 \cdot h^{-1} \cdot g^{-1}$ had reached a maximum of 2.5 nmols $C_2^{H}_4$ ·h⁻¹·mg⁻¹. Conversely nitrogenase activity remained relatively low and constant from replicates kept in the dark at both $15^{\circ}/10^{\circ}$ and 2°C over the entire time series.

The pattern of recovery for <u>P. canina</u> var. <u>rufescens</u> was again similar but in some instances was markedly slower (Figure 7). The rate of acetylene reduction increased in replicates stored at a 12 h thermoperiod of $15^{\circ}/10^{\circ}$ C day/night temperature and a 12 h photoperiod of $300\mu\text{E}$ m⁻²s⁻¹ from the day 1 response and reached a maximum of 3.9

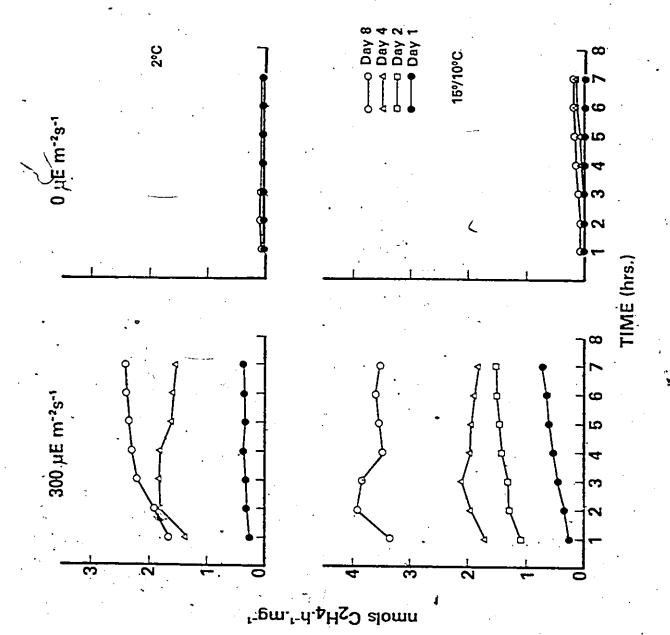
The time course of nitrogenase activity in <u>Peltigera canina</u> var. <u>rufescens</u> in water saturated thalli under 0 and $300\mu E m^{-2} s^{-1}$ illumination and at 5, 15, 25 and 35°C. Experimental replicates which had been collected from under 1 m of snow were initially assayed for nitrogenase activity (\bullet) and then after two weeks of storage at 300 E and $15^{\circ}/10^{\circ}$ (o).



The time course of nitrogenase activity in <u>Peltigera canina</u> var. <u>praetextata</u> in water saturated thalli which had been collected from under snow and stored moist under the following conditions: a 12 hour photoperiod of either $300\mu\text{E m}^{-2}\text{s}^{-1}$ or $0\mu\text{E m}^{-2}\text{s}^{-1}$ with either a 12 hour thermoperiod of $15^{\circ}/10^{\circ}\text{C}$ or at a constant 2°C .



The time course of nitrogenase activity in <u>Peltigera canina</u> var. <u>rufescens</u> in water saturated thalli which had been collected from under snow and stored moist under the following conditions: a 12 hour photoperiod of either $300\mu\text{E m}^{-2}\text{s}^{-1}$ or $0\mu\text{E m}^{-2}\text{s}^{-1}$ with either a 12 hour thermoperiod of $15^{\circ}/10^{\circ}\text{C}$ or at a constant 2°C .



nmols $C_2^{H_4} \cdot h^{-1} \cdot mg^{-1}$ after 8 days. In comparison with <u>P. canina</u> var.

<u>praetextata</u> under identical storage conditions, this lower value suggests a higher light requirement in <u>P. canina</u> var. <u>rufescens</u> for comparable rates of recovery. The rate of recovery at a constant 2°C and a 12 h photoperiod of $300\mu\text{E}$ m⁻²s⁻¹ was again slower than that at $15^{\circ}/10^{\circ}\text{C}$ and identical lighting thus illustrating the effect of low temperature.

Irrespective of storage temperature, lichen thalli kept in continuous darkness exhibited very low levels of nitrogenase activity. Whilst again temperature is intimately involved in the velocity of the recovery, the prime requirement appears to be one of light.

(3.2) Thermal Stress

The very low levels of nitrogenase activity that were observed in summer collections of <u>P. canina</u> var. <u>praetextata</u> during 1975 were interpreted as a response to an unusually hot dry summer. Furthermore, the thermal inactivation of nitrogenase at 35°C which was most pronounced in the woodland species, suggested that temperature stress may figure largely in the seasonal responses of nitrogenase. These depressed summer rates were subsequently induced under controlled conditions in the laboratory by storing lichen thalli air-dry at a continuous temperature of 35°C with a 12 h illumination of $300\mu\text{E}$ m⁻²s⁻¹. After 27 days of this ecologically unrealistic storage treatment, rates of nitrogenase activity were c. 1.0 nmol $\text{C}_2\text{H}_4\cdot\text{h}^{-1}\cdot\text{mg}^{-1}$ and were very comparable with the low summer values observed in the earlier stages of this work (Figure 2). However, replicate thalli of <u>P. canina</u> var. <u>praetextata</u> appeared to be extensively damaged in that leaching of green pigments occured during subsequent hydration and after 40 days,

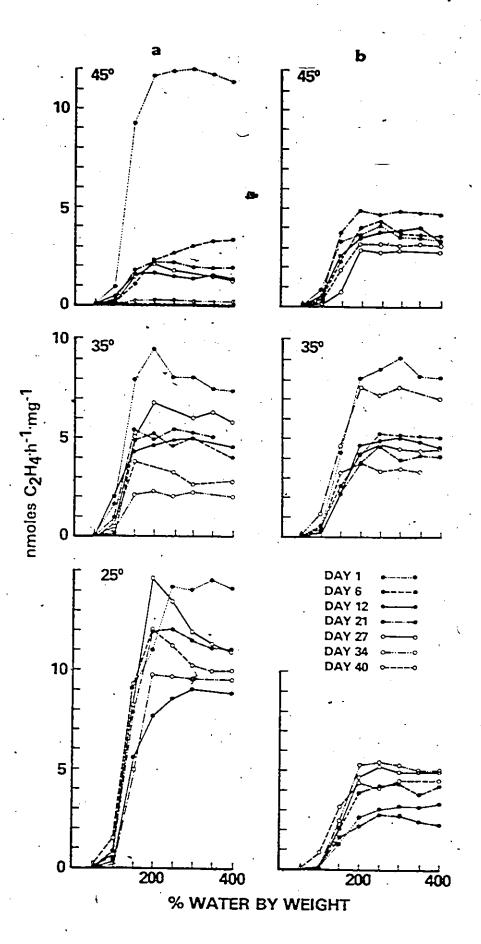
nitrogenase activity could not be detected.

These observations were contrary to the long-held belief that . lichens are completely resistant to high temperature when air-dry and accordingly, a reexamination of this long-standing concept was undertaken (see section 3.2.1).

(3.2.1) Thermal Stress in P. canina var. praetextata and P. canina var. rufescens

The effect of up to 40 days of thermal stress at 25, 35 and 45° C on nitrogenase activity in both populations of Peltigera is given in Figures 8a and 8b. Representative levels of mitrogenase activity in P. canina var. praetextata and P. canina var. rufescens are 10-15 nmols $C_2^{H_4} \cdot h^{-1} \cdot mg^{-1}$ and 5-7 nmols $C_2^{H_4} \cdot h^{-1} \cdot mg^{-1}$ respectively (Figures 2 and 3). Control replicates stored at 25°C for 40 days exhibited closely comparable rates. The activity of the P. canina var. praetextata replicates stored at 45°C however declined rapidly from a maximum of . 12 nmols $C_2^{H_4 \cdot h^{-1} \cdot mg^{-1}}$ on day 1 down to 1.5 nmols $C_2^{H_4 \cdot h^{-1} \cdot mg^{-1}}$ by day 27. Only a trace of activity was present by day 34 and no activity whatsoever could be detected by day 40 (Figure 8a). At 35°C storage, there was also a clear decline in nitrogenase activity in P. canina var. praetextata throughout the experiment but rates of c. 2.0 nmols C2H4 $\cdot h^{-1} \cdot mg^{-1}$ were still evident after 40 days. Conversely, nitrogenase activity in P. canina var. rufescens was maintained at c. 4.0-6.0 nmols $C_2H_A \cdot h^{-1} \cdot mg^{-1}$ throughout the entire experimental storage and time series in marked contrast to the responses of the woodland variety (Figure 8b).

The nitrogenase activity of (a) <u>Peltigera canina</u> var. <u>praetextata</u> and of (b) <u>Peltigera canina</u> var. <u>rufescens</u> after air-dry storage at temperatures of 25°, 35°, and 45°C. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



Net photosynthetic rates in <u>P. canina</u> var. <u>praetextata</u> exhibited a similar pattern of response to the thermal stress treatment (Figure 9a). After 27 days of treatment at 25°C, <u>P. canina</u> var. <u>praetextata</u> was unaffected and its rates of net photosythesis and respiration were consistent with previously reported values (Kershaw, 1977b, c). After 27 days, the 45°C storage treatment had reduced the rate of net photosynthesis to 20 percent of the control, without any concurrent changes in respiration rate (Figure 9a). The data for <u>P. canina</u> var. <u>rufescens</u> (Figure 9b) show clearly that the rates of net photosynthesis and respiration were unaffected by the storage treatment. Thus, the responses of photosynthesis and respiration for both species closely paralleled those observed for nitrogenase activity.

(3.2.2) Microclimatic Factors

The contrasting habitats of <u>P. canina</u> var. <u>praetextata</u> and <u>P. canina</u> var. <u>rufescens</u> and their differential thermal sensitivies appeared to be closely interrelated. It was essential to document the field temperature regimes of these two populations in order to assess this apparent correlation. This information would also indicate the degree of realism of the storage temperatures used in previous experimental designs compared with those actually encountered in the field.

Thallus temperatures in the two diverse habitats measured simultaneously with embedded microthermocouples, showed marked differences. Thallus temperatures for <u>P. canina</u> var. <u>praetextata</u> in the woodland, under full canopy conditions, recorded on June 22 (summer solstice) and June 26, 1977 (Figure 10 and 11) remained below 30°C for most of the

The respiration (Lower pair of curves) and net photosynthetic response (upper pair of curves) of (a) <u>P. canina</u> var. <u>praetextata</u> and (b) <u>P. canina</u> var. <u>rufescens</u> after 27 days of air-dry storage at 25°C (o—o) and 45°C (•—•). Thallus saturation is expressed as a percentage of the . final oven dry weight of each replicate.

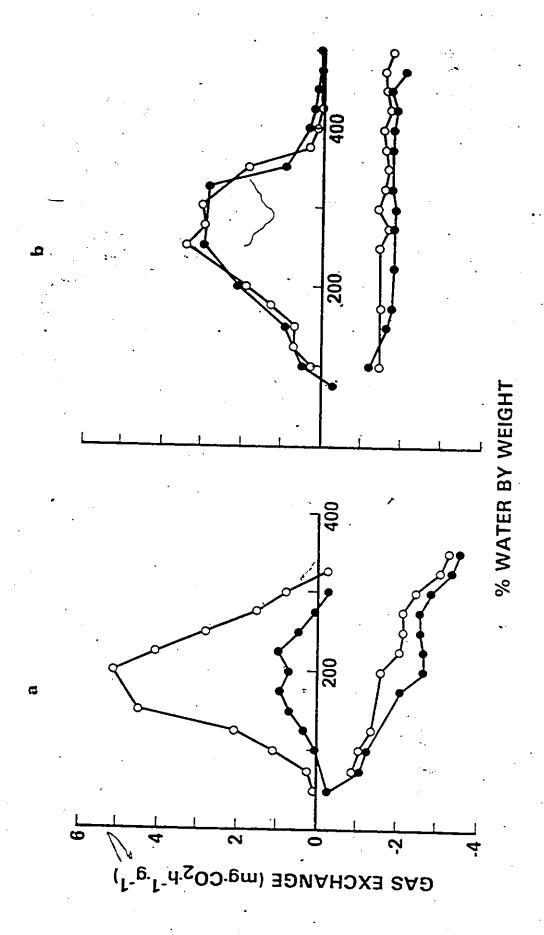
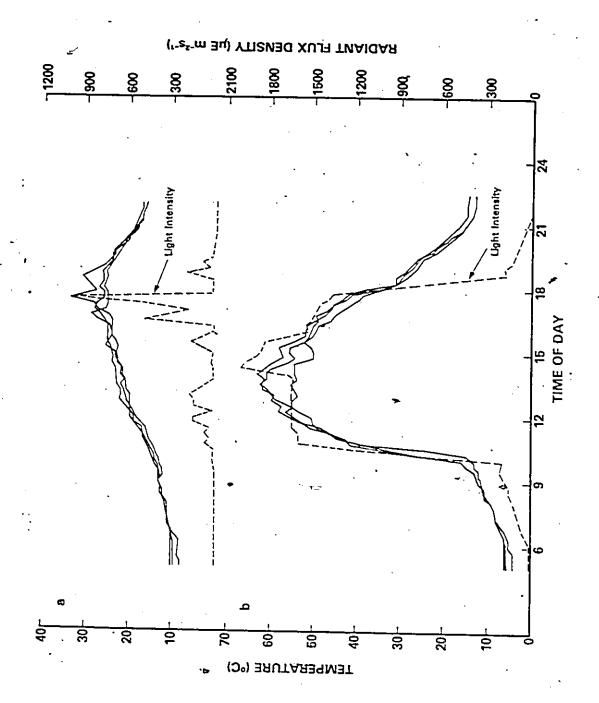


Figure 10 .

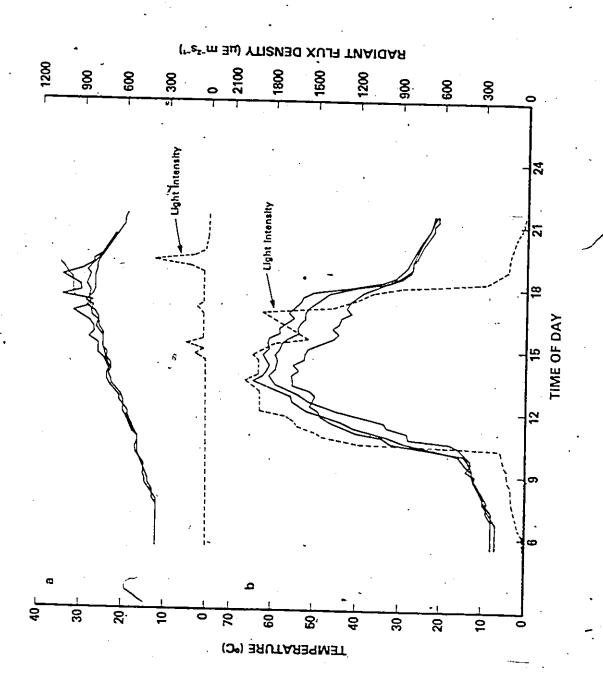
Thallus temperatures and radiant flux density recorded for (a) P. canina var. praetextata and (b) P. canina var. rufesceps on 22 June 1977 for three replicate thalli.



Thallus temperatures and radiant flux density recorded for (a)

P. canina var. praetextata and (b) P. canina var. rufescens on 26

June 1977 for three replicate thalli.



day. In the early evening, thallus temperatures were, for a brief period, slightly in excess of 30°C as a result of sunfleck activity. In contrast, thallus temperatures for <u>P. canina</u> var. <u>rufescens</u> on the adjacent roadside rose rapidly and tissue temperatures in excess of 60°C were recorded at solar noon (Figure 10 and 11). The relative inputs of radiant energy measured in the two habitats merely reinforces these extreme differences. Maximum air temperatures recorded at the Pellston airport for June 22 and 26, 1977 were 27.2°C and 30.6°C respectively. Both days, in fact, were exceptional with bright, sunny skies, very little cloud cover and little or no wind (Appendix A). Thallus temperatures and surface weather observations recorded on July S. 9, 10, and 11, 1977 for Site 1 and on July 17, 22 and August 3, 1977 for Site 2 show a similar pattern and have been summarized in Appendix "A"

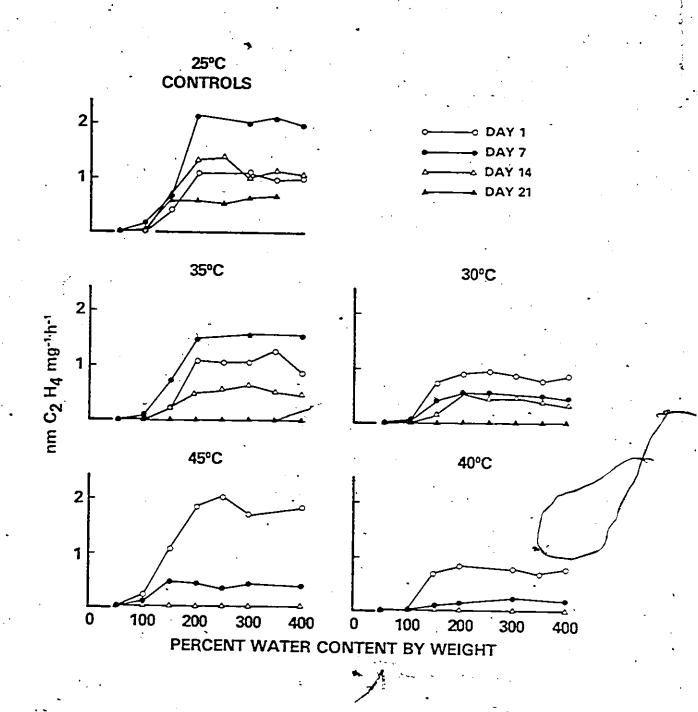
(3.2.3) Therma Sensitivity in Northern Populations of Peltigera and Stereocaulon

The overriding influence of temperature has been commonly cited as having the most profound effect on plant distributions especially on a global scale (i.e. Kellman, 1975). Thus the importance of thermal stress as an ecological parameter influencing the distribution of two populations of Peltigera pointed to the necessity of examining a wider range of lichens from diverse geographical locales. Peltigera scabrosa and P. aphthosa were selected as representative of low-arctic mature spruce-moss woodland where the former is restricted to the cool shaded closed canopy conditions. P. canina var. rufescens growing on an

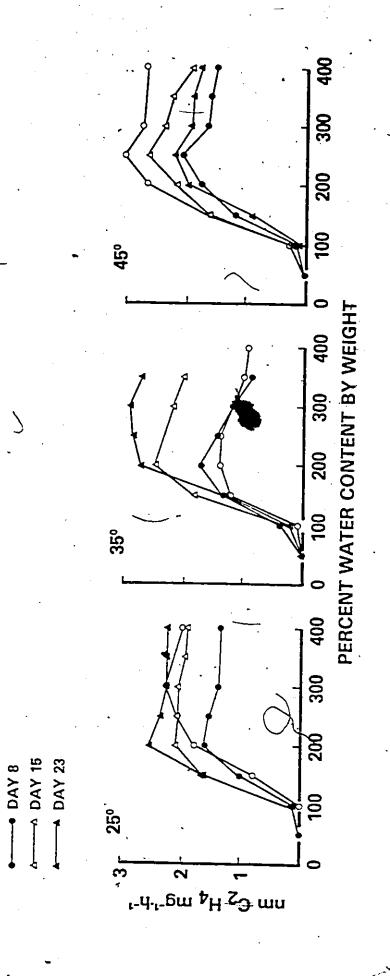
exposed 25 year old burn adjacent to the mature spruce-moss woodland was selected as typical of the early colonizers following forest fire and was expected to be completely analogous to the southern Ontario population both in its response to thermal stress and to the extreme surface microclimate. Stereocaulon paschale forms an integral component of relatively open spruce-lichen woodland covering extensive areas in the Northwest Territories but it does not enter the successional sequence until 60-80 years after the initial burn. Kershaw (1977a, 1978) has emphasized the extreme microclimate of these recently burnt surfaces and the extreme thallus temperature conditions. The inference of the earlier work suggested a level of adaptation in the initial lichen colonizers which enabled them to survive the stressful surface conditions successfully. Accordingly, the potential effect of thermal stress on Stereocaulon paschale also required examination.

