PHYSIOLOGICAL RESPONSES OF CYANOLICHENS TO PHOTOSYNTHETIC PARTNERS

by

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AN ABSTRACT OF THE THESIS OF

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While previous experiments on the physiological activity of primary and secondary cyanolichens have been done, an analysis of photomorphs has never been attempted. Photomorphs of *Peltigera britannica* (Gyelnik) Hotan-Hartw. & Tønsberg were studied to compare the physiological response of a primary cyanolichen to an additional symbiotic partner (green algea) while keeping all species of partners (fungi and cyanobacteria) unchanged. *Peltigera neopolydactyla* (Gyelnik) Gyelnik was examined due to availability. Nitrogenase and photosynthetic activity were measured using the acetylene reduction assay and the Li-Cor 6400, respectively. Measurements were taken in the Willamette National Forest from January to May 2001. *Peltigera britannica* 2° Exhibited lower nitrogenase and slightly higher photosynthetic activity than both primary cyanolichens studied for most of the sample period. The May sampling was the first observation where *P. britannica* 2° exhibited higher nitrogenase activity than its primary photomorph. The May sampling also showed *P. britannica* 2° with twice the photosynthetic activity of *both* primary cyanolichens. The May sampling had the highest observed temperature (21 °C), while lichen water contents remained similar. Further investigation may show secondary cyanolichens remain more physiologically active at higher temperatures (with equal water contents) than primary cyanolichens, due to their additional green algal photosynthetic partner.

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Introduction

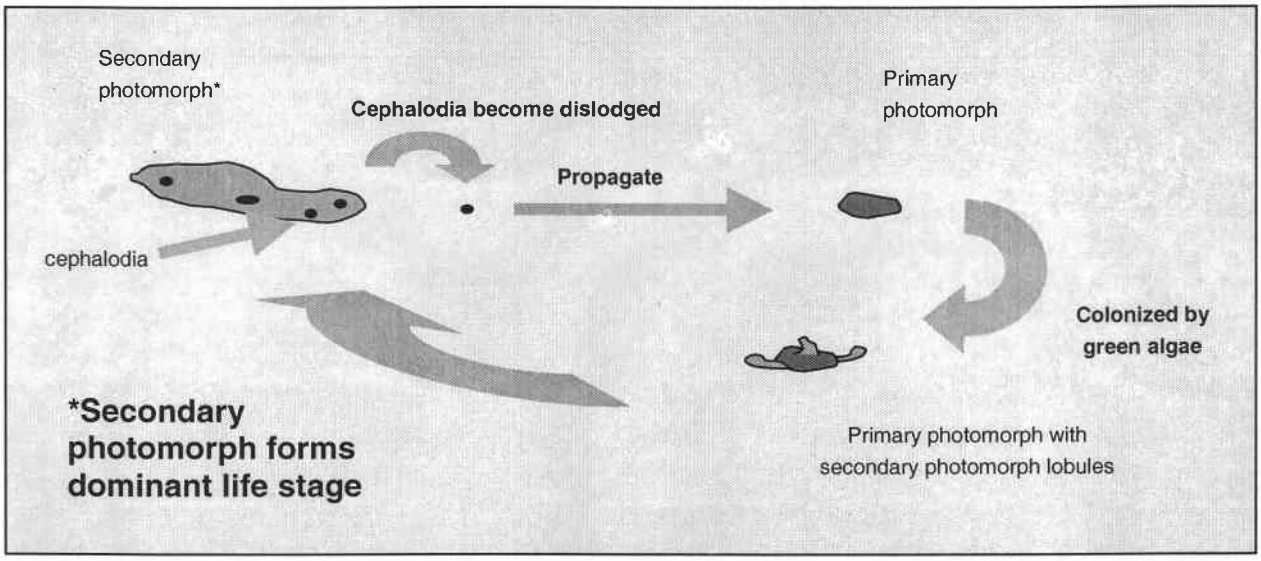
Lichens are symbiotic associations consisting of a fungus and one or two photosynthetic partners: green algae or cyanobacteria, or both. The fungal partner (mycobiont) is constant for a given lichen taxa, while the photosynthetic partner(s) may vary. Photosynthetic partners provide a source of glucose, ATP, and other photosynthetic products to the lichen symbiosis, while the fungal partner provides a microhabitat for the photosynthetic partner(s) in the lichen thallus. Cyanobacteria are unique in their ability to fix atmospheric nitrogen to ammonia products, while also producing photosynthates (see Nash 1996). By fixing and releasing nitrogen to the ecosystem in lichens, cyanobacterial photosynthetic partners provide a valuable limiting e lement in Pacific North-West forests (Dennison 1979, Pike *et al.* 1972).

The term cyanolichen refers to lichens that contain a cyanobacterial photosynthetic partner. Cyanolichens that also contain a green algal photosynthetic partner are referred to as secondary cyanolichens, while primary cyanolichens only contain cyanobacteria as photosynthetic partners. The fungal partner in some secondary cyanolichens can also be found associated with only the cynobacterial partner as a primary cyanolichen. In these cases, the two photosynthetic forms of the cyanolichen are known as photomorphs.

The species *Peltigera britannica* (Gyelnik) Hotan-Hartw. & Tønsberg is mostly found as a secondary cyanolichen, consisting of a fungal, green algal (*Coccomyxa* sp.), and cyanobacterium (*Nostoc* sp.) partner. The cyanobacteria are found on the thallus in isolated pockets surrounded by fungal tissue, termed cephalodia. The cephalodia are located on the laminal surface of the thalli, and become easily dislodged by biotic and abiotic forces. When dislodged the cephalodia will propagate to form independent thalli of the primary photomorph of

P. britannica: *P. britannica* 1°. Eventually green algae will colonize the primary photomorph to form the dominant life stage of *P. britannica*, the secondary photomorph: *P. britannica* 2° (Goward *et al.* 1973; Figure 1). Primary photomorphs are distinguished by the darker color of the photobiont under the cortex, and larger specimens invariably proliferate secondary photomorph lobules, apparently limiting the size and development of the thalli observed.

Figure 1. Photomorph life cycle of cyanolichens with laminal cephalodia, including *Peltigera britannica*



Peltigera britannica 1° contains the same fungal and cyanobacterium species as *P. britannica* 2°. The only distinction between the photomorphs of *P. britannica* is the green algal photosynthetic partner found in *P. britannica* 2°. The photomorphs of *P. britannica* offer a unique opportunity to observe the physiological effect of the green algae photosynthetic partner in cyanolichens.

Peltigera neopolydactyla (Gyelnik) Gyelnik was examined to compare the physiological

activity of the secondary cyanolichen *P. britannica* 2° with a primary cyanolichen of an equal developmental stage. *Peltigera neopolydactyla* contains *Nostoc* sp. as the photosynthetic partner, like *P. britannica*, although it may be a different species. The fungal partners of *P. neopolydactyla* and *P. britannica* are different, but assumed to be closely related, based on lichen thallus morphology.

The results presented here show that both the primary cyanolichens studied, *P. britannica* 1° and *P. neopolydactyla* have higher nitrogenase activity than the secondary cyanolichen *P. britannica* 2° on a lichen biomass scale during wetter and cooler conditions. However, the secondary cyanolichen *P. britannica* 2° was observed to have higher nitrogenase activity than either of the primary cyanolichens studied when environmental conditions were drier and warmer. The differences in nitrogenase activity observed for the cyanolichens under warm, dry environmental conditions (vs. wet and cool periods) suggest the ability of secondary photomorphs/cyanolichens, with their additional photosynthetic partner, to withstand greater dessication stress than primary photomorphs/cyanolichens.

Further, our results show that photosynthetic activity for *P. britannica* 2° was almost always higher (with the exception of one of four sampling dates) than either of the primary cyanolichens sampled. As environmental conditions became warmer and drier (i.e. May sampling), both primary cyanolichens had drastically reduced photosynthetic activity while the secondary cyanolichen, *P. britannica* 2°, experienced no difference in photosynthetic activity, again suggesting the ability of secondary photomorphs/cyanoliches to withstand dessication stress.

Materials & Methods

Lichen samples

The cyanolichens studied, along with their symbiotic components are shown in Table 1. The portions of the thalli that were measured for physiological activity were randomly chosen for all experiments.