The remarkable level of thermal sensitivity of <u>Peltigera scabrosa</u> appears to be strongly correlated with its ecology (Figure 12). Control replicates for the nitrogenase experimental series were stored at 25°/15°C and showed an average level of activity of 1.0 to 1.5 nmols C₂H₄·h⁻¹·mg⁻¹ comparable with the day 1 replicates which had been stressed at 30°/15°, 35°/15°, 40°/15° and 45°/15°C. After 21 days at 30°/15° and 35°/15°C storage temperatures, the potential to fix elemental nitrogen had been completely eliminated. The decline in nitrogenase activity at 40°/15° and 45°/15°C day/night storage temperatures was even more dramatic in that acetylene reduction decreased swiftly after day 1 and ceased in only 14 days under these experimental conditions. The extreme sensitivity observed in this species suggests that

The effects on nitrogenase activity (nmols C₂H₄·h⁻¹·mg⁻¹) as a measure of thermal stress in <u>Peltigera scabrosa</u>. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



The effects on nitrogenase activity (mmols C₂H₄·h⁻¹·mg⁻¹) as a measure of thermal stress in a low arctic population of <u>Peltigera canina</u> var. rufescens. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



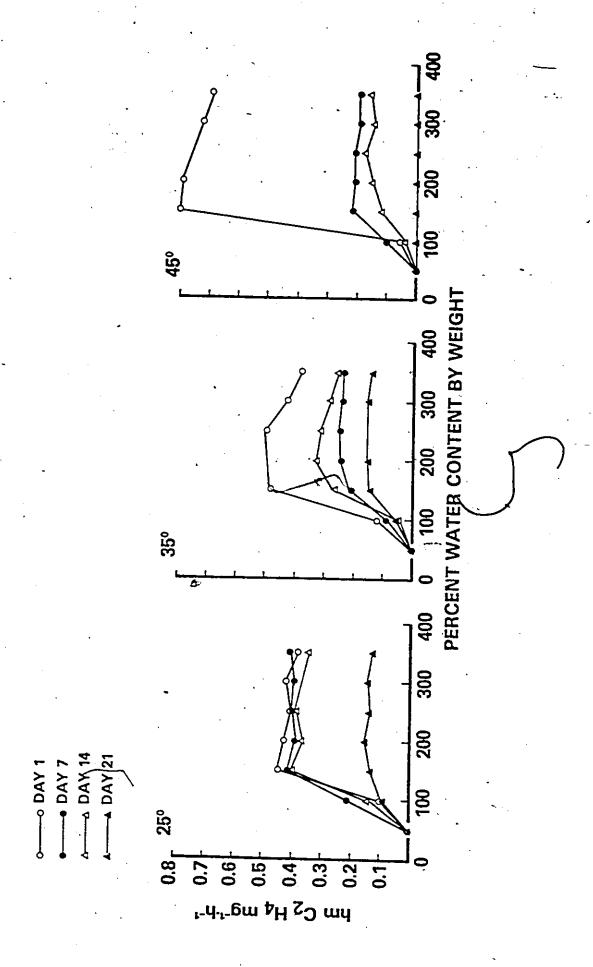
~ DAY 1

even the 21 day control replicates at 25°/15°C were also slightly stressed (see Discussion). Conversely, nitrogenase activity in P. canina var. rufescens collected from the adjacent 25 yr old burn was maintained throughout the 23 day storage time series (Figure 12) and contrasted sharply with the results obtained for the woodland species. The stress treatment was discontinued at this point since, although surface temperatures in excess of 50°C are experienced over burnt surfaces under full radiation conditions in midsonmer (Rouse 1976, Kershaw 1977a, 1978), it highly unlikely that such a stress level would be maintained continuously for more than three weeks. Conversely, the longer experimental stress periods used on the southern populations were chosen to simulate typical summer temperature stress periods which frequently run for 5 or 6 weeks in southern Ontario.

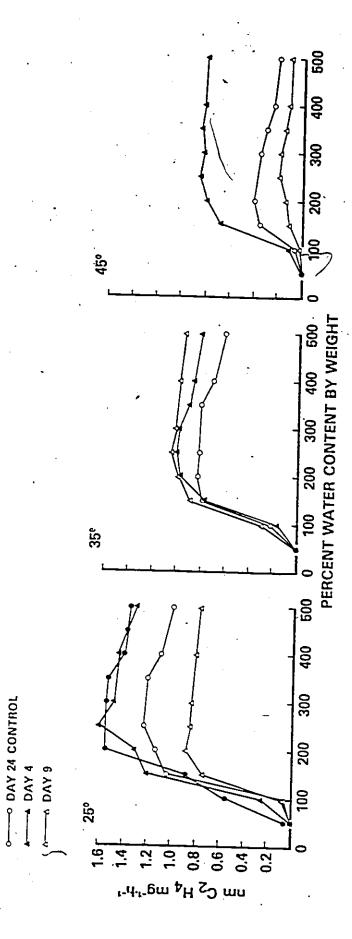
The result of 21 days of thermal stress at day/night storage regimes of 25°/15°, 35°/15° and 45°/15°C on nitrogenase activity of Stereocaulon paschale is given in Figure 14.

After only 7 days stress at 45° C, levels of nitrogenase—activity were reduced from a maximum of c. 0.5 nmols $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ to 0.2 nmols $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ and activity was entirely eliminated after a three week stress period. The replicates stored at 35° C day, 15° C night temperatures showed a gradual decline in nitrogenase activity with the with final values of only 0.15 nmols $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ after 21 days. The apparent decline of activity in the 25° C control replicates probably reflects a considerable degree of variation in nitrogenase activity and the range of cephalodial numbers in this species rather than a real stress response (see Discussion).

The effects on nitrogenase activity (nmols C₂H₄·h⁻¹·mg⁻¹) as a measure of thermal stress in <u>Stereocaulon paschale</u>. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



The effects on nitrogenase activity (nmols C_2H_4 h^{-1} mg⁻¹) as a measure of thermal stress in <u>Peltigera aphthosa</u>. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



→ DAY 1 CONTROL

Storage temperature regimes of 25°/15° and 35°/15° have little effect on the nitrogenase activity of <u>Peltigera aphthosa</u> (Figure 15). However, at 45°/15°C day/night temperatures, nitrogenase activity had declined after 24 days and replicates had lost more than 60 percent of their potential to fix nitrogen. In comparison with the response observed for <u>P. scabrosa</u> which essentially grows in the same habitat, the comparative thermal resistance of <u>P. aphthosa</u> was surprising and indeed unexpected. Presumably, it would require several additional days at this storage treatment temperature to eliminate nitrogenase activity altogether and in this respect, it closely resembles the responses observed for the southern woodland species, <u>P. canina</u> var. <u>praetextata</u>.

(3.2.4) Net Photosynthetic Responses to Thermal Stress in P. canina var. praetextata and P. canina var. rufescens

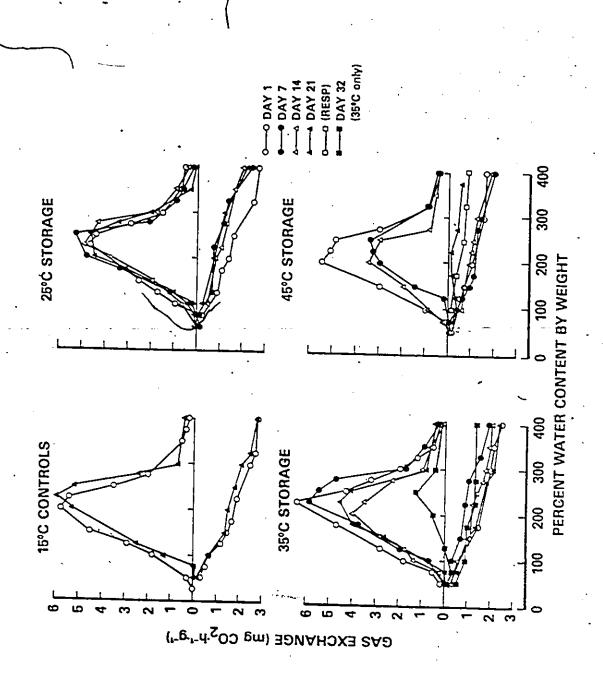
Provisional data for <u>P. canina</u> var. <u>praetextata</u> and <u>P. canina</u> var. <u>rufescens</u> (Figure 9) suggested that net photosynthesis was much more sensitive to thermal stress than was nitrogenase activity.

Accordingly, net photosynthesis and respiration were reexamined over a 22 day stress period in more detail (Figures 16 and 17). The 45°/15°C storage replicates showed a sharp decline in net photosynthetic rates from a normal maximum of 5.5-6.0 mgCO₂·h⁻¹·g⁻¹ down to c. 3.5 mgCO₂·h⁻¹·g⁻¹ by day 7. By day 21 these severely stressed replicates were not even reaching compensation under 150µE m⁻²s⁻¹ illumination. Under a storage stress of 35°/15°C day/night temperatures some reduction in net photosynthesis was evident after 14 and 21 days and consequently, this particular stress

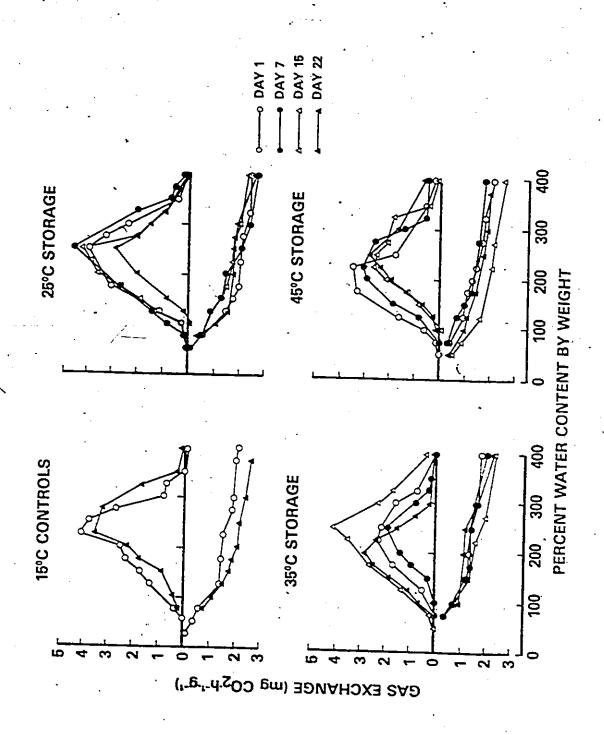
T

Figure 16

The effects on net photosynthesis and respiration (mg CO₂·h⁻¹·g⁻¹) as a measure of thermal stress in <u>Peltigera canina</u> var. <u>praetextata</u>. Thallus saturation is expressed as a percentage of the final oven dry weight' of each replicate.



The effects on net photosynthesis and respiration (mg CO₂·h⁻¹·g⁻¹) as measure of thermal stress in <u>Peltigera canina</u> var. <u>rufescens</u>. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



treatment was extended for an additional 11 days, when a further marked decline of net photosynthesis was observed (Figure 16). Conversely, respiration was largely unaffected by the temperature stress with the exception of the replicates stored at 45°C where, after 21 days, there was a substantial decrease from maximum values of c. 2.0 mgCO₂·h⁻¹·g⁻¹ to 0.8 mgCO₂·h⁻¹·g⁻¹. While the results for P. canina var. rufescens are somewhat more variable (Figure 17) net photosynthesis and respiration remain essentially unaltered after 22 days at all storage treatment temperatures.

(3.3) Light Stress and Light-Mediated Seasonal Adjustment of Net Photosynthetic Capacity

(3.3.1) Light Stress in Peltigera aphthosa

A previous casual observation on apparent chlorophyll photooxidation in thalli of Peltigera aphthosa stored air-dry under a 12 hr photoperiod. of c. 300µE m⁻²s⁻¹ and a constant temperature of 15°C for several weeks pointed to light as a possible stress factor in some lichens. Accordingly, the effect of light stress on pitrogenase activity was examined under controlled conditions (see section 2.4.1) and the results are given in Figure 18. Rates of nitrogenase activity for Peltigera aphthosa after 6 days under a 12 h photoperiod of 450-600µE m⁻²s⁻¹ and a constant 15°C are maximal at c. 1.5 mmols C₂E₄·h⁻¹·mg⁻¹. Thereafter, nitrogenase activity declined to c. 1.0 mmols C₂E₄·h⁻¹·mg⁻¹ by day 14 and by day 30 rates had declined still further to 0.5-0.6 mmols C₂E₄·h⁻¹·mg⁻¹. This gradual decline in fitrogenase activity was accompanied by a progressively bleached appearance of the experimental replicates over

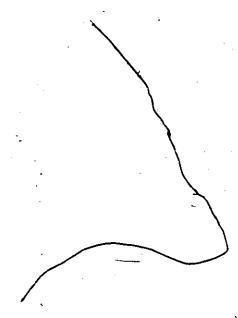
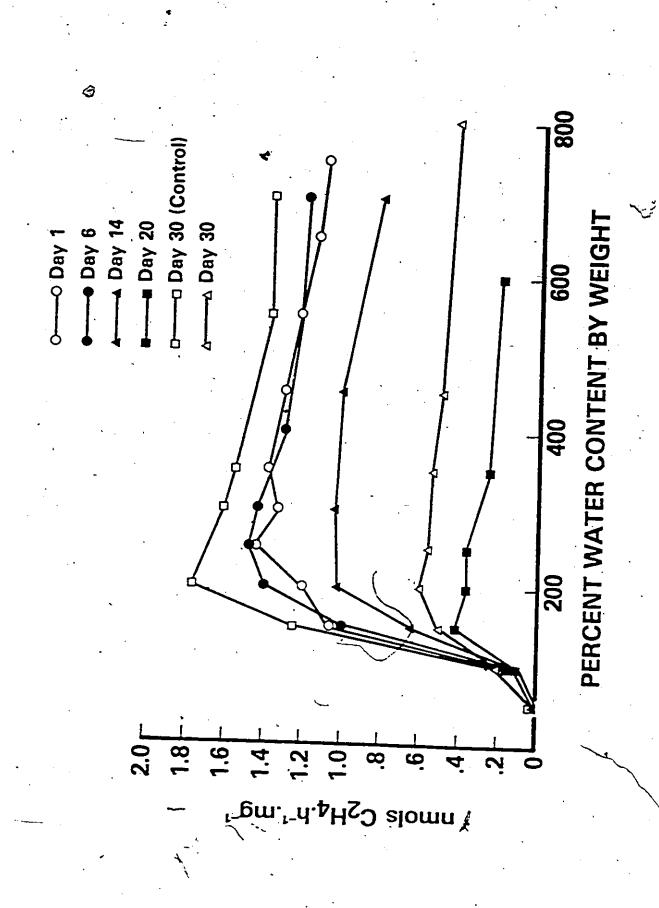
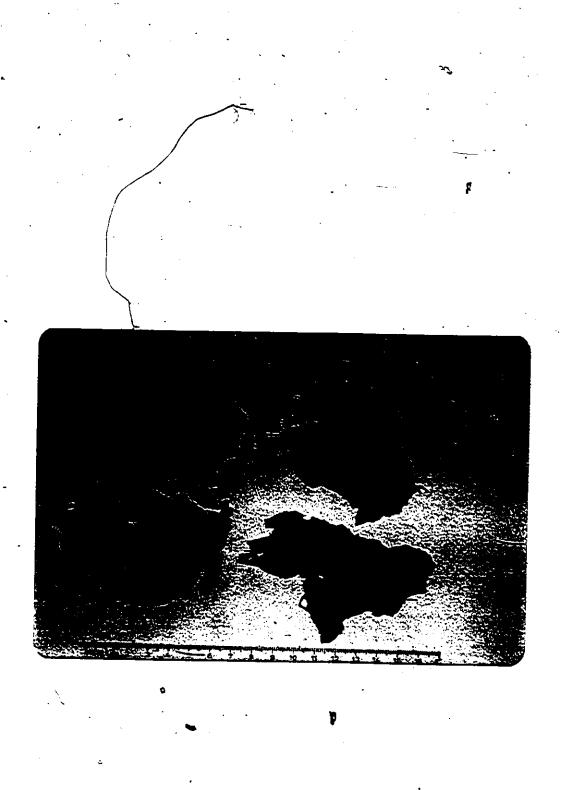


Figure 18

The effects on nitrogenase activity (nmols C₂H₄·h⁻¹·mg⁻¹) as a measure of light stress in <u>Peltigera aphthosa</u>. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



The visible effects of light stress on air-dry thalli of <u>Peltigera</u> aphthosa stored for 30 days under a 12 hour photoperiod of $450-600\mu E$ m⁻² s⁻¹ and a constant $15^{\circ}C$ (right). Control replicates were stored air-dry at an equivalent temperature and photoperiod but at $25\mu E$ m⁻² s⁻¹ illumination.



the time series (Figure 19). Control replicates kept under 25µE m⁻²s⁻¹
light regime and 15°C day/night temperatures were unaffected both in their appearance and their rates of acetylene reduction (Figure 18).

(3.3.2) Light Mediated Seasonal Adjustments of Net Photosynthetic Capacity in Peltigera canina var. praetextata

The rates of both net photosynthesis and respiration observed during the thermal stress experiments on P. canina var. praetextata (section 2.4.3) were considerably different than published values for this species under comparable experimental conditions (see Kershaw, 1977b, c). Unfortunately, Kershaw only collected his material during November-December or April-May and never under full canopy conditions as was the experimental material for the above investigations. The marked differences in the two data sets appeared to bestrongly correlated with the rather distinct light environments to which the experimental material had been exposed and under which it had been collected. It was hypothesized that some adjustment in the capacity of net photosynthesis must take place in concert with the substantial changes in the spectral distribution of incident radiation on the forest floor that occurs annually during leaf fall. It was thus essential to document the rates of net photosynthesis and respiration for P. canina var. praetextata before, during and after leaf fall. Accordingly, experimental material was collected and tested during the first week of September and October, during mid-October and then at

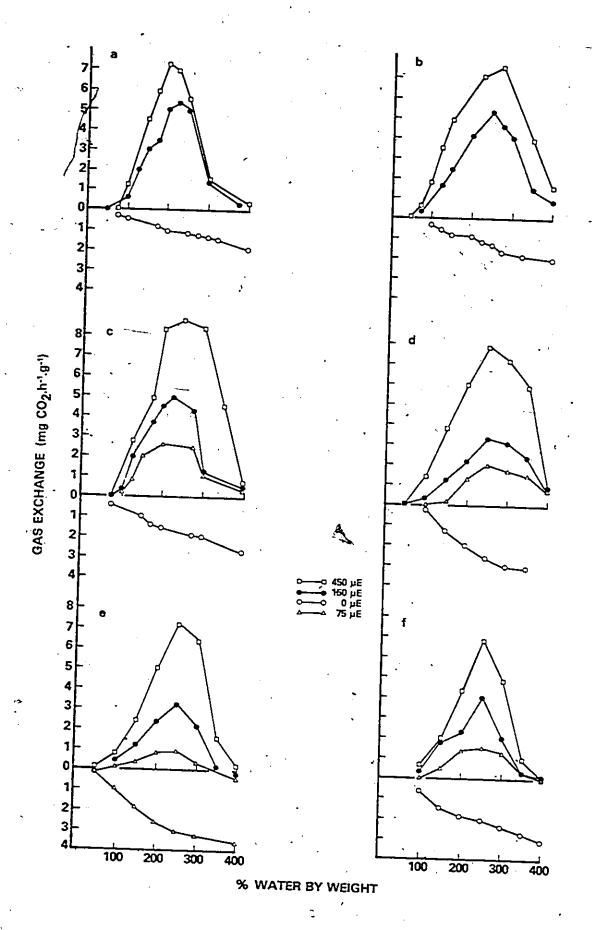
weekly intervals during the last two weeks of October and the first two weeks in November to accommodate the rapid changes that were taking place in the canopy. The results of these experiments are given in Figure 20). Three basic and important points emerge from these data:

First, the rate of respiration increased from September to November which was reflected in and largely responsible for the changing overall pattern of net photosynthesis. At maximum saturation, mean maximal rates of respiration for September 4, October 1, October 19, October 27, November 3 and November 11 were 1.9, 2.2, 2.7, 3.1, 3.6 and 3.2 mg $C0_2 \cdot h^{-1} \cdot g^{-1}$ respectively (Figure 20). Throughout this experimental work, standard errors of the means were in the range of 0.5-0.8 mg $c0_{2} \cdot h^{-1} \cdot g^{-1}$. The mean rate of net photosynthesis at maximum saturation, on the other hand, was just above the compensation point for the September 4, October 1, October 19, and October 27 collections for all light levels. However, as the rate of respiration continued to increase, the rate of net photosynthesis became respiration dominated andwas not compensated at any light level for both the November 3 and November 11 collections. The rates and patterns of net photosynthesis and respiration at maximum saturation would now be comparable to those reported by Kershaw (1977b, c) who noted mean maximal rates of respiration of 4.0 $\mbox{mgCO}_{2} \cdot \mbox{h}^{-1} \cdot \mbox{g}^{-1}$ and heavily depressed rates of net photosynthesis at maximum saturation for experimental replicates collected during early December.

Secondly, this seasonal trend in the rate of respiration was matched by concurrent trends in the rates of net photosynthesis at the lower light levels of 75 and 150 μ E m⁻²s⁻¹ but not at 450 μ E m⁻²s⁻¹.