Table 1. Lichen species studied. Lichen nomenclature utilizes the fungal species as it's own genus and specific epithet. Though *Peltigera neopolydactyla* and *P. britannica* (1° & 2°) both contain the same genera of cyanobacteria, the species of *Nostoc* is assumed to be more uniform between the photomorphs of *P. britannica* since one (*P. britannica* 1°) is vegetatively reproduced from the other (*P. britannica* 2°)

Lichen/Fungal Partner	Photosynthetic Partner(s)	Species
Peltigera neopolydactyla	Cyanobacteria	*Nostoc* sp.
P. britannica 1°	Cyanobacteria	*Nostoc* sp.
P. britannica 2°	Cyanobacteria & Green Algae	*Nostoc* sp. & *Coccomyxa* sp.

All lichen samples were collected in the Western Cascades Menagerie Wilderness area located in the Willamette National Forest, Sweet Home District, Oregon. All lichen samples were collected along the Trout Creek trail in an open-canopy forest dominated by *Pseudotsuga menziesii*, *Acer macrophyllum* and *A. circinatum*. The understory included *Xerophyllum tenax, Gaultheria shallom*, *Berberis nervosa*, *Polystichum munitum*, *Rhododendron macrophyllum*, and *Asarum caudatum*. Established approximately 80 years ago by forest fire (Smith, personal communication) the forest was well dominated with *Lobaria oregana*, with other cyanolichens *L.*

pulmonaria and *Pseudocyphellaria* sp. present. Elevation was determined at approximately 1700 ft. using topography maps and highway signs (Road Atlas, 2000).

Lichen samples were collected in areas approximately 1.5 m in diameter designated hereon as "collection areas". If one study species was found, the other two study species were collected from a distance of no more than 1.5 m from the original lichen collected. This was done so that lichens analyzed for physiological activity were assumed to have undergone similar micro-habitat conditions (i.e. light, temperature, and humidity). More than one collection area was used during each sampling date. All lichens sampled throughout the entire study period were found no more than three quarters of a mile from one another.

The total number of lichen samples analyzed for each physiological process, along with collection dates are given in Table 2. A late-winter/early-spring period was determined as the period when physiological activity would be greatest, when lichens are fully hydrated after a wet rainy winter (Kershaw 1985, McCune personal communication).

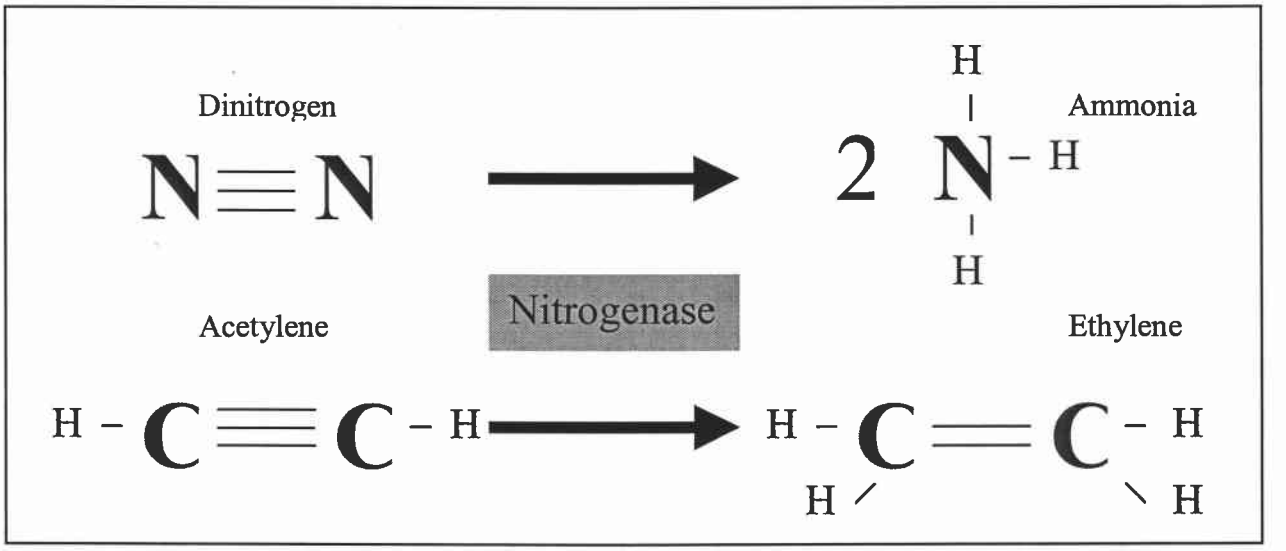
Table 2. Lichen samples collected from the Menagerie Wilderness.

	Acetylene Reduction Assay				
	# of Samples used				
Species of *Peltigera*	1/20/01	2/18/01	4/22/01	5/13/01	Total # sampled
P. britannica 2	4	11	6	7	28
P. britannica 1	4	8	6	7	25
P. neopolydactyla	4	8	6	7	25
	Photosynthesis				
	# of Samples used				
Species of *Peltigera*	1/20/01	2/18/01	4/22/01	5/13/01	Total # sampled
P. britannica 2	5	4	5	5	19
P. britannica 1	5	3	4	7	19
P. neopolydactyla	5	5	5	4	19

Nitrogen Fixation

The acetylene reduction assay (ARA) was used to quantify nitrogen fixation rates by measuring nitrogenase activity. Nitrogenase, the enzyme responsible for catalyzing the conversion of atmospheric dinitrogen to ammonia, also catalyzes the reduction of acetylene to ethylene (Figure 2).

Figure 2. The chemistry behind the acetylene reduction assay. Nitrogenase attacks the triple bonds between the two heavy molecules in the case of dinitrogen and acetylene.



The ARA is a common tool for measuring nitrogenase activity (Stewart et al. 1968, Hardy et al. 1968, Dennison 1979). Today ratios are generally constructed between acetylene reduction and actual nitrogen fixation rates, using $^{15}N_2$ fixation to provide accurate nitrogen fixation rates (Millbank 1981, Nash 1996). The current study was not conducted to analyze the contribution of nitrogen to the ecosystem. Differences of nitrogen fixation rates or nitrogenase activity between lichens were expressed (nmoles C_2H_4 reduced gdw^{-1} hr^{-1}).

The acetylene reduction assay followed the general guidelines presented by Stewart et al. 1968. A mixture of 14% of acetylene to air was used to incubate the lichen samples for one hour in an air-tight container at atmospheric pressure (ca. 1 atm).

Different incubation containers were used during the first sampling of January than all other sampling dates. These containers had a volume of 370 ml and after initial experimentation, it became clear that smaller incubation containers would be more appropriate for the size of (*P. britannica* 1°) lichen thalli available. Thus, 23 mL incubation containers were used for the February, April, and May samplings. All incubation containers used throughout the study were equipped with septa for gas exchange.

After completion of the incubation period, 1.5 mL replicate ARA gas samples were withdrawn from each incubation container and held in air-tight transfer vials. The ARA gas samples were stored for no more than 48 hrs at –11 to –12 °C before gas chromatographic analysis for acetylene and ethylene concentrations were measured. A Perkin Elmer Autosystem flame-ionized Gas Chromatograph (GC) was used at the U.S. EPA Western Ecological Division lab in Corvallis, Oregon.

Gas chromatograph results were used to calculate the nmoles of ethylene produced during the one hr incubation period. The GC provided a percentage of the ethylene present to the total amount of acetylene. Using the molecular mass of the known volume of acetylene used (Ideal Gas Law) during the ARA incubation, the nmoles of ethylene produced were calculated (Figure 3). The nmoles of ethylene produced in an hour was then related to the lichens biomass expressed in grams dry weight (gdw).