Seasonal adjustments of net photosynthetic capacity (mg CO₂·h⁻¹·g⁻¹) for <u>Peltigera canina</u> var. <u>praetextata</u>. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.

Collection dates were as follows: (a) September 4, 1978; (b) October 1, 1978; (c) October 19, 1978; (d) October 27, 1978; (e) November 3, 1978; (f) November 11, 1978.



This suggests that only some of the adjustment in the rate of net photosynthesis can be explained by concurrent changes in the rate of respiration. Mean maximal rates of net photosynthesis at a quantum flux density of 150 μ E m⁻² s⁻¹ for September 4, October 1 and October 19 were 5.3, 5.2 and 4.9 mgCO₂·h⁻¹·g⁻¹ respectively. Thereafter, mean maximal rates of net photosynthesis declined sharply and on October 27, November 3 and November 11 were 3.3, 3.3 and 3.9 mgCO2.h-1.g-1 respectively. These values are regarded as typical rates of net photosynthesis for this time of year (cf. Kershaw, 1977b, c). This abrupt decline in net photosynthesis was correlated with complete leaf fall, which occurred between October 19 and October 27. The pattern of response at 75 μ E m ${\rm m}$ s illumination is again similar but is not as marked (Figure 20). Mean maximal rates of net photosynthesis declined from 2.5 and 2.0 $mgCO_2 \cdot h^{-1} \cdot g^{-1}$ on October 19 and 27 respectively to 0.9 and 1.5 mgC- $_2$ ·h⁻¹·g⁻¹ on November 3 and 11. Under 450 $_{
m LE}$ m⁻²s⁻¹ illumination, the mean maximal rate of net photosynthesis remained very high and relatively constant. Values were observed in a range from 6.8 mgCO₂·h⁻¹·g⁻¹ to 7.8 $mgCO_2 \cdot h^{-1} \cdot g^{-1}$ for all collections except on October 19 when an exceptionally high mean rate of 8.7 $mgCO_2 \cdot h^{-1} \cdot g^{-1}$ was recorded (Figure 20).

Thirdly, it should be noted that these very high rates of net photosynthesis, particularly at a quantum flux density of 450 and 150 μ E m⁻²s⁻¹, have not been reported previously and are exceptional in this regard. However, only a portion of these elevated rates of photosynthesis can be attributed to a decreased rate of respiration (see below).

Recent evidence by Kershaw (1977b) has shown the existence of thermal acclimation in <u>P. canina</u> var. <u>praetextata</u> to temperatures which corresponded to equivalent ambient temperature levels in the field.

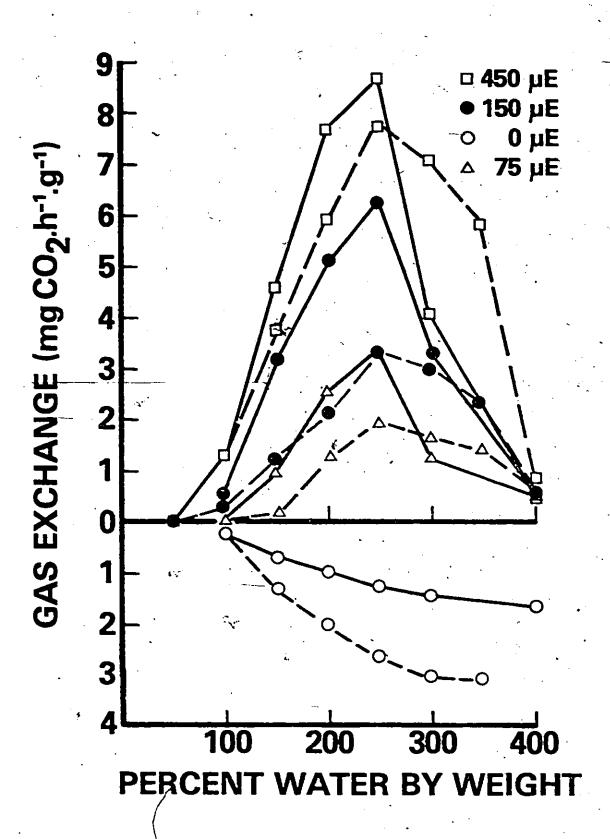
This temperature acclimation of net photosynthetic optima was achieved without any significant change in respiration rate and showed a remarkable degree of homeostasis during spring, summer and early winter. Kershaw (1977c) further showed that this thermal acclimation could be induced experimentally under controlled conditions in the laboratory but only during the spring and fall. Thus, there appeared to be a strong seasonal component to the direction of the induced thermal acclimation of photosynthesis in that low temperature acclimation could not be induced during mid-summer. Similarly, high temperature acclimation could not be induced during mid-winter.

Therefore, taking into consideration the high level of physiological plasticity of P. canina var. praetextata exhibited at this time of year, an attempt was made to induce an adjustment of net photosynthetic capacity in the laboratory. Lichen material collected on October 27 that had been exposed to naturally high levels of incident radiation was examined for its rates of net photosynthesis and respiration (Figure 20) and then stored for two weeks at a constant temperature of 15°C and a 12 hr photoperiod of $25\mu E$ m⁻²s⁻¹ which effectively simulated the low light full canopy conditions that prevailed a few weeks earlier. This was also a realistic light storage treatment in another sense; clones of P. canina var. praetextata growing on the forest floor are frequently covered with fallen leaves or a light snowfall for brief periods during this time of year. Fourteen days

later, net photosynthesis and respiration were examined as before at radiant flux densities of 450, 150, 75 and 0 μE m $^{-2}$ s $^{-1}$

After two weeks under the experimental storage conditions (15°C, 25 μ E m⁻²s⁻¹), the rates of net photosynthesis and respiration changed markedly except under $450\mu\text{E}~\text{m}^{-2}\text{s}^{-1}$ illumination where the rate remained at c. 8.0 mgCO₂·h⁻¹·g⁻¹ (Figure 21). Mean maximal rates of net photosynthesis under $75\mu E m^{-2} s^{-1}$ illumination increased from 2.0 to 3.4 mgCO₂-h⁻¹-g⁻¹ while under $150\mu\text{E m}^{-2}\text{s}^{-1}$ illumination, the equivalent figures were 3.3 to 6.2 mgCO2.h-1.g-1. Rates of net photosynthesis under 150 μ E m $^{-2}$ s $^{-1}$ illumination had returned to the high values in excess of 5 mgCO₂·h⁻¹·g⁻¹ characteristic of the rates observed both on September 4, October 1, and October 19 (Figure 20) and in the 15° and 25°C storage temperature treatment of the thermal stress series on P. canina var. praetextata (Section 3.2.1., Figure 15). The mean maximal rate of respiration at maximum saturation had decreased from 3.1 to 1.7 $mgCO_2 \cdot h^{-1} \cdot g^{-1}$ and was comparable with the rates observed during early September. The adjustment of photosynthetic capacity had almost doubled the rate of net photosynthesis under $150\mu E$ m $^{-2}$ s $^{-1}$ illumination and nearly halved maximal rates of respiration at maximum saturation. While the effects of this storage treatment (15°C, 25 μ E m⁻²s⁻¹) on subsequent respiration contributed to some increase in net photosynthetic rates, in view of the substantial adjustments in photosynthetic capacity under 75 and $150\mu\text{E}\text{ m}^{-2}\text{s}^{-1}$ illumination (Figure 20, 21), these changes could largely be photosynthetic in nature. This interpretation is reinforced by the constancy of the response at $450\mu\text{E m}^{-2}\text{s}^{-1}$ illumination (Figure 20, 21).

The induction of changes in net photosynthetic capacity (mg ${\rm CO_2} \cdot {\rm h}^{-1} \cdot {\rm g}^{-1}$)' for <u>Peltigera canina</u> var. <u>praetextata</u>. Experimental replicates which had been collected on October 27, 1978 (-----) were stored moist for two weeks under low light conditions ($25\mu {\rm E~m}^{-2} {\rm s}^{-1}$) and reexamined (-----). Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.



DISCUSSION

The environmental control of nitrogenase activity in lichens has not been examined previously in any depth. The lack of a multivariate approach has largely obscured any collinearity between environmental factors and any seasonal adjustments of nitrogenase activity. The work reported here establishes the existence of an innate seasonal rhythm of nitrogenase activity which is quite different to the seasonality of nitrogenase activity described previously by other authors. It would suggest that a considerable degree of metabolic complexity exists in Peltigera which may be adaptive. This level of physiological adaptation may provide for relatively high growth rates which ultimately allow Peltigera to compete successfully with closely associated moss species on the woodland floor or equally to establish and exploit the harsh regime of a roadside environment prior to its final elimination by the slow establishment of higher plants.

Classically, the explanation of the ecology of a lichen has been sought largely in terms of its net photosynthetic response to temperature, moisture and light although other physiological aspects have been considered. Studies by Bliss and Hadley (1964), Adams (1971a, b), Rundel (1972), Lange (1969); Kärenlampi (1971) and Lechowicz et al., (1974) attempted to demonstrate the adaptation of physiological patterns to large scale climatic differences in selected lichen populations. Other investigations such as those by Harris (1972), Lechowicz and Adams (1973, 1974), Larson and Kershaw (1975a, b, c) and Kershaw (1977b, c) also attempted to understand the spatial distribution of lichens based

on photosynthetic patterns but only in relation to small scale microclimatic differentation. Unfortunately, many of these studies were univariate in their experimental approach and many have not adequately demonstrated clear relationships between the distribution of plants, their physiological characteristics and the physical parameters of the environments in which they grow. Rowever, the considerable ecological implications of the concept of thermal stress and equally light stress which have been examined in this thesis provide a superior understanding of the distribution of lichens on both a microclimatic and macroclimatic scale.

The seasonal adjustment of net photosynthetic capacity in <u>Peltigera</u> merely reemphasizes the often quoted but not clearly demonstrated fact that some lichens are particularly well adapted to the environments in which they are growing.

(4.1) The Environmental Control of Nitrogenase Activity

The selection of the appropriate experimental design to demonstrate seasonal differences was extremely important as discussed in Section 1.

Peltigera canina var. praetextata and P. canina var. rufescens were the species best suited to this approach. It was necessary to examine the response of nitrogenase activity in both populations as a function of thallus moisture content, thallus temperature, radiant flux density, the time of year and the influences of snowcover with the experiments arranged factorially. With some simple modifications, the available methods for the examination of the response of nitrogenase activity in lichens to environmental factors proved to be very suited

to a study of this type. Physiological data matrices were produced for each collection. The degree of variability among replicates was large on occassion but clear responses of enzyme activity to environmental influences could be discerned throughout the year and a considerable volume of ecologically interpretable data was obtained.

Although light is a general requirement for nitrogenase activity and there is a marked increase in activity with increasing light levels up to c. $75\mu E m^{-2} s^{-1}$ (Cox and Fay, 1969), in P. canina var. praetextata and P. canina var. rufescens, there is apparently no further marked rate increase at higher light levels of $75-450\mu E$ m s (Figure 2, 3). Other previous studies of the relationship between light and nitrogenase activity in both free living blue-green algae (Stewart, 1965; Fogg and Stewart, 1968; Henriksson et al., 1972; Englund and Meyerson, 1974; Jones, 1977c) and in lichens (Kallio et al., 1972; Kallio, 1973; Hitch and Stewart, 1973; Crittenden and Kershaw, 1979) also show little interaction between high light and nitrogenase activity. Stewart (1974) summarizes the information for the effects of light intensity on terrestrial blue-green algae and states that there is a general but not always a very direct correlation between light intensity and nitrogen fixation in natural ecosystems. Thus the ability to fix nitrogen at low light intensities suggests that this may be an adaptation to shade or darkness. For instance, Kershaw et al. (1977) have found that nitrogenase activity continues in darkness for several hours at temperatures of 15°C and less. They note that in temperate latitudes when periods of darkness are less than 12 hours in duration, nitrogen fixation could continue throughout the night, unimpaired by the absence of light,

given that an adequate carbon reserve is available. At night, moist thalli would not dry further, temperatures typically would be $10^{\circ}-15^{\circ}\mathrm{C}$ and a considerable proportion of the annual nitrogen budget could be accumulated under these conditions. Nevertheless, the ultimate source of reductant for the nitrogenase reaction is derived from photosynthesis which provides a pool of substrates (Stewart, 1977). The subsequent processes, some of which are dark reactions, supply electrons for nitrogenase and thus any factor which regulates the size of this pool may affect nitrogenase activity.

Maximum rates of nitrogenase activity can be sustained at relative moisture contents above 200% for both P. canina var. praetextata and P. canina var. rufescens (Figure 2, 3). Data presented by Larson (1977) suggest that thallus moisture in excess of c. 200% of the dry weight is held as superficial films and droplets. It is probable therefore that rates of nitrogenase activity are dependent upon variation in the mass of water held within the interstices of the thallus but independent of the extent of surface moisture. The response of nitrogenase activity in Peltigera canina var. praetextata and P. canina var. rufescens to variation in thallus moisture content contrasts markedly with that of net CO, exchange (Figure 16, 17) which may often be strongly dependent upon both internal and external water loading. The low diffusivity of CO, in water compared with air has been implicated by Crittenden and Kershaw (1979) as an important rate-limiting factor in very wet thalli. Large quantities of surface water have been noted by these authors to be correlated with depressed CO fixation rates published for a number of species (i.e. Smith 1962; Larson and Kershaw 1975b, c; Kershaw 1977b, c; Kershaw and Smith 1978). However, at maximum saturation, the rate of respiration is also at a maximum and this certainly must be taken into account in any discussion of the apparently depressed rates of net photosynthesis when experimental thalli are completely saturated. An optimum water content may also play a role in the shape of the net photosynthetic response curve. The high partial pressure of C_2H_2 in the gas exchange cells and its high solubility in water probably accounts for the relatively unimpaired rates of acetylene reduction at equally high levels of thallus moisture.

P. canina var. praetextata and P. canina var. rufescens arevery similar both in their water holding capacity and the threshold water content at which nitrogenase ceases. Hitch and Stewart (1973) found similar threshold values for nitrogenase activity in Collema crispum, Lichina confinis and Peltigera rufescens with nitrogenase activity ceasing at water contents of less than 80-90% of the oven dry, weight. Kershaw (1974), investigated the moisture relations of four Peltigera species with respect to nitrogenase activity and found threshold values ranging from approximately 60-100% relative moisture content. Similar threshold values have been reported for Peltigera aphthosa (Kallio et al., 1976) and Stereocaulon paschale (Crittenden and Kershaw, 1979) and this concurs with data presented in the thesis for Peltigera scabrosa (Figure 12), P. canina var. rufescens from Hawley Lake (Figure 13), Stereocaulon paschale (Figure 14) and Peltigera aphthosa (Figure 15, 18). Clearly, there is a minimum moisture requirement for nitrogenase activity in lichens of about 75% of their oven dry weight. This minimum value is independent of temperature, quantum flux density, seasonal variation and habitat preference. The absence of any difference in the pattern of

response of nitrogenase activity to this threshold value in the several.

populations used throughout these experiments is puzzling, particularly in view of their distinct and contrasting habitats.

Maximum nitrogenase activity has generally been observed between 20° and 30°C for a variety of nitrogen-fixing organisms and the data presented for P. canina var. praetextata and P. canina var. rufescens is no exception (Figure 2, 3). Waughman (1977) has found a temperature optimum of 20°-30°C for several leguminous and non-leguminous plants, while Pattnaik (1966) and Fogg and Than-Tun (1960) reported a temperature optimum in the range of $30^{\circ}-35^{\circ}\mathrm{C}$ for free living blue-green algae. Similar optimum temperatures for nitrogenase activity have been reported in a range of lichen species: for example, 20°C in Lichina confinis and 35°C in Peltigera rufescens (Hitch and Stewart, 1973); 15°C in Solorina crocea and Nephroma arcticum (Kallio et al., 1972); 16-21°C in Peltigera canina (Maikawa and Kershaw, 1975); 30°C in Lobaria pulmonaria, Sticta weigellii and Leptogium cyanescens (Kelly and Becker, 1975); 25°-35°C in Stereocaulon paschale (Kallio, 1973; Crittenden and Kershaw, 1979) and 20°-30°C for Peltigera aphthosa (Kallio and Kallio, 1978; Englund, 1978). However, two aspects of the data call for additional comment: firstly, the different pattern of nitrogenase activity at 35°C with respect to moisture and light compared with the pattern at all other temperatures and secondly, the complete absence of any seasonal temperature adjustment of optimal rates. The normal relationship between thallus moisture content and nitrogenase activity at e.g. 5-30°C shows that activity is maintained during thallus drying until approximately 200% thallus saturation. The activity then decreases rapidly. At 35°C however, the activity is not maintained but

falls rapidly in an almost linear fashion. Since this same pattern of response is evident if the experimental replicates are simply held at a constant level of thallus saturation, the rapid linear decrease of nitrogenase activity is interpreted as resulting solely from high temperature stress. A similar deactivation of nitrogenase activity at 35°C has also been reported by Kershaw et al., (1977) and Jones (1977d).

The constancy of the temperature optimum of nitrogenase activity irrespective of the season is surprising in view of the very substantial seasonal adjustment of net photosynthetic temperature optima found in some Peltigera species (Kershaw, 1977t, c). In mid-summer, the temperature optimum for net photosynthesis is at 30°-35°C, in early summer the optimum is at 25°C and/in spring and fall at 15°C, corresponding with the prevailing ambient temperatures. The data presented show no evidence of any seasonal change in the optimum temperature for nitrogenase activity but point to the very, marked temperature sensitivity of the nitrogenase reaction. This is a fact of some considerable and practical importance in field work where the temperature cannot be controlled (see below). The range in temperature employed in these studies covers much of the temperature variation within which nitrogenase operates in nature. Thus the pronounced response to temperature elicited under controlled laboratory conditions suggests that temperature could potentially be a principal rate determinant of nitrogen fixation in the field.

There are considerable changes in the absolute rate of nitrogenase activity at different times of the year. Seasonal variation of nitrogen fixation in lichens has been reported by Hitch and Stewart (1973) and Kallio and Kallio (1975) and in free living blue-green algae

by Horne and Goldman (1972) and Horne and Carmiggelt (1975). All of these studies measured nitrogenase activity in situ and the rate changes were directly related to light, temperature and moisture. Since these environmental parameters have a marked seasonal distribution, there was a corresponding seasonal distribution of nitrogenase activity. However, the data presented in Figures 2 and 3, obtained under rigorously controlled experimental conditions reveal a seasonal pattern of activity independent of the direct effects of the field levels of light, temperature and moisture. This intrinsic rhythm may be ultimately related to environmental patterns throughout a season but not in an immediate and direct sense. The mechanism of this marked rate change is obscure; Harris (1972) has suggested that seasonal changes in algal numbers in Parmelia caperata may be significant but no evidence has been found for seasonal changes in blue-green algal cell numbers in P. canina var. praetextata. There is, however, a considerable body of evidence documenting seasonal changes in plants. The marked seasonal levels of net photosynthesis in sub-arctic lichens (Larson and Kershaw, 1975b, c, d) and temperate lichens (Kershaw 1977b, c) and the equally marked seasonal changes of dark nitrogenase activity in Peltigera polydactyla (Kershaw et al., 1977) are of particular significance as are the seasonal changes of dry weight, carbohydrate content, glucose and asparagine absorption and nitrogen content reported by Smith (1961) for P. polydactyla and the seasonal changes in nitrogenase activity reported for Stereocaulon paschale by Huss-Danell (1977b) and Crittenden and Kershaw (1979). Kershaw (1977b, c) and Kershaw et al., (1977) have emphasized the importance of the supply of endogenous carbohydrate at particular times of the year. The large

increase in endogenous pools of carbohydrate normally seen in higher plants in response to cold temperatures and reduced daylength (Levitt, 1972) may also be evident in lichens. This could, in part explain the seasonal difference both in the response to the addition of glucose and mannitol in the light/dark interactions with nitrogenase activity (MacFarlane et al., 1976; Kershaw et al., 1977) and in the large increases in the winter and spring rates of acetylene reduction for P. canina var. praetextata and P. camina var. rufescens. With an increase of stored carbohydrate, there is presumably more energy available for the nitrogen fixation process both in the light and in the dark.

The very low rates of July 1975 which were especially pronounced in <u>P. canina</u> var. <u>praetextata</u> occurred after a very hot and dry period whereas the equivalent data for July 1976 with much higher rates of nitrogenase activity (Figure 2) were representative of a much cooler and wetter summer. Thus temperatures of 30°C with continuously dry conditions could be largely responsible for the low levels of activity in the summer, effectively representing the high temperature sensitivity of the nitrogenase system (see Section 4.2).

The influence of snowcover and snowmelt on subsequent rates of nitrogenase activity in lichens has largely been ignored and rarely controlled for in both laboratory and field settings. The problem seems to have revolved around the inability of some investigators particularly in the field, to appreciate and/or dissociate the complexity and interrelations of snowcover and the resultant changes in environmental parameters which ultimately control the physiology of the organisms in question. In general, the two fundamental effects of snow cover are to lower the

surface temperatures to c. -5.0 to 0°C and either to eliminate or drastically reduce ambient light levels. The ocurrence of low temperatures will also severely limit the availability of liquid moisture and this, in itself, will severely impair most physiological processes. These considerations must also be integrated over the entire winter period of snowcover which is typically of several weeks in duration at this latitude. Obviously, the relative levels of both temperature and radiant energy under snow will be governed by ambient air temperatures and the depth of the snowpack. For instance, as little as 5cm of powdery snow reduces the level of irradiance to 25% of uncovered surface values (Huss-Danell, 1977b).

The rate of recovery of nitrogenase activity of P. canina var. praetextata and P. canina var. rufescens after snowmelt was, like most physiological processes, very temperature dependent (Figure 5, .6, 7). The constant but insignificant level of nitrogenase activity from dark replicates for both species can be attributed to the light pretreatment prior to the first assay. There is some evidence that the nitrogenase enzyme system is unstable at cold temperatures (Dua and Burris, 1963; Haystead et al., 1970; Kallio et al., 1972; Kallio, 1973), and this has been implicated by Crittenden and Kershaw (1979) in the loss of functional nitrogenase activity in lichens during winter months. These authors have strongly suggested that only low temperatures or prolonged frost episodes are involved in nitrogenase inactivation before snowfall and after snowmelt and this is interpreted as a key factor causing the seasonal vafiation in potential nitrogen fixing activity in S. paschale. While some of the seasonal activity reported by these authors is closely linked with the seasonal distribution of environmental factors, no clear

evidence was presented to suggest that low temperatures per se were Solely responsible for the observed patterns of seasonal nitrogen fixation. Unquestionably, nitrogenase activity will be reduced by low temperatures (Figures 2, 3, 5, 6, 7) providing, of course, that there exists sufficient liquid moisture. In constrast to nitrogenase activity, it has been shown that there is a marked photosynthetic adaptation to temperature in lichens which closely corresponds to the prevailing seasonal temperatures (Larson and Kershaw 1975c, d; Kershaw 1977b, c; Kershaw and Smith, 1978). Again, providing that sufficient liquid moisture is available, net photosynthesis will continue to supply the needed reductant and carbon reserves for the nitrogenase reaction although at probably reduced rates. While the effects of temperature will figure largely in the decline of nitrogenase activity following a thick snowcover, the abrupt elimination of radiant energy is more likely to be the dominant influence. Thus MacFarlane et al., (1976) have shown that nitrogenase activity declines sharply in the absence of light while Kershaw et al., (1977) extended the above work to show that this decline of nitrogenase activity in the dark is temperature dependent. Further, these studies clearly illustrated that nitrogenase activity can continue in the dark for extended periods of time at 5° and 15° C and that dark nitrogenase activity was dependent on aerobic conditions. This strongly implicates respiration as a source of energy in the dark (see Bottomley and Stewart 1977). The immediate physiological impact of a deep snowfall will therefore be an abrupt cessation of photosynthesis concurrent with a sharp decline in nitrogenase activity followed by a more gradual reduction in the rate of nitrogenase activity with time. Low temperatures will reduce the rate of respiration to minimal levels

severely reducing the energy supplies to the nitrogenase enzyme. Integrated over a period of several weeks under snow, very little activity of the three basic processes of photosynthesis, respiration or nitrogenase activity will be in evidence for P. canina var. praetextata and P. canina var. rufescens until early spring. This is corroborated by previous studies indicating that nitrogenase activity in lichens beneath snowbeds is completely suppressed (Alexander and Kallio, 1976; Huss-Danell, 1977b; Crittenden and Kershaw, 1979) or that activity beneath snow was below the sensitivity of the assay techniques employed (Englund and Mayerson, 1974). Following snowmelt, physiological reactivation in the lichen will ensue rapidly. The recovery rate of nitrogenase activity will be impeded by prevailing low temperatures (Figure 6, 7) and continued intermittent frost, a view also shared by Kallio et al., (1976), Alexander and Kallio (1976) and Crittenden and Kershaw (1979). That reestablishment of activity can be rapid suggests that the mechanism involved could largely be one of enzyme synthesis or light activation of the enzyme or both and may not be due to an increase in the numbers of the blue-green phycobiont (cf. Harris, 1971).

The effect of snowcover on seasonal nitrogenase activity in P. canina var. praetextata and P. canina var. rufescens was not discovered in the earlier phases of the work because neither of these species was collected from under the snow. December collections were made before the first snowfall to facilitate the selection of the experimental material. The only "winter" collection was made in March 1976 for P. canina var. praetextata and this followed a two week

thaw which exposed collected lichen clones to moderately cool temperatures, full saturation and full daylight.

Clearly, the investigation of the environmental control of nitrogenase activity demands as a prerequisite a laboratory based multivariate approach. The lack of control in field studies of, for instance, temperature and previous thallus history (MacFarlane et al., 1976) could account for the discrepancies in the reported temperature optima for nitrogenase activity in lichens. Field studies of nitrogenase activity must be coupled with more stringent determinations under controlled conditions in the laboratory. The seasonal components of nitrogenase, comprising both an endogenous and exogenous rhythm, will contribute very significantly to the marked variation in the absolute rates of acetylene reduction for a single species reported in the literature. Thus some of these published rates of nitrogenase activity and the estimates of total nitrogen fixed per year are of questionable value. Rarely have previous studies considered the multivariate nature of the environmental control of nitrogenase activity nor has a proper conversion factor been determined. It has become customary to use a conversion factor of 1/3 N_{2} reduced per acetylene reduced based on the fact that acetylene reduction is a 2 electron and N_2 reduction a 6 electron transfer. However, experimentally a value of 1/3 seldom, if ever, is found. Values of the ratio reported in the literature vary widely (1/4-1/3) so it is apparent that no standard conversion value is reliable for interpreting acetylene reduction in terms of nitrogen fixation. The primary reason for the variable conversion factor appears to be related to the unusual

efficiency of electron transfer to acetylene as employed experimentally (see Burris, 1974 for an account). Unless a conversion factor is determined under the specific experimental conditions it is unwarranted to attach quantitative significance to the nitrogen fixation rates based on acetylene reduction alone.

(4.2) Thermal Stress as an Ecological Factor in Lichens

Temperature has long been recognized as having a most pronounced influence on plant distributions largely through its effect on the availability of moisture and its relation to physiological and biochemical reaction rates; this subject has been adequately treated in several publications (Watts, 1971; Larcher, 1975; Kellman, 1975; Bannister, 1976 etc.), the details of which need not be elaborated here. An important consideration in plant distribution mechanisms however is the concept of thermal extremes since they may not only limit physiological processes but may also cause the death of the whole or part of the plant and thus either completely eliminate it from a particular habitat or reduce its competitive vigour. Consequently, a study of the resistance of plants to, for instance, high temperatures provides insights into their ecology.

The apparent high temperature sensitivity of the nitrogenase enzyme and the sensitivity of <u>P. canina</u> var. <u>praetextata</u> to continuous but moderate levels of thermal stress necessitated an examination of these factors to assess their role in controlling the distribution of the species. The preliminary observations of thermal stress confirmed

the interpretation of the low summer rates of nitrogenase activity in Peltigera but also pointed to thermal stress as an ecological parameter of considerable importance. The literature documenting the effects of thermal stress on plants is extensive and has been reviewed by Levitt (1972), Kappen (1973) and Alexandrov (1977). However, the published works pertaining to the lichens and mosses (Lange 1953, 1955, 1959; Biebl 1967e; 1968) which have been widely acclaimed and acknowledged have largely obscured the ecological significance of thermal stress as an ecological factor (see below). Historically, the problem has always revolved around the choice of an exact criterion which could be used to measure the effects of heat stress on plants in some absolute way (Levitt, 1972). Further difficulties have also arisen over the method of application and duration of the heat stress period. Typically, stress temperatures of 70° - 100° C have been used on the lichens for a duration of 30 minutes followed by assessment of the heat injury several days later. . Thus, the temperature of a 30 minute heat treatment that suppressed respiration intensity in the lichens by 50% was chosen as a criterion for the assessment of thermal injury. Consequently, Lange (1953) showed a correlation between the thermal resistance of lichen species from the exposed Fulgensietum continentale, characteristic of open, dry, hot places in southwest Germany which tolerated up to 100°C. This result contrasted with that obtained for the mountainous epiphytes (Usneetum barbatae) which were already severely damaged at around 75°C. The actual stress temperatures that were used were all highly unrealistic ecologically and probably seldom occur naturally. Thus, in the air-dry state, the heat resistance of most lichens has been summarized by many

authors (e.g. Kappen, 1973; Farrar, 1973; Hale 1974; Raven et al., 1976) as "extreme and exceptional" or "poikilohydric plants in this state are insensitive and can survive any temperatures occurring naturally on the earth", (Larcher, 1975). On the other hand, hydrated lichen thalli do not differ from other kinds of plant tissue; their limits of heat resistance range from 35° to 46°C (Lange, 1953). Alexandrov (1977) has extended the above conclusions and suggests that the dramatic enhancement of the thermostability of dessicated mosses and lichens can readily be explained by the well-known difference in the response of solubilized and dehydrated proteins to thermal denaturation. A heat treatment in the order of 120-150°C is required to denature dehydrated proteins, whereas these same

In contrast, the ecologically-based approach used throughout this thesis has taken any severe decline in one or any of the three basic lichen metabolic processes, (photosynthesis, respiration or nitrogenase activity) over a three to four week stress period as indicative of a thermal stress response. Death may not ensue directly from the stress and recovery may be possible but the resultant reduction of competitive ability sequentially integrated over a field season will lead to the potential elimination of such non-adapted species from a stressful environment. Based on the microclimatic data, experimental stress temperatures were deliberately chosen at a realistic level. Surface temperatures of 45°C in the open are commonly encountered for periods of 3-4 weeks during hot, dry summers. Consequently, the data presented demonstrated the severe loss of metabolic capacity in some lichen species and hence a reduction in their ability to compete at a maximal level

or to survive when stressed by quite moderate temperatures.

The microclimatic temperature measurements on the open roadside indicated maximum surface temperatures of c. 60°C which agrees well with maxima reported by Lange (1953), Rouse (1976) and by Kappen (1973) who has summarized the literature on a great number of measurements of temperature maxima in poikilohydric plants. Kappen concludes that, for exposed boundary layer conditions in temperate climates, temperatures range from 50° - 60° C. While these temperatures may seem at the outset to be excessive and abnormally high, the microclimatic considerations of open and exposed situations can account for these extremes and contrast markedly with the microclimatic features characterizing and northern woodlands. The single largest and most important difference between the two habitats is in their reception of solar energy. The shielding of the plant canopy drastically reduces the incident solar radiation reaching the forest floor to c. 2-5% (Figure 22). The difference in reception of solar radiation at the two distinct surfaces has important ramifications in a number of respects. From the energy balance equation, solar radiation or Q* is partitioned between the latent heat of evapotranspiration LE, sensible heating of the atmosphere H and heating of the subsurface layer G in the form:

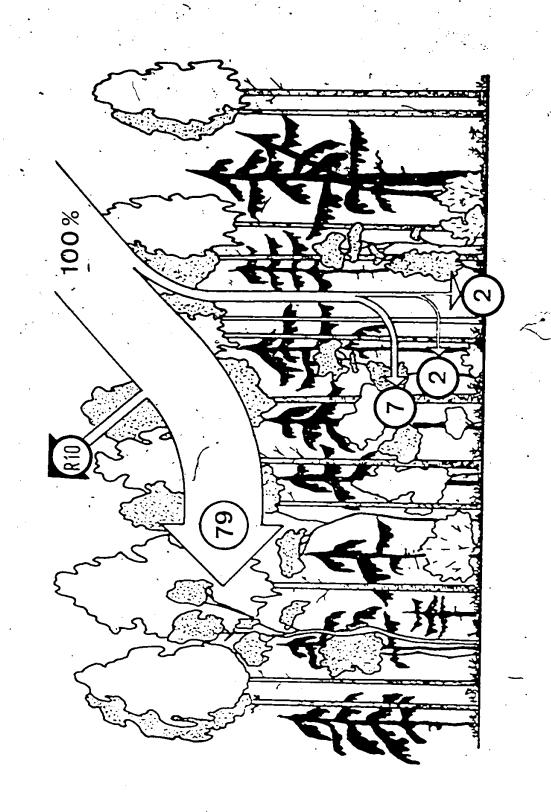
$$Q^* = LE + H + G \tag{1}$$

It is evident that any differential in Q* must be accompanied by an equivalent change in the terms on the right side of equation (1).

A greater absorption of solar radiation in the open exposed areas will create very high surface radiative temperatures and will result in large effective long-wave radiation losses. Rouse (1976) has reported that

Figure 22

The attenuation of radiation in woodland and in adjacent exposed areas. Of the incident photosynthetically active radiation, 10% is reflected (R) from the upper sruface of the canopy. Different amounts of the radiation that penetrates the woodland are absorbed in each layer, depending on the structure. In the forest, the greatest absorption of radiation occurs at the crowns of the trees, while in the open and acceptable areas, maximum absorption incident radiation above the canopy reaches the woodland floor. (After Larcher, 1975).



-,

1

surface temperatures can rise as high as 65°C over fresh burns in the Northwest Territories and diurnal temperature ranges are extreme at up to 45°C. The development of high soil-surface temperatures is enhanced by dry soil-surface conditions and low evaporation rates. Both of these effects lead to a strong sensible heating of the soil. In contrast, less solar radiation will be absorbed in temperate and northern woodlands and there will be a much lower long-wave radiation loss. This/latter feature arises from the shielding of the surface by the plant/canopy and the fact that plants can lessen their radiational heat load by latent and sensible heat losses so that their relative temperatures will remain near the ambient air temperature. Soil temperatures, prior to snowmelt will likely not differ substantially between the two different habitats thus the high soil temperatures which are achieved in the early summer in exposed areas are probable due to a large subsurface heat flux during and immediately after snowmelt. Since winter soil temperatures are much the same, the strong early summer soil heating is matched by a late summer cooling so that over the year, the G term from equation (1) tends to zero (Rouse, 1976). The depth of snow in the two different areas will also be important. There will, on average, be less snow in the open areas than in the woodland and this will result in less meltwater for soil replenishment in the spring. The snow will melt earlier so that the soil is exposed to the evaporative processes sooner. Evaporation rates will initially be high thus depleting the surface soil moisture by early to mid-summer; all of the incoming radiant energy will now be realized in the sensible heat term H of equation (1). Clearly, the sensible heat flux in mid-summer will be considerably greater over exposed surfaces than over the forest floor. Rouse (1976) notes that this would be expected from the very large temperature gradients which develop between these exposed surfaces and the overlying atmosphere.

All biological activity at the exposed surface and within the first few centimeters of soil must the to terms with these temperature extremes. Thus a maximum stress tempeyature of 45°C would not be excessive and is certainly not stressful to populations adapted to high surface temperatures. However, it is highly stressful to some low arctic species or temperate species characteristic of shaded locations. Conversely, above the boundary layer and under shade conditions, temperatures in excess of 35°C are rarely encountered and in this case it is not surprising to find marked stress responses at 45°C. The indications of severe stress at 25° and 35°C correlate well however with the microclimate of some northern shade species (see below).

The degree of thermal sensitivity of the two populations of P. canina, assessed by the examination of net photosynthesis, respiration and nitrogenase activity integrated over a period of 3-4 weeks correlates exactly with their contrasting ecology and microclimatic considerations discussed above. Larcher (1975) notes that the resistance to temperature extremes is an advantage to any plant, but especially to those that must avoid competition; these cannot establish themselves under favourable temperature conditions and are found only in open and therefore, microclimatically extreme habitats. Thus P. canina var. rufescens as a result of its relatively high growth rate (Hale 1974) and its tolerance to high temperatures can establish and fully exploit the harsh roadside

environment prior to its final elimination by the slow establishment of higher plants. Alexandrov (1977) suggests that the biological significance of such differential thermal sensitivity would appear to be that the heat resistant species must possess more thermostable proteins in order to avoid thermal denaturation since the latter would eventually either seiously impair the function of the organism or eventually result in its death. The nature of the damage due to heat stress is obscure but appears to be associated with extensive rupturing of algal cell membranes in P. canina var. praetextata. Leaching of green pigments occurs immediately during subsequent hydration and would be interpreted by Alexandrov (1977) as a lack of conformational flexibility of membrane proteins in this species. The low summer rates of nitrogenase activity observed for P. canina var. praetextata were interpreted as a response to a hot dry summer (MacFarlane and Kershaw, 1977) and the data presented in this thesis adequately confirms this observation. The low summer rates subsequently returned to normal values by early winter; this observation points to a potential for recovery from natural levels of stress.

Stereocaulon paschale, a species of open low arctic spruce-lichen woodland in the Northwest Territories is a dominant component in the successional sequence following forest fire. The thermal sensitivity of S. paschale in the air-dry state is of considerable interest in this regard. The post fire recovery period in lichen woodland has been documented by Maikawa and Kershaw (1976) and it is apparent that there is a delay of c. 60 years before Stereocaulon enters the succession. Kershaw (1977a) has suggested that the relatively extreme surface

microclimate of the burnt surfaces (Rouse, 1976; Kershaw and Rouse 1976; Kershaw et al., 1975) is largely responsible for this long delay in the development of the mature lichen surface of these low-arctic woodlands. Kershaw (1978) has documented air temperatures up to 48°C at a height of 2mm above the crystose lichen Biatora granulosa which colonizes recently burnt surfaces. Conversely, in the open lichen woodland, temperatures only briefly exceeded 40°C but with the usual level of canopy shading, temperatures remained below 30°C (see Kershaw, 1978 for details). Kershaw and Smith (1978) have suggested that the surface temperature of the earlier stages of the succession is too excessive for the survival of S. paschale and its entry into the succession is delayed for 60-80 years during which time the extreme surface microclimate is ameliorated by the following processes: The gradual accumulation of an organic layer which is accompanied by an enhanced retention of soil moisture thus resulting in a substantial reduction of the sensible heat flux (Rouse 1976; Kershaw 1977a). A concurrent tree growth leading to a sharp decrease in solar radiation. The combination of these two processes results in a much cooler average surface temperature which can now be successfully colonized by S. paschale. Thus the long successional sequence leading to the final establishment of Stereocaulon woodland is seen by Kershaw and Smith (1978) as primarily a function of the thermal sensitivity of Stereocaulon.

These microclimatic observations correlate extremely well with the response of nitrogenase activity to thermal stress for <u>S. paschale</u> (Figure 14). Kershaw and Smith (1978) and MacFarlane and Kershaw (1979) have shown that <u>S. paschale</u> cannot maintain its photosynthetic capacity

after even 12 hours of 45°C stress and thus it is not likely that it, would survive in a surface regime where temperatures in excess of 45°C have been recorded. Similarly, the long term stress effects which are evident at 35°C offer a reasonable explanation for the 60-80 year delay of the appearance of Stereocaulon into the succession following forest fire. The slow establishment of Alectoria ochroleuca and A. divergens which are important components in the final development of lichen heath in the low arctic has also been attributed to an ameliorated surface microclimate (see Kershaw and Smith, 1978 for details).

The extreme thermal sensitivity of Peltigera scabrosa, a species which is restricted to closed canopy conditions in low arctic woodland. was initially surprising but in retrospect strongly correlates with the observed distribution of this species. The low arctic closed canopy spruce-moss woodland represents the final successional stage in the recovery following forest fire (Maikawa and Kershaw 1976). Temperatures will rarely be above 20°C and the observed sensitivity to a thermal stress of only 25°C (see also MacFarlane and Kershaw, 1978, 1979) presumably will limit Peltigera scabrosa to these cool shaded conditions. Conversely, the thermal resistance of Peltigera aphthosa, a species which grows actually intermingled with P. scabrosa was again unexpected. However, it does enter the successional sequence after fire much earlier than P. scabrosa where it initially becomes established in the vicinity of seedling spruce trees. Thus, it may have to withstand ambient temperatures of 35°C under periodic full radiation conditions and apparently retains this level of thermal tolerance in mature closed canopy woodland while losing

its ability to tolerate even moderate levels of illumination (see below).

Physiological plant ecology is concerned fundamentally with the modification of plant metabolic processes by the fluctuating external influences of the environment. During the last two decades, a considerable amount of work has been done in the autecologicalphysiological field of lichens and a great bulk of information has accumulated. The experimental approaches employed have attempted to explain the ecology of a lichen in terms of its net photosynthetic (and to a lesser extent nitrogenase) response to temperature, moisture and light (e.g. Stalfelt, 1939; Ried, 1960; Lange, 1965; 1969; Harris, 1969, 1972; Lechowicz and Adams, 1973, 1974; Lechowicz et al. 1974; Larson and Kershaw 1975b, c, d; Kershaw 1975, 1977b, c etc.). Despite the absence of a unified approach in experimental design or the lack of control of, for instance, seasonal acclimation of net photosynthesis in lichens (Stalfelt 1939; Larson and Kershaw 1975a, b, c; Kershaw 1977b, c), the collective results represent a remarkably similar set of multivariate responses of net photosynthesis for a considerable array of species. It would seem that the level of differences that has been reported has not on most occasions, been sufficiently large to explain more fully the ecology of a species. The similarity of responses suggests that perhaps more intensive physiological or, in fact, biochemical investigation is required to best dissect the excessively complex multifactorial nature of ecological interrelationship. A neglected and alternative avenue involves the examination of the resistance to

thermal extremes (both high and low temperatures) ranging from complete thermal tolerance to extreme thermal sensitivity; this presents another continuous variable which considerably advances the understanding of the physiological ecology of lichens on several geographical scales. For instance, the interpretation of the physiological ecology of lichens ranging from the tropics to the high arctic becomes more tenable in light of the dominant nature of thermal stress as an ecological parameter while the responses of net photosynthesis, respiration and nitrogenase activity to moisture, temperature, light and season within which ecologists have been attempting to seek a complete explanation of a lichen's ecology may, in fact, play an inferior role. However, although thermal tolerance may be a dominant factor, other attributes of the metabolic processes of the lichens in question will most certainly be involved.

From the data presented in this thesis, it appears that the most sensitivite indicator of thermal stress is photosynthesis, the capacity for nitrogenase activity being maintained much longer. Respiration is much more resistant still to heat stress with rates only being affected by the most extreme temperatures used and then only after a integrated period of 2-3 weeks. These observations concur with comments by Larcher (1975) who notes that photosynthesis is particularly vulnerable to heat stress while respiration is disturbed primarily by cold temperatures. Moreover, since a lichen is approximately 90% fungal tissue on a dry weight basis, respiration can be considered to be thus largely fungal in origin. Thus the mycobiont would appear to possess considerably greater tolerance to thermal stress than the algal component.

Either the resistance of the respiratory process to heat or the differential thermal resistance of the mycobiont and phycobiont or both may well account for the very extreme temperatures required by Lange (1953) to obtain any stress response at all from the many lichens he examined simply because thermal stress was being assessed by measuring the rate of respiration only.

(4.3) Light as an Ecological Factor in Lichens

The effects of moderate levels of illumination on nitrogenase activity of air-dry thalli of Peltigera aphthosa were very surprising indeed in view of recent statements by Raven and Curtis (1970) and Raven et al., (1976) who have summarized the biology of lichens in the following manner: "when the lichen dries out, photosynthesis ceases and in this state of suspended animation, blazing sunlight or great extremes of heat and cold can be endured". The decline of nitrogenase activity was paralleled by a similar response in net photosynthesis; Kershaw and MacFarlane (1979) showed that the rate of net photosynthesis for P. aphthosa was barely compensated after 21 days under the experimental stress conditions (15°C; 450-600µE m⁻²s⁻¹) while respiration remained unaffected. As noted previously, P. aphthosa enters the succession at Hawley Lake, Ontario following forest fire at a comparatively early stage but it is also very abundant on the floor of the mature and adjacent spruce-moss woodland where it grows intermingled with Peltigera scabrosa and the moss Ptilium crista-castrensis. It has been demonstrated (Kershaw and MacFarlane, 1979) that the general level of thermal

tolerance for P. aphthosa remains essentially unchanged despite the gross microclimatic differences between the contrasting surfaces (see Section 4.2). The independence of the spatial and temporal considerations of the thermal sensitivity of this species contrasts, in part, with the effects. of radiant flux density. Since P. aphthosa initially becomes established on recently burnt surfaces in the vicinity of seedling spruce, it may periodically be necessary to tolerate full radiation conditions. In contrast, light levels in the mature spruce-moss woodland will probably range from c. 25-100 μ E m⁻²s⁻¹, and levels of illumination higher than this will result in progressive photooxidation and bleaching of thalli of P. aphthosa in the air-dry state. It should be emphasized here that the lichen thalli wore at very low levels of thallus moisture content and the results must be qualified in these terms (see below). Similarly preliminary evidence suggests that the closely associated moss, Ptilium crista-castrensis; is substantially more light sensitive than P. aphthosa (Kershaw and MacFarlane, 1979). This agrees with comments by Levitt (1972) who notes that the most extreme sensitivity to light is shown by the shade plants belonging to the lower group of plants, the algaes and mosses. The relatively low but constant rates of nitrogenase activity that were in evidence even after 30 days under moderate light stress levels point to an potential for recovery in P. aphthosa. Unfortunately, a moist series was not run in parallel. This would have been of considerable interest since these experimental results would provide additional data on the extent and rapidity of the injury or the possibility of complete repair, recovery and adaptation to the elevated light levels.

The detrimental effects of growing higher plants from shaded habitats in strong light including the destruction of chlorophyll and changes in the chloroplast morphology have been well established (Gauhl 1969, 1970; Montfort, 1950; Munding, 1952) but to this author's knowledge no previous physiological data exists which illustrates the concept of light stress as an important environmental factor in the ecology of some lichens. The physiological and morphological protective mechanisms separating the sun and shade races of P. aphthosa, are at present, obscure. Pigmentation has been interpreted as being a protection against high levels of incident radiation (Bifter, 1901; Galloe, 1908; Laudi et al., 1969) while Hampton (1973), showed differential amounts of the photosynthetic pigments chlorophyll a, phycocrythrin and phycocyanin between sun and shade forms of P. canina. Morphologically, the thickness of the upper cortex has also been implicated in influencing the light intensity reaching the alga and hence is regarded as a protective mechanism against chlorophyll oxidation. For instance, the same lichen species growing in full sunlight developed an upper cortex about twice as thick as that of a specimen from a shaded habitat as shown by Bitter (1901) for Hypogymnia physodes, Tobler (1925a) for Xanthoria parietina, Galun (1963) for Buellia canescens and Looman (1964) for Lecanora reptans. Ertl (1951) extended these investigations and demonstrated a relationship between cortex thickness and habitat for Peltigera praetextata, Peltigera canina and Solorina saccata. In view of these observations, it would be of interest to examine aspects of pigmentation in the sun and shade races of both Peltigera-aphthosa and P. canina. Equally, an ultrastructural

study and a more rigorous examination of light stress in the two ecotypes for both species might be enlightening.

The seasonal adjustment of net photosynthetic capacity in Peltigera canina var. praetextata correlates extremely well with concurrent changes in the canopy and the subsequent changes in the radiant energy spectrum incident on the forest floor. Under full canopy, low light conditions, very High maximal rates of net photosynthesis were observed and were accompanied by low maximal rates of respiration. In concert with leaf fall and the opening of the canopy, maximum rates of net photosynthesis declined to approximately one-half of the previous rates while the maximum rate of respiration increased almost two-fold. It should be borne in mind that the relationship between these two processes is not linear, i.e. a doubling of the respiration rate does not necessarily result in the reduction of net photosynthesis by a factor on one-half. The existence of photorespiration (CO, compensation point $\tau=50-70$ ppm CO₂ at 25°C and 300 μ E m⁻²s⁻¹) and the non-linear and differential responses of net photosynthesis and respiration in relation to thallus moisture content negate any comparison of this kind. However, the high rates of net photosynthesis observed under full canopy conditions, can, in part, be attributed to a decrease in the rate of respiration. Harris (1969) has shown the existence of a greater photosynthetic efficiency (based on respiratory changes) in tree base forms of Parmelia physodes and regards this as a positive adaptation to growth under shade conditions. Conversely, Hampton (1973) has found that concentrations of the photosynthetic pigments phycoerythrin, phycoyanin and chlorophyll a were

substantially higher in specimens of P. canina collected under dense shade than sun specimens of <u>P. canina</u> growing on an open roadside.

Beneath the full canopy and thus in green-rich (or red deficient) light or in light of low intensity, synthesis of phycoerythrin which absorbs maximally in the green region of the spectrum is stimulated (e.g. Holm-Hansen, 1968; Haury and Bogorad, 1977). Recent evidence by Scheibe (1972) indicates that a phytochrome-like pigment may regulate the responses of blue-green algae to light quality.

The correlation between the magnitude of the rates of net photosynthesis and respiration and the light environment under which the plant was either grown or exposed has been observed previously. For example, Björkman (1968), in a study of sun and shade species representing different taxonomic groups of herbaceous plants, found that the shade plants exhibited low dark respiration rates and low compensation points compared with the sun species, which showed dark respiration rates 5-8 times higher and comparatively higher compensation points. Berry (1975) also supports the contention that leaves of plants developing in high light have high rates of respiration. Patterson (1975) in a study of photosynthetic acclimation to irradiance in Celastrus orbiculatus noted that the low irradiance plants had respiration rates less than half as great as the high irradiance plants. The lower respiration rates in the plants grown under low illumination contributed to the higher net photosynthetic rates of such plants when they were measured at low light intensity. The reduced respiration rate was also reflected in the low light compensation values for these plants. With high irradiance during growth, the respiration rate

increased and this resulted in higher light compensation values. Harris (1969) found some evidence for seasonal variation in respiration rates for Parmelia caperata and P. physodes which appeared to be strongly correlated with changes in the canopy. He hypothesized that these changes could be attributed to a prolonged period of high photosynthetic activity brought on by high water availability and high light intensity before the canopy closed. In this regard, it is completely analogous to the situation described here for P. canina var. praetextata.

The laboratory-induced increase in net photosynthetic capacity in P. canina var. praetextata illustrated a remarkable degree of physiological plasticity. Maximal rates of net photosynthesis were almost doubled while maximal rates of respiration (at maximum saturation) were reduced by a factor of approximately one-half. Thus, the rates of both net photosynthesis and respiration were comparable with those observed earlier under low light full canopy conditions but the induced physiological changes occured in a considerably shorter period of time. Unfortunately, the induction experiment involving net photosynthetic capacity was only performed once and only after the experimental material had been stored at low light (25 μ E m⁻²s⁻¹) for two weeks. The shortage of experimental material and above all, the lack of time precluded any other approach. It would be of interest to determine the rapidity of the response and the extent of any seasonal limitations. This undertaking would involve the examination of the rates of net photosynthesis and respiration in the spring after snowmelt, during bud break and on into full canopy development. A pigment analysis and a documentation of the changes in the spectral distribution on the forest floor might also prove to be

enlightening. The entire investigation would then, of necessity, require repetition during the fall. While no comment can be made regarding the rapidity and the seasonal limitation of the adjustment in net photosynthetic capacity in P. canina var. praetextata, there is some evidence to suggest that such physiological changes can be expeditious in other species with both seasonal and directional restrictions. Kershaw and MacFarlane (1979) discovered an identical response in Peltigera scabrosa. Replicate air-dry thalli which had been stored at high light levels $(300\mu E m^{-2} s^{-1})$ and a constant temperature of 15°C were subsequently stored at low levels of irradiance (25 μ E m⁻²s⁻¹) at the same temperature. Maximal rates of net photosynthesis doubled while maximum rates of respiration also increased almost two-fold when compared to the rates observed for the controls. Experimental replicates were subsequently returned to the high light treatment whereupon the rates of net photosynthesis and respiration reverted to the previous control levels. These changes occured in a period of 2 days. Kershaw (1977b, c) has observed that P. canina var. praetextata can acclimate its net photosynthetic optimum to a range of temperatures in either direction at critical periods during the spring and fall. These rapid alterations in net photosynthetic optima were subsequently induced under laboratory conditions within 24 hours and without any concurrent changes in the rate of respiration. In the case of white clover, lowering the illumination from 70 to 11 W/m^2 resulted in a gradual drop of the respiration rate to less than half its original level (McCree and Troughton, 1966). Raising the illumination to the original level brought the respiration rate back to its initial value. The adaptations occurred

within 24 hours of the light change and permitted the plants to grow at light levels which were below the compensation point for the unadapted plants but not below that of the adapted plants. Hatch et al., (1969) showed that the photosynthethic rates of fully expanded maize and Amaranthus leaves changed over a period of 6 days on transfer of the plants from low to high light intensity, or vice versa, to become comparable with the rate of control plants grown continuously under the same light intensitiy. In these experiments, changes in the levels of PEP carboxylase and pyruvate dikinase accounted for the altered photosynthetic rates.

Thus it seems that several physiological factors are modified when plants are exposed to light of different quality and level of illuminance. However, there is no consensus of opinion concerning any one specific factor as the prime cause of the altered photosynthetic capacity and its relation to concurrent changes in the rate of respiration. Nevertheless, the adjustment of net photosynthetic capacity to irradiance appears to be straightforward. The ability to adapt to different irradiance levels is clearly an advantage since this results in either the availability of a greater number of habitats or the ability to grow successfully under a continually fluctuating light environment. This may be a strategy that is especially well developed in <u>Peltigera</u> to compensate for the otherwise extremely varied and limiting environmental growth conditions. The maximum rates of net photosynthesis reported here for <u>P. canina</u> var. <u>praetextata</u> are apparently in excess of all of the previously documented rates in lichens (see Kershaw, 1977b, c and

and Kallio and Karenlampi, 1975). These high rates of net photosynthesis are essential since P. canina var. praetextata appears to compete actively with a number of woodland mosses which have rates in excess of 3 mg CO₂·h⁻¹·g⁻¹ (Stalfelt, 1937). For <u>P. canina</u> var. praetextata to be successful in maintaining a relatively high growth rate and to compete successfully with the associated moss species of the woodland floor will demand not only-a continual adjustment of the optimal photosynthetic rate but also a high rate of protein synthesis compared with other lichen species. Hale (1974) notes that it is axiomatic that lichens grow slowly and he presents a number of published records which, taken as a whole, provide some basis for this generalization. However, some of the highest growth rates ever published for lichens have been recorded for Peltigera canina and P. rufescens (Frey, 1959). In view of Frey's work and in light of the substantial physiological data presented in this thesis and in related publications, Peltigera canina is probably an exception to this statement and as a lichen, it may be unique physiologically. This might explain its widespread and continental distribution which ranges from southern Latin America to subarctic Canada (see Hale, 1969).

Section 5.

SUMMARY

1. A multivariate examination of nitrogenase activity was undertaken in two populations of the lichen Peltigera canina collected from deciduous woodland and from an open and exposed xeric roadside in southern Ontario. The responses of nitrogenase activity were examined as a function of thallus moisture, thallus temperature, radiant flux density, the time of year and the influences of snow cover with the experiments arranged factorially to produce a physiological data matrix for each collection. Only a small increase in nitrogenase activity with increasing radiant flux density was evident but a marked temperature optimum at 25° - 30° C was observed for both varieties. This temperature optimum was independent of light intensity, thallus moisture and seasons. Maximum nitrogenase activity could be sustained at relative moisture contents greater than 200% of the oven dry weight. Despite the contrasting habitats, the threshold water content at which nitrogenase ceased was approximately 75% of the oven dry weight and was independent of radiant flux density, temperature, season and habitat preference. Of outstanding interest however were the very large rate changes occurring throughout the year. The seasonal changes in nitrogenase activity were clearly evident for both populations at all temperatures and light levels. The influence of radiant energy was identified as a prerequisite for the initial recovery of nitrogenase

activity in <u>Peltigera canina</u> after snowmelt while the effects of temperature were directly linked to the velocity of this recovery.

- 2. A secondary interaction between seasonal nitrogenase activity and elevated temperatures pointed to the potential importance of high temperatures and the contrasting microclimates of the two respective habitats. Responses to thermal stress in terms of net photosynthesis, respiration and nitrogenase activity were documented for both P. canina var. praetextata and P. canina var. rufescens. Subsequently, thallus temperatures were measured with embedded microthermocouples simultaneously in the two diverse habitats. The differential level of thermal sensitivity correlated exactly with the ecology of these two populations. The examination of thermal resistance was consequently extended to northern populations of Peltigera and Stereocaulon from equally diverse Habitats. Thermal sensitivity was identified as a dominant influence governing the succession of lichen species following forest fire and was found to be strongly correlated with the gross microclimatic changes accompanying this natural process. Furthermore, the studies suggested that thermal sensitivity could be one of the dominant parameters in the ecology of lichens and could equally be important for other poikilohydric plants.
- 3. Dessicated thalli of <u>Peltigera aphthosa</u> were found to be very susceptible to quite moderate levels of radiant flux density as indicated by a progressive loss of net photosynthetic potential which was matched by photooxidation of the chlorophyll of air-dry experimental replicates. Thus the multivariate theme was developed further with the recognition of light stress as another ecological parameter of

considerable importance to some lichens.

4. Seasonal adjustments of net photosynthetic capacity were documented for the woodland variety of <u>Peltigera canina</u>. These alterations were largely manifested in substantial changes in both the pattern and the maximum rates of net photosynthesis and respiration and the strongly correlated with a progressive opening of the canopy with leaf fall. The adjustment of net photosynthetic capacity was subsequently induced in the laboratory. This potential for seasonal, adjustment of net photosynthetic rates was considered to be essential to the success of the organism which must adapt to a continually changing light environment.

REFERENCES

- Adams, M.S. 1971a. Temperature response of CO₂ exchange in Cladonia rangifering from the Wisconsin pine barrens, and a comparison with an Alpine population. Am. Mid. Nat. 86: 224-227.
- Adams, M.S. 1971b. Effects of drying at three temperatures on CO₂

 exchange of <u>Cladonia rangiferina</u> L. Wigg. Photosynthetica 5:

 124-127.
- Alexander, V., and S. Kallio. 1976. Nitrogenase activity in <u>Peltigera</u>

 <u>aphthosa</u> and <u>Stereocaulon paschale</u> in early spring. Rep. Kevo

 Subartc. Res. Stn. 13: 12-15.
- Alexandrov, V. Ya. 1977. Cells, Molecules and Temperature. Ecological Studies 21. Springer-Verlag, Berlin.
- Bannister, P. 1976. Introduction to Physiological Plant Ecology.

 Blackwell Scientific, Oxford.
 - Berry, J.A. 1975. Adaptation of photosynthetic processes to stress.

 Science 188: 644-650.
 - Biebl, R. 1967e. Temperaturresistenz tropischer Urwaldmoose. Flora 157: 25-30.
 - Biebl, R. 1968. Über Wärmehaushalt und Temperaturresistenz arktischer Pflanzen in Westgrönland. Flora B, 157: 327-354.
 - Bitter, E. 1901. Über die Variabilität einiger Laubeflechten und Über den Einflußaußeren Bedingungen auß ihr Wachstum. Jahrb. Wiss. Bot. 36: 421-492.

- Björkman, O. 1968. Further studies on differentiation of photosynthetic properties of sun and shade ecotypes of Solidago virgaurea.

 Physiol. Plant 21: 84-99.
- Bliss, L.C. and E.B. Hadley. 1964. Photosynthesis and respiration of alpine lichens. Am. J. Bot. 51: 870-874.
- Bond, G. and G.D. Scott. 1955. An examination of some symbiotic systems for fixation of nitrogen. Ann. Bot. 19: 67-77.
- Bottomley, P.J. and W.D.P. Stewart. 1977. ATP and nitrogenase activity in nitrogen-fixing heterocystous blue-green algae.

 New Phytol. 79: 625-638.
- Burns, R.C. and R.W.F. Hardy. 1975. Nitrogen Fixation in Bacteria and Higher Plants. Springer-Verlag. New York.
- Burris, R.H. 1974. Methodology. Chapter 2 in The Biology of Nitrogen Fixation (A. Quispel ed.). North-Holland Publishing Co., Amsterdam.
- Cox, R.M. and P. Fay. 1969. Special aspects of nitrogen fixation by blue-green algae. Proc. R. Soc. Lond. B. 142: 357-366.
- Crittenden, P.D. and K.A. Kershaw. 1979. Studies on lichen dominated systems. XXII. The environmental control of nitrogenase activity in <u>Stereocaulon paschale</u> in spruce-lichen woodland. Can. J. Bot. 57: In Press.
- Dilworth, M.J. 1966. Acetylene reduction by nitrogen-fixing preparations from Clostridium pasteurianum. Biochim. Biophys. Acta 127: 285-294.

- Dua, R.D. and R.H. Burris. 1963. Stability of nitrogen-fixing enzymes and the reactivation of cold labile enzyme. Proc. Natl. Acad. Sci. U.S.A. 50: 169-175.
- Englund, B. 1978. Effects of environmental factors on acetylene reduction by intact thallus and excised cephalodia of <u>Peltigera</u>

 <u>aphthosa</u> Willd. in Environmental Role of Nitrogen-fixing Blue-Green Algae and Asymbiotic Bacteria (U. Granhall ed.) Ecol. Bull.

 (Stockholm) 26: 234-246.
- Englund, B. and H. Meyerson. 1974. In situ measurement of nitrogen fixation at low temperatures. Oikos 25: 283-287.
- Ertl, L. 1951. Uber die Lichtverhältnisse in Laub-flechten. Planta 39: 245-270.
- Farrar, J.F. 1973. Lichen physiology: progress and pitfalls. Ch. 12 in Air Pollution and Lichens, (B.W. Ferry, M.S. Baddeley and D.L. Hawksworth ed.) Univ. of Toronto Press.
- Field, G., D.W. Larson and K.A. Kershaw. 1974. Studies on lichen-dominated systems. VIII. The instrumentation of a raised-beach ridge for temperature and wind speed measurements. Can. J. Bot. 52: 1927-1934.
- Fogg, G.E. and W.D.P. Stewart. 1968. In situ determinations of biological nitrogen fixation in Antarctica. Bull. Br. Antarct. Surv. 15: 39-46.
- Fogg. G.E. and Than-Tun. 1960. Interrelations of photosynthesis and assimilation of elementary nitrogen in a blue-green algae. Proc. R. Soc. B. 153: 111-127.

- Frey, E 1959. Ergbn. wiss. Unters. schweiz. Natn. parks 41: 239.

 Galloe, O. 1908. Dansk likeners oekologie. Dan. Bot. Tidskr. 28:

 285.
- Galun, M. 1963. Autecological and synecological observations on lichens of the Negev, Israel. Isr. J. Bot. 12: 179-187.
- Gauhl, E. 1969. Differential photosynthetic performance of Solanum

 dulcamara ecotypes from shaded and exposed habitats. Carnegie

 Inst. Washington Yearb. 67: 482-88.
- Gauhl, E. 1970. Leaf factors affecting the rate of light saturated 'photosynthesis in ecotypes of Solanum dulcamara. Carnegie Inst. Washington, Yearb. 68: 633-36.
- Hale, M.E. 1969. How to Know the Lichens. Brown Publishers, Toronto.
- Hale, M.E. 1974. The Biology of Lichens. Edward Arnold, London.
- Hampton, R.E. 1973. Photosynthetic pigments in <u>Peltigora canina</u> (L.) Willd. from sun and shade habitats. Bryologist 76(4): 543-545.
- Hardy, R.W.F., R.D. Holsten, E.K. Hackson and R.C. Burns. 1968. The acetyelene-ethylene assay for N₂ fixation: laboratory and field evaluations. Plant Physiol. 43: 1185-1207.
- Harris, G.P. 1969. A study of the ecology of corticolous lichens. Unpublished Ph.D. thesis, Imperial College, London.
- Harris, G.P. 1971. The ecology of corticolous lichens. II. The relationship between physiology and the environment. J. Ecol. 59: 441-452.

- Harris, G.P. 1972. The ecology of corticolous lichens. III. A simulation model of productivity as a function of light intensity and water availability. J. Ecol. 60: 19-40.
- Hatch, M.D., C.R. Slack, and T.A. Bull. 1969. Light-induced changes in the content of some enzymes of the C₄-dicarboxylic acid pathway of photosynthesis and its effect on other characteristics of photosynthesis. Phytochem. 8: 697-706.
- Haury, J.F. and L. Bogorad. 1977. Action spectra-for phycobili . , protein synthesis in a chromatically adapting Cyanophyte, Fremyella diplosiphon. Plant Physiol. 60: 835-839.
- Haystead, A., R. Robinson and W.D.P. Stewart. 1970. Nitrogenase activity in extracts of heterocystous and non-heterocystous blue-green algae. Arch. Mikrobiol. 74: 235-243.
- Henriksson, E., and B. Simu. 1971. Nitrogen fixation by lichens.
 Oikos 22: 119-121.
 - Heariksson, E., B. Englund, M.B. Heden and I. Waas. 1972. Nitrogen fixation in Swedish soils by blue-green algae. In Taxonomy and Biology of Blue-Green Algae (T.V. Desikachary ed.) Madras.
 - Hitch, C.J.B. and W.D.P. Stewart. 1973. Nitrogen fixation by lichens in Scotland. New Phytol. 72: 509-524.
 - Holm-Hansen, O. 1968. Ecology, physiology and biochemistry of blue-green algae. Ann. Rev. Microbiol. 22: 47-70.
 - Horne, A.J. and C.R. Goldman. 1972. Nitrogen fixation in Clear Lake, California. I. Seasonal variation and the role of heterocysts. Limnol. Oceanogr. 17: 678-692.

- Horne, A.J. and C.J.W. Carmiggelt. 1975. Algal nitrogen fixation in Californian streams: seasonal cycles. Freshwat. Biol. 5: 461-470.
- Huss-Danell, K. 1977a. Nitrogen fixation by <u>Stereocaulon paschale</u> under field conditions. Can. J. Bot. 55: 585-592.
- Huss-Danell, K. 1977b. Nitrogenase activity in the lichen <u>Stereocaulon</u>

 <u>paschale</u>: recovery after dry storage. Physiol. Plant. 41: 158
 161.
- Jones, 1977c. The effects of light intensity on acetylene reduction by mats of blue-green algae in sub-tropical grassland. New Phytol. 78: 427-431.
- Jones, K. 1977d. The effects of temperature on acetylene reduction by mats of blue-green algae in sub-tropical grassland. New Phytol. 78: 433-436.
- Kallio, P. and S. Kallio. 1978. Adaptation of nitrogen fixation to temperature in the <u>Peltigera aphthosa</u> group. In Environmental Role of Nitrogen-fixing Blue-green Algae and Asymbiotic Bacteria. Ecol. Bull. (Stockholm) 26: 225-233.
- Kallio, P. and L. Kärenlampi. 1975. Photosynthesis in mosses and lichens. In Photosynthesis and Productivity in Different Environments (P.J. Cooper, ed.) Cambridge Univ. Press, Cambridge.
- Kallio, P., S. Sukonen and H. Kallio. 1972. The ecology of nitrogen fixation in Nephrona arcticum and Solorina crocea. Rep. Kevo Subarctic Res. Stn. 9: 7-14.

- Kallio, S. 1973. The ecology of nitrogen fixation in <u>Stereocaulon</u>

 paschale. Rep. Kevo Subarctic Res. Stn. 10: 34-42.
- Kallio, S. and P. Kallio. 1975. Nitrogen fixation in lichens at Kevo,

 North Finland. In Fennoscandian Tundra Ecosystems (F.E. Wielgolaski,

 ed.) Part 1. Ecol. Studies 16: 292-304. Springer-Verlag, Berlin.
- Kallio, S., P. Kallio, and M.-L. Rasku. 1976. Ecology of nitrogen fixation in <u>Peltigera aphthosa</u> (L.) Willd. in North Finland.

 Rep. Kevo Subarctic Res. Stn. 13: 16-22.
- Kappen, L. 1973. Response to extreme environments. In The Lichens (V. Ahmadjian and M.E. Hale, eds.) Academic Press, New York.
- Karenlampi, L. 1971. Studies on the relative growth rates of some fruticose lichens. Rep. Kevo Subarctic Res. Stn. 7: 33-39.
- Kellman, M.C. 1975. 'Plant Geography. Methuen and Co., London.
- Kelly, B.B. and V.E. Becker. 1975. Effects of light intensity and temperature on nitrogen fixation by Lobaria pulmonaria, Sticta weigelii, Leptogium cyanescens and Collema subfurvum. Bryologist 78(3): 350-355.
- Kershaw, K.A. 1974. Dependence of the level of nitrogenase activity on the water content of the thallus in <u>Peltigera canina</u>,

 <u>P. evansiana</u>, <u>P. polydactyla</u>, and <u>P. praetextata</u>. Can. J. Bot. 52: 1423-1427.
- Kershaw, K.A. 1975. Studies on lichen-dominated systems. XIV. The comparative ecology of <u>Alectoria mitidula</u> and <u>Cladina alpestris</u>.

 Can. J. Bot. 53: 2608-2613.

- Kershaw, K.A. 1977a. Studies on lichen-dominated systems. XX. An examination of some aspects of the northern boreal lichen woodlands in Canada. Can. J. Bot. 55: 393-410.
- Kershaw, K.A. 1977b. Physiological-environmental interactions in lichens. II. The pattern of net photosynthetic acclimation in Peltigera canina (L.) Willd. var. praetextata (Floerke in Somm.)

 Hue and P. polydactyla (Neck.) Hoffm. New Phytol. 79: 377-390.
- Kershaw, K.A. 1977c. Physiological-environmental interactions in lichens. III. The rate of net photosynthetic acclimation in Peltigera canina (L.) Willd var. praetextata (Floerke in Somm.)

 Hue, and P. polydactyla (Neck.) Hoffm. New Phytol. 79: 391-402.
- Kershaw, K.A. 1978. The role of lichens in boreal tundra transition areas. Bryologist 81(2): 294-306.
- Kershaw, K.A. and P.A. Dzikowski. 1977. Physiological-environmental interactions in lichens. VI. Nitrogenase activity in <u>Peltigera</u>

 <u>polydactyla</u> after a period of dessication. New Phytol. 79: 417-421.
- Kershaw, K.A. and J.D. MacFarlane. 1979. Physiological-environmental interactions in lichens. X. Light as an ecological factor in Peltigera aphthosa (L.) Willd. and P. canina var. praetextata Hue. New Phytol. (in preparation).
- Kershaw, K.A. and W.R. Rouse. 1976. The impact of fire on forest and tundra ecosystems. Indian and Northern Affairs Publ. No. QS-8117-000-EE-Al. pp. 1-54.

- Kershaw, K.A. and M.M. Smith. 1978. Studies on lichen-dominated systems.

 XXI. The control of seasonal rates of net photosynthesis by moisture, light and temperature in Stereocaulon paschale. Can.

 J. Bot. 56: 2825-2830.
- Kershaw, K.A., J.D. MacFarlane and M.J. Tysiaczny. 1977. Physiological-environmental interactions in lichens. V. The interaction of temperature with nitrogenase activity in the dark. New Phytol. 79: 409-416.
- Kershaw, K.A., W.R. Rouse, and B.T. Bunting. 1975. The impact of fire on forest and tundra ecosystems. Indian and Northern Affiars Publ. No. QS-8038-000-EE-Al. pp. 1-81.
- Lange, O.L. 1953. Hitze und Trockenresistenz der Flechten in Beziehung zu ihrer Verbreitung. Flora 140: 39-47.
- Lange, O.L. 1955. Untersuchungen über die Hitzeresistenz der Moose in Beziehung zu ihrer Berbreitung. Flora 142: 381-399.
- Lange, O.L. 1959. Untersuchungen über Wärmehaushalt und Hitzeresistenz mauretanischer Wusten-und Savannenpflanzen. Flora 147: 595-651.
- Lange, O.T. 1965. Der CO Gaswechsel von Flechten bie tiefen Temperaturen. Planta 64: 1-19.
- Lange, O.T. 1969. Experimentell-okologische Untersuchungen an Flechten der Negev-Wuste. I. CO₂-gasweschel von <u>Ramalina maciformis</u> (Del.)

 Bory unter kontrollierten Bedingungen in Laboratorium. Flora

 (Jena), Abt. B. 158: 324-359.
- Larcher, W. 1975. Physiological Plant Ecology. Springer-Verlag, Berlin.
- Larson, D.W. 1977. A method for the in situ measurement of lichen moisture content. J. Ecol. 65: 135-145.

- Larson, D.W. and K.A. Kershaw. 1975a. The measurement of CO₂ exchange in lichens: a new-method. Can. J. Bot. 53: 1535-1541.
- Larson, D.W. and K.A. Kershaw. 1975b. Acclimation in arctic lichens.

 Nature (London), 254: 421-423.
- Larson, D.W. and K.A. Kershaw. 1975c. Studies on lichen-dominated systems. XIII. Seasonal and geographical variation of net CO₂ exchange of <u>Alectoria ochroleuca</u>. Can. J. Bot. 53: 621-626.
- Larson, D.W. and K.A. Kershaw 1975d. Studies on lichen-dominated systems. XVI. Comparative patterns of net CO₂ exchange in Cetraria nivalis and Alectoria ochroleuca collected from a raised-beach ridge. Can. J. Bot. 53: 2884-2892.
- Laudi, G., P. Bonatti, and L.D. Trovatelli. 1969. Differenze ultrastrutuali di alcune specie di <u>Trebouxia</u> poste in condizioni di illuminazione differenti. G. Bot. Ital. 103: 79-107.
- Lechowicz, M.J. and M.S. Adams. 1973. Net photosynthesis of <u>Cladonia</u>

 <u>mitis</u> from sun and shade sites on the Wisconsin pine barrens.

 Ecology 54: 413-419.
- Lechowicz, M.J. and M.S. Adams. 1974. Ecology of <u>Cladonia</u> lichens.

 II. Comparative physiological ecology of <u>C. mitis</u>, <u>C. rangifernia</u> and <u>C. unicialis</u>. Can. J. Bot. 52: 411-422.
- Lechowicz, M.J., W.P. Jordan, and M.S. Adams. 1974. Ecology of Cladonia lichens. III. Comparison of C. carolineana, endemic to southeastern North America, with three northern Cladonia species. Can. J. Bot. 52: 565-574.
- Levitt J. 1972. Responses of Plants to Environmental Stress. Academic Press, New York.

- Looman, J. 1964. Ecology of lichen and bryophyte communities in Saskatchewan. Ecology 45: 481-491.
- MacFarlane, J.D., and K.A. Kershaw. 1977. Physiological-environmental interactions in lichens. IV. Seasonal changes in the nitrogenase activity of Peltigera canina (L.) Willd var. praetextata

 (Floerke in Somm.) Hue, and P. canina (L.) Willd var. rufescens

 (Weiss) Mudd. New Phytol. 79: 403-408.
- MacFarlane, J.D. and K.A. Kershaw. 1978. Thermal sensitivity in lichens. Science 201: 739-741.
- MacFarlane, J.D. and K.A. Kershaw. 1979. Phsyiological-environmental interactions in lichens. IX. Thermal sensitivity in the lichen genera Peltigera and Stereocaulon. New Phytol. (In Submission).
- MacFarlane, J.D., E. Maikawa, K.A. Kershaw, and A. Oaks. 1976.

 Physiological—environmental interactions in lichens. I. The interaction of light/dark periods and nitrogenase activity in Peltigera polydactyla. New Phytol. 77: 705-711.
- Maikawa, E. and K.A. Kershaw. 1975. The temperature dependence of thallus nitrogenase activity in <u>Peltigera canina</u>. Can. J. Bot. 53: 527-529.
- Maikawa, E., and K.A. Kershaw. 1976. Studies on lichen-dominated systems. XIX. The postfire recovery sequence of black-spruce lichen woodland in the Abitau Lake Region, N.W.T. Can. J. Bot. 54: 2679-2687.
- McCree, K.J. and J.H. Troughton. 1966. Prediction of growth rate at different light levels from measured photosynthesis and respiration rates. Plant Physiol. 41: 559-566.

- Millbank, J.W. 1972. Nitrogen metabolism in lichens. IV. The nitrogenase activity of the Nostoc phycobiont in Peltigera canina.

 New Phytol. 71: 1-10.
- Millbank, J.W. 1975. Aspects of nitrogen metabolism in lichens.

 Chapter 18, pp. 441-457 in Lichenology: Progress and Problems

 (D.H. Brown, D.L. Hawksworth and R.H. Bailey, eds.) Academic

 Press, London.
- Millbank, J.W. 1977. Lower plant associations. Chapter 4, pp. 125152 in A Treatise On Dinitrogen Fixation, Section III, Biology
 (R.W.F. Hardy and W.S. Silver ed.) John Wiley and Sons, New York.
- Montfort, C. 1950. Photochemische Wirkungen des Hohenklimas auf die Chloroplasten photolabiler Pflanzen in Mittle-und Hochgebirge.

 Z. Natur-forsch. 5b: 221-26.
- Munding, H. 1952. Untersuchungen zur Frage der Strahlenresistenz des Chlorophylls in den Chloroplasten. Protoplasma 41: 212-32.
- Patterson, D.T. 1975. Photosynthetic acclimation to irradiance in Celastrus orbiculatus Thumb. Photosynthetica 9(2): 140-144.
- Pattnaik, H. 1966. Studies on nitrogen fixation by Westelliopsis prolifica Janet. Ann. Bot. 30: 231-238.
- Pieters, G.A. and K. Schurer. 1973. Leaf temperature measurement. I.

 Thermocouples. Acta Bot. Neerl. 22: 569-580.
- Purnell, H. 1962. Gas Chromatography. John Wiley and Sons, New York.
- Raven, P.H., and H. Curtis. 1970. Biology of Plants. Worth Publishers,
 New York.

- Raven, P.H., R.F. Evert and H. Curtis. 1976. Biology of Plants.
 Worth Publishers, New York.
- Ried, A. 1960. Stoffwechsel und Verbreitungsgrenzen von Flechten.
- II. Wasser-und Assimilationschaushalt, Entquellungs-und
 Submersionsresistenz von Krusten Flechten benachbarter Standorte.
 Flora (Jena) 149: 345-385.
- "- Rouse, W.R. 1976. Microclimatic changes accompanying burning in subarctic lichen woodland. Arctic and Alpine Res. 8: 351-376.
 - Rundel, P.W. 1972. CO₂ exchange in ecological races of <u>Cladonia</u> <u>subtenuis</u>. Photosynthetica 6: 13-17.
 - Scheibe, J. 1972. Photoreversible pigment: occurrence in blue-green algae. Science 176: 1037-1039.
 - Schimmelpfennig, H. 1975. Basic thermocouple thermometry. Application Note W-15. Wescor Inc. Utah.
 - Schollhorn, R. and R.H. Burris. 1966. Study of intermediates in nitrogen fixation. Federation Proc. (abstr.) 25: 710.
 - Scott, G.D. 1956. Further investigation of some lichens for fixation of nitrogen. New Phytol. 55: 111-116.
 - Sloger, C. and W.S. Silver. 1967. Bact. Proc. (abstr.) p. 112.
 - Smith, D.C. 1961. The physiology of <u>Peltigera polydactyla</u> (Neck.)

 Hoffm. Lichenologist 1: 209-225.
 - Smith, D.C. 1962. The biology of lichen thalli. Biol. Rev. 35: 537-570.

- Stalfelt, M.G. 1937. Der Gasaustauch der Moose. Planta 27: 30-36.
- Stalfelt, M.G. 1939. Der Gasaustauch der Flechten. Planta 29: 11-31.
- Stewart, W.D.P. 1965. Nitrogen turnover in marine and brackish habitats.
 - I. Nitrogen fixation. Ann. Bot. (N.S.) 29: 229-239.
- Stewart, W.D.P. 1967. Nitrogen turnover in marine and brackish habitats.

 II. The use of ¹⁵N₂ in measuring nitrogen fixation in the field.

 Ann. Bot. 31: 385-406.
- Stewart, W.D.P. 1974. Blue-green algae. Chapter 7 in The Biology of Nitrogen Fixation (A. Quispel ed.). North-Holland Publishing Co. Amsterdam.
- Stewart, W.D.P. 1977. Blue-green algae. Chapter 3 in A Treatise on Dinitrogen Fixation, Section III: Biology (R.W.F. Hardy and W.S. Silver eds.). John Wiley and Sons, New York.
- Stewart, W.D.P., G.P. Fitzgerald and R.H. Burris. 1967. In situ studies of N₂ fixation using the acetylene reduction technique.

 Proc. natn. Acad. Sci. U.S.A. 58: 2071-2078.
- Tanner, C.B. 1963. Basic instrumentation and measurements for plant environment and micrometereology. Soils Bull. No. 6. Univ. of Wisconsin.
- Tobler, F. 1925a. Zur Physiologie der Farbunterschiede bei <u>Xanthoria</u>.

 Ber. Deut. Bot. Ges. 58: 301-305.
- Watanabe, A. and T. Kiyohara. 1963. Symbiotic blue-green algae of lichens, liverworts and cycads. In Studies in Micro-algae and Photosynthetic Bacteria (Japanese Society of Plant Physiologists, eds.) pp. 189-196. Univ. of Tokoyo Press, Tokyo.

Watts, W.A. 1971. Postglacial and interglacial vegetation history of southern Georgia and central Florida. Ecol. 52: 676-90.

Waughman, G.J. 1977. The effect of temperature on nitrogenase activity.

J. Exp. Bot. 28(105): 949-960.

Section 7.

APPENDIX "A"

TABLE I

KEY TO SURFACE WEATHER OBSERVATIONS

SKY AND CEILING

Sky cover contractions are in ascending order. Figures preceding contractions are heights in hundreds of feet above station. Sky cover contractions are:

CLR Clear: Less than Ø.1 sky cover
SCT Scattered: Ø.1 to Ø.5 sky cover
BKN Broken: Ø.6 to Ø.9 sky cover
OVC Overcast: More than Ø.9 sky cover

- Thin (When prefixed to the above symbols)
- -X Partial obscuration: 0.1 to less than 1.0 sky hidden by precipitation or obstruction to vision (bases at surface)
- X Obscuration: 1.0 sky hidden by precipitation or obstruction to vision (bases at surface)

Letter preceding height of layer identifies ceiling layer and indicates how ceiling height was obtained. Thus:

E Estimated height Measured Indefinite

V Immediately following numerical value, indicates a variable ceiling

VISIBILITY

Reported in statute miles and fractions (V=Variable)

CODED PIREPS

Pilot reports of clouds not visible from ground are coded with ASL height data preceding and/or following sky cover contraction to indicate cloud bases and/or tops, respectively. UA precedes all PIREPS

WEATHER AND OBSTRUCTION TO VISION SYMBOLS

A BD BS D F GF H	Hail Blowing dust Blowing sand Blowing snow Dust Fog Ground fog Haze	IC IF IP IPW K L R	Ice crystals Ice fog Ice pellets Ice pellet showers Smoke Drizzle Rain Rain showers	SP SW T T+ ZL	Snow Snow grains Snow pellets Snow_showers Thunderstorms Severe thunderstorms Freezing drizzle Freezing rain
------------------	--	--------------------------------------	---	---------------------------	--

Precipitation intensities are indicated thus: -- Very Light; -- Light; (no sign) Moderate; + Heavy

WIND

Direction in tens of degress from true north, speed in knots. \$600 indicates calm. G indicates gusty. Peak speed of gusts follows G or Q when gusts or squall are reported. The contraction WSHFT followed by GMT time group in remarks indicates windshift and its time of occurrence. (Knots X 1 15 statute mi/hr.)

EXAMPLES: 3627=360 Degrees, 27 knots; 3627G40=360 Degrees, 27 knots, peak speed in gusts 40 knots

DECODED REPORT

Kansas City: Record observation, 1500 feet scattered clouds, measured ceiling 2500 feet overcast, visibility 1 mile, light rain, smoke, sealevel pressure 1013.2 millibars, temperature 580F, dewpoint 560F, wind 1800, 7 knots, altimeter setting 29.93 inches. Runway 04 left, visual range 2000 feet variable to 4000 feet. Pilot reports top of overcast 5500 feet.

RUNWAY VISUAL RANGE (RVR)

RVR is reported from some stations. Extreme values during 10 minutes prior to observation are givin in hundreds of feet. Runway identification precedes RVR report

TYPE OF REPORT

The omission of type-of-report data identifies a scheduled record observation for the hour specified in the sequence heading. An out-of-sequence, special observation is identified by the letters "SP" following station identification and a 24-hour clock time group, e.g. "PIT SP Ø715-X M1 OVC". A special report indicates a significant change in one or more elements.

AREA FORECASTS

are 18-hour availation forecasts plus a 12-hour categorical outlook prepared 2 times/day giving general descriptions of clour cover, weather and frontal conditions for an area the size of several states. Heights of cloud tops, and icing are referenced ABOVE SEA LEVEL (ASL); ceiling heights, ABOVE GOUND LEVEL (AGL); bases of cloud layers are ASL unless indicated. Each SIGMET or AIRMET affecting an FA area will also serve to amend the Area Forecast.

SIGNET OR AIRMET

messages warn airmen in flight or potentially hazardous weather as squall lines, thunderstorms, fog, icing and turbulence. SIGMET concerns severe and extreme conditions of importance to all aircraft. AIRMET concerns less severe conditions which may be hazardous to some aircraft or to relatively inexperienced pilots. Both are broadcast by FAA or NAVAID voice channels.

TERMINAL FORECASTS

contain information for specific airports on expected ceiling, cloud heights, cloud amounts, visibility, weather and obstructions to vision and surface wind. They are issued 3 times/day and are valid for 24 hours. The last six hours of each forecast are covered by a categorical statement indicating whether VFR, MVFR, IFR or LIFR conditions are expected. Terminal forecasts will be written in the following form:

CEILING: Identified by the letter "C" CLOUD HEIGHTS: In hundreds of feet above the station (ground) CLOUD LAYERS: Stated in ascending order of height VISIBILITY: In statute miles but omitted if over 6 miles WEATHER AND OBSTRUCTION TO VISION: Standard weather and obstruction to vision symbols are used SURFACE WIND: In tens of degrees and knots; omitted when less than 10

EXAMPLE OF TERMINAL FORECAST

DCA 221010: DCA Forecast 22nd day of month - valid time 102-102. 10 SCT C18 BKN 5SW - 3415G25 OCNL C8 X ISW: Scattered clouds at 1000 feet, ceiling 1800 feet broken, wind 330 degrees 12 knots Gusts visibility 5 miles, light snow showers, surface wind, 340 degrees 15 knots Gusts to 25 knots, occasional ceiling 8 hundred feet

sky obscured, visibility l mile in moderate snow showers. 122 C5Ø BKN 3312G22: At 12Z becoming ceiling 5000 feet broken, surface to 22. Ø4Z MVFR CIG: Last 6 hours of FT after Ø4Z marginal VFR due to ceiling.

WINDS AND TEMPERATURES ALOFT (FD) FORECASTS

are a 12-hour forecasts of wind direction (nearest 10° true N) and speed (knots) for selected flight levels. Temperatures aloft ($^{\circ}$ C) are included for all but the 3000-foot level.

EXAMPLES OF WINDS AND TEMPERATURES ALOFT (FD) FORECASTS:

FD WBC 121745
BASED ON 121200Z DATA
VALID 130000Z FOR USE 1800-0300Z. TEMPS NEG ABV 24000

FT 3000 6000 9000 12000 18000 24000 30000 34000 39000 BOS 3127 3425-07 3420-11 3421-16 3516-27 3512-38 311649 . 292451 283451 3026 3327-08 3324-12 3222-16 3120-27 2923-38 284248 285150 288749 At 6000 feet ASL over JFK wind from 330° at 27 knots and temperature

TWEB (CONTINUOUS TRANSCRIBED WEATHER BROADCAST)

Individual route forecasts covering a 25 nautical mile zone either side of the route. By requesting a specific route number, detailed en route weather for a 12 or 18-hour period (depending on forecast issuance) plus a synopsis can be obtained.

TABLE II

Surface weather observations recorded at Pellston, Michigan, U.S.A. for

June 22, 1977

10000000000000000000000000000000000000	10.5 10.5 10.5 10.5 10.5 10.5 10.5 10.5	17 87 17 87 17 87 17 803 17 803 17 803 17 803 17 7 87	
ग्रिक्षेत्र विस्त्र स्व कर्तिः	. (YO 0.10 0.10 0.10 0.12 0.14 0.14 0.14 0.14 0.14 0.14 0.14 0.14	16 803 12 803 125 CU	29.320 524, 123 2 29.320 524, 123 2 29.350 72.0 40.10 29.350 72.0 40.10 29.370 420 57.0 67.0 20.370 420 57.0 67.0 20.370 51.0 67.0 20.370 51.0 20.370 51.0 67.0 20.370 51.0 67.0 20.37
भेज बिर्म शब्द अस व करो	010 010 010 014 014 014 014 015 017 017 017	37 36 200 562 3 662 3	29.12.5 Thus West of the of th
अब्राध्यक्ष सम्बद्ध	010 012 3 014 FRW 014 FRW 014 WW 015 WW 015 WW 017 WW	37 16 803 105 3 105 CU	98.35 74:0 40.10 98.35 74:0 40.10 15.370 15.0 11.0 4 97.370 16.0 11.0 6 97.370 16.0 11.0 6 27.345 11.0 16.12 27.345 11.0 10.0 10.0 10.0 10.0 10.0 10.0 10.
स्रिश्चिक्ष स्र न न न	0/3 60 0 0/4 60 0 0/4 60 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	37 14 803 125 CU 714	26.350 26.0 55.2 0 26.350 26.0 55.2 0 26.370 42.0 41.6 71.6 71.6 71.6 71.6 71.6 71.6 71.6 7
भारीक्षेत्र सम्मानित	0/4 Free 0/4 O/4 Press	37 36 803 602 714	29,360 55.0 51.0 11.0 4 25,370 51.0 11.0 4 27,300 12.0 15.0 15.0 5 27,300 12.0 15.0 15.0 5 27,300 12.0 15.0 15.0 5 27,300 12.0 15.0 15.0 15.0 15.0 15.0 15.0 15.0 15
शक्त्रा स्व कर्ताः	0/4 Few old	37 12 803 125 CU 3	25,370 H3.0 Alle from 51,270 51,270 51,270 51,250 51,450 51,270 51,450 51,570 51,450 51,570 5
असे त्र न न न	014 minu (16 803 102 3 105 CU 3	09.370 51.4 71.4 7.4 62.4 5.2 51.4 71.4 7.4 62.4 5.2 51.4 7.4 62.4 5.2 51.4 7.4 62.4 5.2 51.4 7.4 62.4 5.2 51.4 7.4 62.4 5.2 51.4 5.2 51.4 5.2 51.4 5.2 51.4 5.2 51.4 5.2 51.4 5.2 51.4 5.2 51.4 51.4 51.4 51.4 51.4 51.4 51.4 51.4
अस्ति न जले	014 WW 0	200 3 /25 3 /25 514	20,370 (400 1720 6 20,3345 12.0 (4.1.2) 20,3420 23.5 1.74 1.10 1.10 1.10 1.10 1.10 1.10 1.10 1.1
रू न जुले स्रम्	67 614 W. W. C.	16 803 002 3 15 50 50	20,3% 11.6 (6) 2 20,3% 11.6 (6) 2 20,3% 12.5 (14) 1 19,3% 180 184 3 19,3% 180 184 3 21,3% 79.0 (3) 2 21,3% 79.0 (3) 2 21,3% 77.0 (3) 2 21,3% 77.0 (3) 2 21,3% 77.0 (3) 2
र्भ के हो। स कहा	6/0 0/3	803 002 3	27.34c 71.0 (6.1.2 29.31c 75.3 (1.6) 29.365 72.4 (2.6) 19.350 :80 584 2 29.335 72.0 (5.6) 21.325 72.0 (5.6) 21.325 74.0 (5.6) 21.325 77.0 (6.6) 21.325 77.0 (6.6)
300	610 a7	6200 3 FEW CV	29.470 23.57.741 19.314753 61.61 19.350 380 584 2 19.350 380 584 2 69.335766 3665 69.325 79.063 6 29.335 81.063 0 29.335 81.063 0
52.30		000 3 12 CU	29 365 72,42261 19,353 78,92261 29,335 78,02563 29,335 78,02563 29,335 78,00363 29,335 78,0036
	7/0/7	12 000 3 12 00 214	29.365 76.49 (26.6) 19,350 :80 5843 69,335 78.06 50:5 29,335 81.0630 77 29,335 76.0630 77 29,335 76.0630 77
-	013 Eaw	L'EW	75.0 584 3 77.0 56 3 78.0 630 7 18.4 610 7
4133	210		75.0 6.3 6 7 7 6 7 7 6 7 7 6 7 7 6 7 7 6 7
M4434	08 1011 WWD VRAC		73.07.50 3 81.043.07 11.07
5307	0HM 1000		81.06307 18.46107
12 30	42 010 WWD		7.0 12.0 27.
22 55	600 01		75.0 (2:0)
	600 101	18 000	
नि	00		0 %
00 15	00	•	, 'S
00 1	210 017	308	12012
200	710 00		100
000000000000000000000000000000000000000	00 00 a		25.7
			-
-			
STATION FREITURE CONPUTATIONS		SUNERT OF DAT REARTS	ATUATES, NOTES AND MIKCELLANGOUS PITENDERA
20 Sto 0 2500	-	\vdash	
	(1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Sorry Park Park	
ı	N. 1841	NA 647 774	
14.43c 26.370 W	370	0.0	
-, 2/0,-	10.510.		

TABLE III

Surface weather observations recorded at Pellston, Michigan, U.S.A. for June 26, 1977

	# 5 2.17 X : 5							a stratut	V. 1. 1	V. athaintat of cestred	doiret Dajer.					Ţ		
	161100001		<u>ت</u>	URFACE YEAT	HER OBSERY	ATIONS			•	10007 1000		Soul	1	nein	()	7		
11.	1 2 2	5	- 1							į	3	1		`				
Column			1 (F. pe)	::	2 3441							┢	100	_	17.01 -01			
15.1 C. L. R. L. L. R. L.	_	4.00 10 00 000		10177110	i	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	13	<u>-</u>	714121	I WAS THULL	1,100,1100,1				4 8 5		:	
	J	dia	+		11 11 11		Ξ			-		•		111	- 2	3 :	:	
255 C. L. R		0'0	1			₹ ? ?	Ţ		00	74	&	*1	_T	575763	200	¥		
25	T	6/10/	1		45.5	20,100	T						5/10 5		0	Z		
22. C. L. R. S.					1	2 2	1320		-	5		3		C2.E 52.	ં	R	~~~	
State Stat		0 / 0			; ای	3	882		<u>त</u>	27		<u>- q</u>		a Fload		Ş	_	
		- 1	1		157		223	<u> </u>		1115 61	4			1.5 5%	0 0	S	• ••	
State Stat	T				カナスか		283	Pehis	2/9		Ì	1	1	6		0,4		
	_ {	2/2	250		25.25	.~.	263			207	20		S ohill	77	ठ	Į.	•	
	7	71/	55,		5.05	~ ~	17.0					Š	1. Ye. K	50110	-	5	•	
25		C4.K	35		8767k		1661	र हो।				2	12/10	4/14	1_	5		. •
	255	-	35		37.7%		990	上たい	i	£03		2	T.	<u>X</u>	₹ -	ار ار		
15 250 - 510 15 15 15 15 15 15 15	27.27	250-307	125		名があ	1	1700	<u> </u>	-	1	TRBL	12	10 32	<u> </u>	20	识	`	
15. 25 - 317.	1657		45			12209	1967	Ļ				16	Y V	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	र्नेह	1/2		
13 15 15 15 15 15 15 15	古	3 I	25.		13/8/	11/52	326	$\overline{}$	VREL		812			2	T	Ţ		
455 25C - 217	25.	- 1	2.5		<u>प्रस्थित</u>	8/12	486	$\overline{}$				63	0708	67	7	13		
13 250 - 807 319 170	1523	2 SC - BKW	7.0		12 25 6	26.18	686	- ∔	מעער			7	3000	300	7	1		
12 25 25 25 25 25 25 25	538 3	350-56	7.12		12/11/21		913				612		7,050,7	\sim	0	_		
250 - 3474	آلہ	250.50	157		17/18/10	1//10	984						9,020		ا	· /\		
155 250 - 65 M 15	П.	25c - 11KM	バ			12613	573					2	2 10 20 C	_	, ,	Ţ		
15 15 15 10 10 10 10 10	j	25C-BKN_	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		10/ Bes [4	2/10	5%			707		1	Q	7	200	<u> </u>		
15 15 15 15 15 15 15 15	F	750 - GAN	115		V	121.03	2.80	Foul	Ted NW	, ,		2	1,010,1	17.77	シング	J.		٠
17 17 17 17 17 17 17 17	-i	255 Oh!!	15		į	00 00	ء 2	TCU	N. W. F.			4	7.00.7		+-	13		
	7	1.25 BKN	13-	77	3	3	583	*		202		(ह	3070			73		
		250-8124	15			00:00	1.86			2	-	1/2	``\			10		
		1350 BIN	12/		1	6001	484					700	21 ?			<u>3.</u>	•	
							-/						2	285		บ		
													-	-	+	Τ		
															-	·		
					-	_						-	-			Τ		
					+	-										Γ		
	-			·	+	-				-								
C C C C C C C C C C C C C C C C C C C	:	***	\vdash		100 100 100				tonana t	*****			-			_		
	•	1	įį						or 142 . vy (A)	Model (ch.)		BIEALTS, HOTI	13 44 B 13 C C	LAUTOUL PHE	appeter.			
0 0 0 83 57 "" 37100 371140 171101 29070 " " " " " " " " " " " " " " " " " "	-	1	!	8	- +	77		 	<u> </u>	├							•	
0 0 0 0 58 50 0 0 0 0 0 0 0 0 0 0 0 0 0	Ŀ	力の十		7						<u> </u>	Į			}				
0 0 0 83 51 700 37160 17120 2900 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	-	0	5.0	2001				- 1		F						_		
		00	2/2		27/1/2	77.57	ئ كى	03	<u> </u>	_	2							
	Z:		7			١,	•	\ \ \	<u>)</u> د)						T-	,	
							•				•	1.1. Con me 1	resultate over	((: 1)/(-U.S-C	TANKE PARE] ;	7	IJ

Thallus temperatures and radiant flux density recorded for (a)

- P. canina var. praetextata and (b) P. canina var. rufescens on July
- 8, 1977 for three replicate thalli (Site 1).

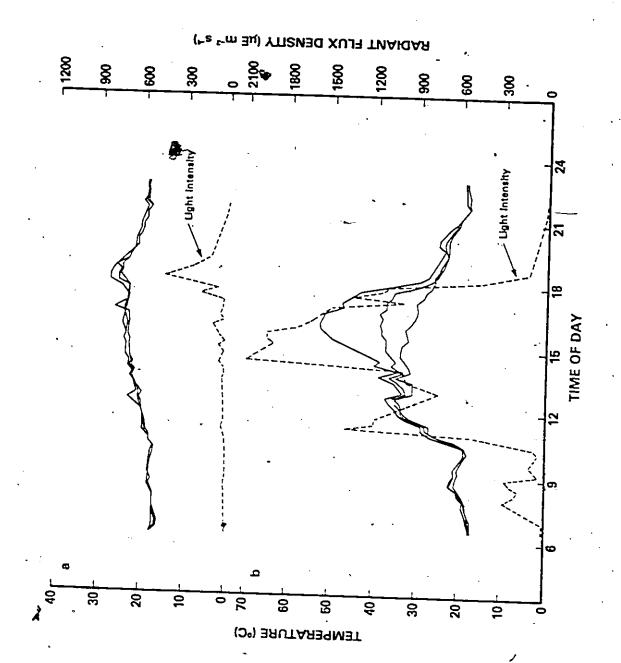


TABLE IV

Surface weather observations recorded at Pellston, Michigan, U.S.A. for
July 8, 1977

Thallus temperatures and radiant flux density recorded for (a)

P. canina var. praetextata and (b) P. canina var. rufescere on July

9, 1977 for three replicate thalli (Site 1).

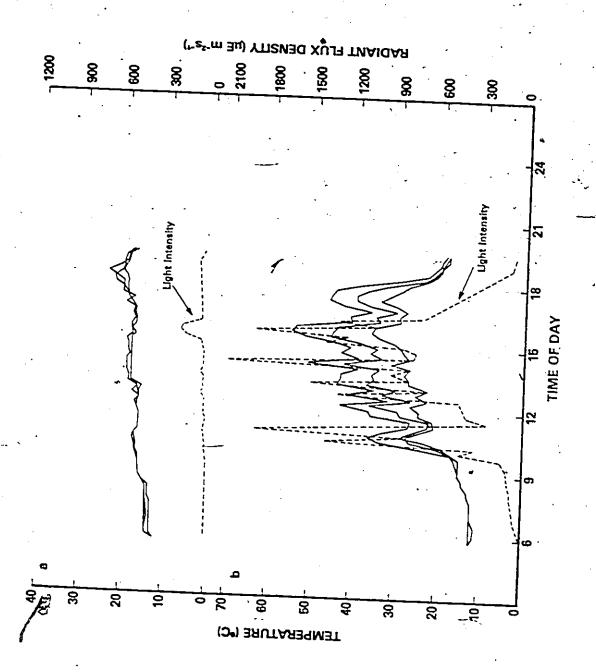


TABLE 🔻 .

Surface weather observations recorded at Pellston, Michigan, U.S.A. for July 9, 1977

,	1	¥.								11.10	thurs if tas	0	0.0			1996	·
			[1]		SURFACE WEATHER OBJERVATIONS	IER OBIERY	ATIOHS	,			11	# # # # # # # # # # # # # # # # # # #	2	50	,	3	·
				911144V	111111	111						1	4		197		_,
	:		34 F 45 B E44 14 B Frank 56 41 Fr 15	1111	******	ξį	***) (m m m				: : :			40.
-	Ē	=		#1 F	=	11 11 11		Ξĺ					=	=		<u>ئ</u>	_
	وأمر		>	(N. 62)		ろうだ	3330		37.6	82	-		273	以	200	100	<u>/</u>
÷	*		ł	33		\$5000 \$10000	0						14.3	5591	500	77.7	\geq
	₹	خ	٦٣	90		32.30	70 62	ĺ					67.6	15/20	12 S S S S S S S S S S S S S S S S S S S	グン	
-	<u>ئ</u> ے	3	15000	2,50	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	11/13/	727.00	700	٦			¥	1943.	07.75	10 S	10	·
	~		CLK	کې د د د			77.0%	101	EE'W	007	公氏	•	77.5	15.43	0 /3	et/	2
	<u></u>	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	2 2 2 2 2	200	0 3	イボス	2002	570	186	Mi				11.00	0,007	0.9%	À
			1	7,7		22.50	7.50 7.7.7 7.7.7.7.7.7.7.7.7.7.7.7.7.7.7.7.	700	\$ \$ \$ \$	7				7/200	<u>0</u> ,	7	
•	2		3.5 SeT	300	2 4	2017	7 2	010	12.17	111-111			77	10 % C	500	0 °	<u>}</u>
	-		635 11(h)	36		12775	7,0		BKAI	187	2116		27.4	27.2		기 . 당	<u>.</u>
. ,		6832		30	3	30/18	15/10	027	7077	, 1/4 /	7		750	200		4 C	.
-			\$	30		2336655	3618	0.32		160			S4.72	Q'77	5,7	9	
			3 :	2015		3	~ -	5.50		0/10	59	-	38.46	060,0	7692	7	_
	_		3 ;	2,00		<u>.</u>	3 3	6.7 5	ᆜ.				35.460	0190	7 53	2/9	
, ,	_		L.	100	<u>مد</u>			- 1023	-WADVAGE	484			27,460	. 4	15007	7	
>	_	((2)	100 COLOR 100 CO	7.00	1		ء ا	5		400			05% 5%		<u> </u>	<u>र</u> इ.	
·	2		5	2,2		3		75.7					24.6		~	3	
<u> </u>				1		\sum_{i}	_	·/-					33.57	7,53	3 6 65	<u>(</u>)	
-			7.50	8	2.2		<u> </u>	026		(03	22		27.476	2620	120	\$	٠.
	<u>)</u>	200	7 0.0	200		3	3	- 02/					22.63	0 % 0	3	Corre	* *
,	او		2000	70.				233		1			39.460	77.7	53.9	8	
-			101,01	* /	٠,	<u>~</u>	S	200		303		,	0/25	773	17 72	0	
	4	- 5//	230 501	12.		\$ 5	10	0.77	-				137.5cm	1000	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	190	
•		5	CCK	30	3	5500	0000	027		-		,	21,500		00%	Q o	_
		-					- 	-				-			1		
	İ	1					-	-									
- .`		-					-						-		+	\dashv	
		+						1				ğ .	1	1	†	-	
					-	-							<u> </u>	-	+		
														1	+	T	_
-	= = =	:	Page 1 to 1 t		<u>"</u>	ISATION PRELIUSE CONFUTA	* CONFUTATION!			SUBMART OF DAY	1 No. 2	=		1	Wi Prition		
-	=			· -	1 Minne	PHAT		┢				1	.				
		×				3/	7 (7)	3567		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	¥ 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	12.11					
	5	-	C	14	3 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1							<u> </u>				-	
•		-1 -1	2	14.7	111111111111111111111111111111111111111			1 1 1 1 1 1	Ž	111	±	3					
		-	÷	., o,	>/; :]:	\gtrsim	2		1	./.		\ <u>\</u>					·- •
	7.	X	 -	(2)		10 10 10 10 10 10 10 10 10 10 10 10 10 1	12/2/2018 12/2/14/2018	() 20:(0)	7	S S	0						/
y ^		Ē					i	>									ا ہے
Li			~					· '	_						•		

Thallus temperatures and radiant flux density recorded for (a)

P. camina var. praetextata and (b) P. camina var. rufescens on July

10, 1977 for three replicate thalli (Site 1).

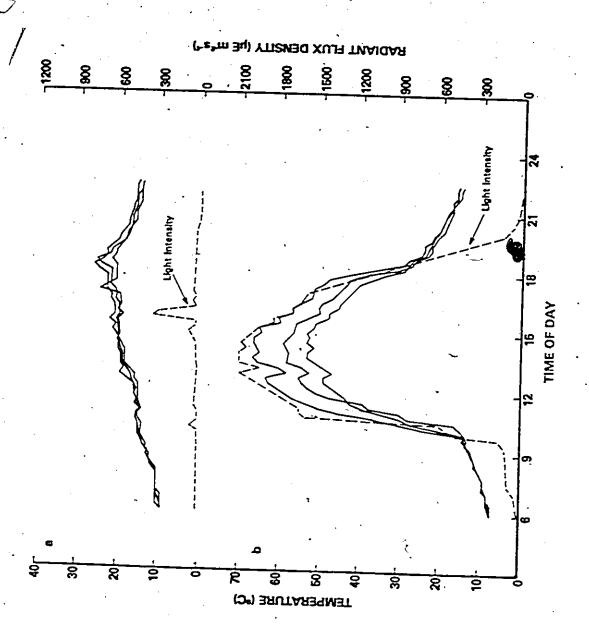


TABLE VI

Surface weather observations recorded at Pellston, Michigan, U.S.A for July 10, 1977

10-11	100 100 100 100 100 100 100 100 100 100	0 5 7 6 7 6 9 7	795/544.0 MO O O CA	11/10/2	875/0 1/5/1/2 P 2 C	1000	301381 73	62011072	2 / 1/18 2.7	× × × × × × × × × × × × × × × × × × ×	140 HO7 5	15.010.57 3	7	89400 421/11/7 7 K	20,7 (22.1/2/	3	07 25 TES 10	637517 10/6	25,400 GL 8 18.Cholog 14)	(0.02.59 d.			Stradel, wotes and miscellantous prenonera	-				e.l. Cathern minic other interestated bythe
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	111 M 141 M 141 M 141 CO 114 C	2/0 72	lo mad	, (303 40		202		04 01/1			// 9	fer. att	16 14 27	Blure Rupe		00%			//		SUMMAY OF BAY (Minigh to Winigh)	Park Place Control of the Park Control of the	Wood Gal	154 167 New Net 1784	77 10 0 0 0	* 9.1. Cotter
IURFACE VEATHER OBSERVATIONS (Marked for sea at devicement Conditional)	11 11 11 11 11 11 11 11 11 11 11 11 11	05.00 00 018	114100 60 029	1 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	280	030000000000000000000000000000000000000	JX 60.00	100	2 2 2 2 2 2 2 2 2 2	7.43 15 07	74 55 15 08	57/3 05	232 mx 9 6 d /2. 018	1361 06 12 017	1.10 10 01.1	10/10/01/2	80 11	07 77 55	1/0 8/0/1/0/0/0/0/0/0/0/0/0/0/0/0/0/0/0/0/0/	7507			HATION PREHIUSE COMPUTATIONS	2811 15787 25790 55300		"" 77 - 11 77 C ES 34 dV 30 das	27.51 27.520 77.785	1
	B LING CONTROL CONTROL OF ANY DESCRIPTION OF ANY DE	50 50 50 50 50 50 50 50 50 50 50 50 50 5	2	250-50r 30	\$50 - 8KV 30	1	1745	30.7	10.81 035-864 30	1	250- BK	250 80	45 1ct 250-000 30	- BK11	\neg	- i	C12001C	ברות נאנר. בי	170351 ENODOC 10	Without 1			(fac) (ac) (ac) (ac) (ac) (ac) (ac) (ac) (1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	26 67 0 0	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 77 70	
10 10 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	FAY7) 1944	22 X	K 1255	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	닞	27.8	75.80	1	A 1155		<u>. 1</u>	0 000	7.59/2		4185		× (3)	1	於		1.		TAN	11 C	0 - 13.60	0 1 75:10	X X X X X X X X X X	

Thallus temperatures and radiant flux density recorded for (a)

P. canina var. praetextata and (b) P. canina var. rufescens on July

11, 1977 for three replicate thalli (Site 1).

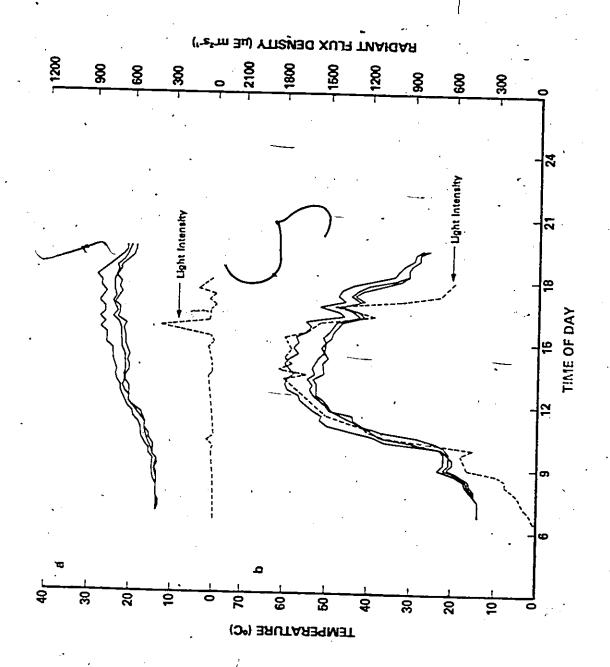


TABLE VII

Surface weather observations recorded at Pellston, Michigan, U.S.A. for ¹July 11, 1977

•			125
2000 11 V.	1000 0000 000 000 000 000 000 000 000 0	27.365.51.8 5.45.7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	SERAISI, MOTES AND MISCELLANEDUS PHEMOMENA. MA PAS, CONTEMNO PHINING OFFICE 1911-413-61-37/ECL Parism 4.
2) A 110 2) A 121 117 1 T 10 1 T 1 T	STEAMS AND TOTAL COSTS OF STATE	563 56	The first of all the fi
SURFACE WEATHER OBSERVATIONS (AL. wed to use of the squared Code as user)	100 Per 100 Pe	10 00 00 00 00 00 00 00 00 00 00 00 00 0	111100 TILLY CONTITION 1 10055 0655 125 1864 1 27.36571722 51.009/400 7.36571732 51.009/400
(err	Extraction desired to the second of the seco	## 240 BKN 25 ## 200 BKN 35 ## 200 BKN 30 ## 200 BKN 40 ## 200	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
10.00 (10.00)	(12)	できなるないなるよう。	

.

Thallus temperatures and radiant flux density recorded for (a)

P. canina var. praetextata and (b) P. canina var. rufescens on July

17, 1977 for three replicate thalli (Site 2).

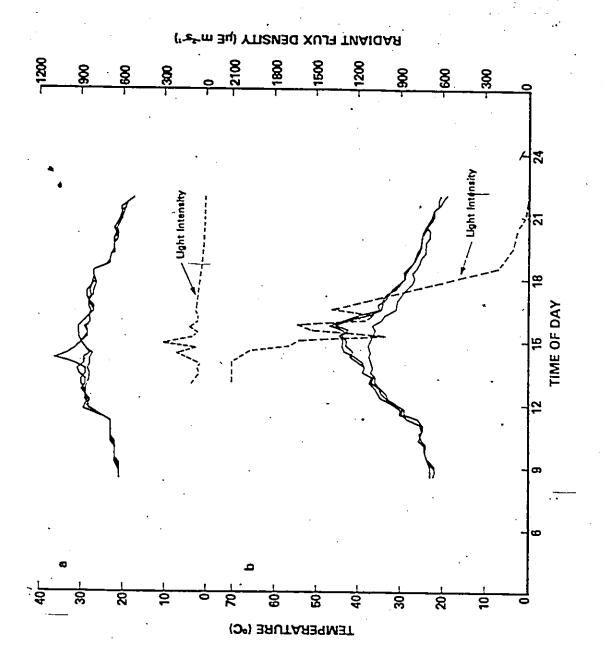


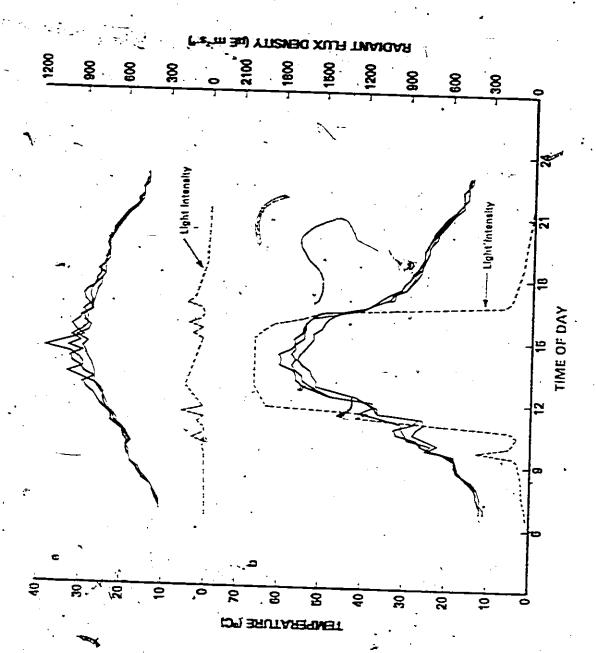
TABLE VIII

Surface weather observations recorded at Pellston, Michigan, U.S.A. for July 17, 1977

Thallus temperatures and radiant flux density recorded for (a)

P. canina var. praetextata and (b) P. canina var. rufescens on July

22, 1977 for three replicate thalli (Site 2).



- TABLE IX

Surface weather observations recorded at Pellston, Michigan, U.S.A. for
July 22, 1977

•.		125
Portstow Michigan	25.50	* P.1. CALESTANT HILLING PRINCE INTO B
And thing distances of the second sec	Few CU 808 45 Few CU 804 45 Few CU 804 45 FEEW CU 80 811 FEEW CU 80 811 FEW	WS C C
ER OBSE	22/87/4/2000 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	21.010, = 500, - 500, - 50,000, - 50
SUR.		72 5.2 min.
annak Itana		79.200 0000
	= ====================================	<u> </u>

Thallus temperatures and radiant flux density recorded for (a)

P. canina var. praetextata and (b) P. canina var. rufescens on August

3, 1977 for three replicate thalli (Site 2).

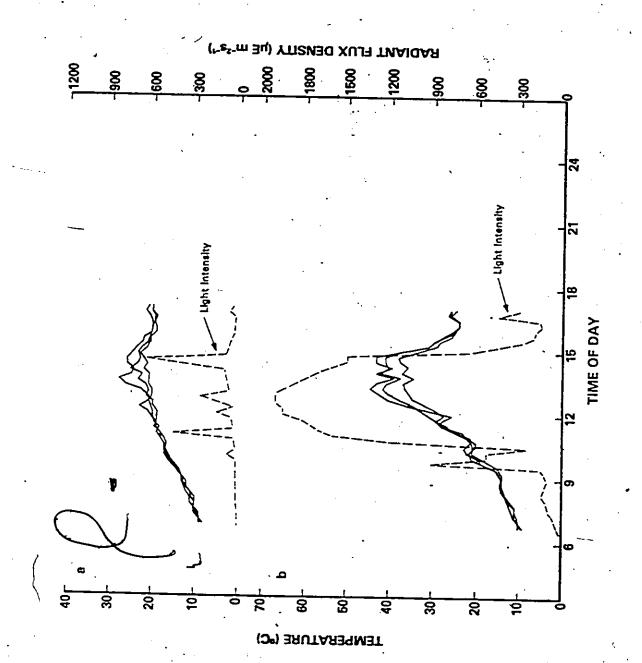


TABLE X

Surface weather observations recorded at Pellston, Michigan, U.S.A. for August 3, 1977

,	ę Ę	11-711		1		Halls	ATEM ALT	900	74.500		\	- ATTOREAL .	TA *** 3:47230	V.A. DEFARTMENT OF AND ATMODES INC. BECADES	13114700	7000			(
	, x 2	N	be SUBTRACT	4		4	(Abrigad he are of designated Circl Bullians)	1		.		•			-	3		3	۲I !	1	\top
, 144		100	pr 429,614m9		tryal Lagran		9641474			•						3	31.		$\overline{}$	¥ .	T:
· —		. <u>=</u>	A Care of Person				18 W 594 (B)	î =		100 PE			1111	HIME AID MIPLEMENTAL COSESSINA	TAL COSTS 841		1	ξĒ		111	111
	;		940	1	200			100 //	2	00 00	999	105		20	đ		09311	11.77	1	= 5	٠,١,٠
	1	र्देश	7040	+	2	+		w -		00 00	953						36.36	3 3	ه د) \{ ;	J.
			G. 5.	1	g c	-		Ŝ.	3	00 8	1361	\perp					83.765	9.3	2 C	70	J:
	ه ساد	100	140-80-1	1	3 7	+		1221	۶,۷	200		-	33				SIF7E	3%	3	3	12/
**	9	3	355.1.200-1314	+-	300	+		10 m	7.	100 00	7,7,7	16 W SC			-		36%	37.2	6	Ž U	1
	र्वे	22.30	Jetse 1 -300-51	-	200	-		12	13	V		ζ, <i>(1)</i>	100		1	J	35,335	77.7	35.2	1/2/2/2	J
	2	583	272		2	$ \cdot $		155 50		700	566	┿	1.	7 0,007	20 50		68.155	7,37	**	7	7.
		7	250-567		2			17 75		T 📆 "	997	1		-			016,(0	3,72	0 (א א	اد
- -	<u>জ</u>	355	250 - 50 1	,	8	+				i	25%			2/4			33,64			+	. Tx
•	<u> </u>	550	450 - S.F	+	361	+		17.77		2///	995						67.67	3 2	1>	7 7	Τ,
	T			\dagger		+			\supseteq	2/3	99.3	Sew Sc	J				29,11,50	23.2	12	+-	_
		25.5	3056 10000	6 1/2		\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \		1850 1200 1200 1200 1200 1200 1200 1200 12		77.70	250	Eem	٦.	196	77	9 37	39.770	1,7	6	14	τ.
-	\ <u>\\</u>	<u> </u>	3007 6 10000	12		┿	Τ.	7		1	727	3,1000.	3 27				29,730	77.0-	11/1/11	K	_
		100	305 CT E100 KKN 100	107		1_	2	7000	1 1 1 2	2/2	0000	٠ اج	27000	S	DAKKSU		29.130	077	33/6/2	S B	_
	8	$\overline{}$			-	સ	/	10 1	•	٠١ `	1000	320	지 장 장			0.3	05/ 62	13.8	হা	<u>र्थ</u> इ	+
-			WO SC FEIOU OVE		2			10/21	22	<u> </u>	100		,				01/1/2	7%	<u>u </u>	N (. 1
• .	4		1005CT 25001	7	720			100 73	2773	Ž	000			00	00,00	C			3Ľ	4	_
	N. S.	<i>_}</i> /	\mathbf{L}	┧	7	-		13	6.120	19019	686			2	7		2017	2 0	27.7	1	.
	外	1	Ľ	7	5	-		_	7	10,7	6//2	ングス	ن	2 0 %	3		24120		1	4	_1
		7557	1005ct 1505cm	} →	15/			13084	2	50	265]	}	7			20,100			10	-
	<u>;</u> ;	77.43		7	6	-	7	1341	20115	1515	63.5			. 1	1		1016) i	3 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	`	_
	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	2233	air	7	200	_		3.46	17.77	_	992						2,7,3	7 2 2		\$ \frac{1}{2} \fra	ੁ
	+	+		+	+	\dashv		+						 			.• 1	4		-}	-, -
	+	+		+	+	$\frac{1}{1}$	-	+	-										-	_	_
<u> </u>	+	+		+	+	 -	+	1	+	- - -									-		_
-	+	+		+	+	+	\dagger	+	+				-								,
	\vdash	-		°.	-	-	+	+	+	- -											,
1	-	-		+	+	+	+	+	1				1				1				
1	ſ:	+		-	L	1	1	1	$\frac{1}{2}$				7			·					
	1.5.1	r e	(M.) (M.) (M.)	1	ijē		l	THOM PART	SURE COM	STATION PACISURE CONFUTATIONS	-		SURGEST OF DAY	r DP DAY		4 Feather	STRABES, HOTEL AND MISCELL ANTOUS PREMOMENA	CILLANIOUS	PRÍMOMÍNA		
1.	5 2			Į.	<u> </u>	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	75 07 7	0	635	1255	1855	\vdash	-	\vdash	1		*			T	
<u>1~1</u>		1	+-	80	* * *	1	1	+				ije		N. C.	iį					\prod	
_1:	11.7	4	υ,	27	.4	18 H		1 L				7	É	_		+				T	
<u> </u>	1252	3 7	0 0	12	7 / 2		75,210	\$\\\ \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	26365	39.140	29.140 29.15	14 21	7	۵	E	4					
;	1	X:	0000	7.71	\$	# FE. 1		1		1601	1	0	_ _ _	>	. ————————————————————————————————————						
				•			>	•							! 	• r.1. con	e.5. Contivent beinting perior the 44-piving legion	DITICL 1916.	HS-DVIN	1112	_