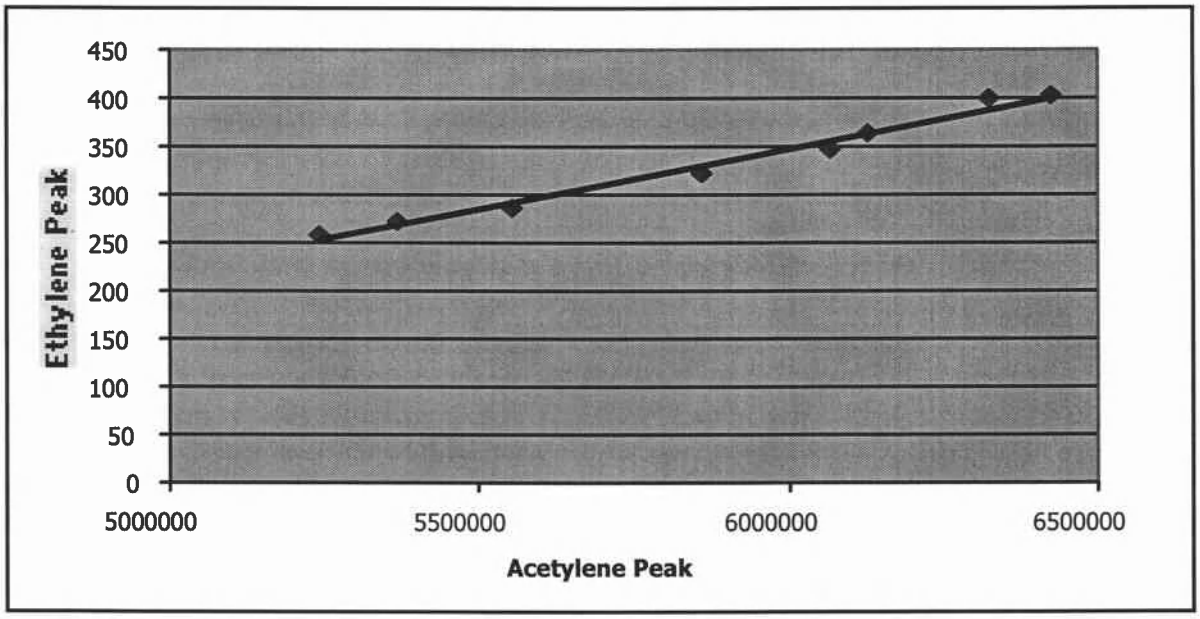
Figure 3. Calculation of the nmoles of ethylene produced.

$$\text{nmoles } C_2H_4 = \frac{C_2H_4^*}{(C_2H_2 + C_2H_4)} \text{ (nmoles } C_2H_2)$$

*ratio from gas chromatograph

The acetylene source used (OXARC chemical) consistently contained traces of background ethylene. The level of ethylene inherent in the acetylene source was assessed using three blank controls incubated with a 14% acetylene to air mixture for the same one hr period exposed to the lichens. Chemical analysis of the control ARA gas samples followed the same procedures outlined earlier. Regression analysis was used to determine the concentration of ethylene found in any concentration of acetylene for the environmental conditions (e.g. temperature & relative humidity) present for each sampling date (Figure 4). The amount of ethylene in the three blank controls was averaged and subtracted from the experimental values.

Figure 4. Regression analysis of control samples from May 13, 2001. Values represent peak values from gas chromatographic analysis. The formula y = 1.2623E-4x - 409.97927, where y = ethylene peak and x = acetylene peak was constructed to analyze the amount of ethylene that was present from the acetylene source and not the acetylene reduction assay (ARA). P-values for x and y variables are 2.74E-5 and 8.41E-7 respectively.



Biomass – Grams Dry Weight (gdw)

Lichen dry weights for each species were determined using two to three lichen samples to establish an estimate for all other lichen samples. Samples were collected and weighed (i.e. fresh weight) on the date of sampling, along with the other lichen samples, using a 0.01 g digital balance. The samples were dried overnight in an oven drier at 105 °C. A ratio was calculated using the averages of the fresh and dry weights. The ratio was applied to fresh weights of all other lichen samples to estimate their gdw.

Photosynthesis

The Li-cor LI-6400 portable photosynthesis system is an infrared gas analyzer that uses an internal reference of CO_2 to measure the CO_2 concentration flux that enters and leaves the

enclosed "leaf" chamber. The LI-6400 controls the irradiance, photosynthetic active radiation (PAR), relative humidity (RH), and temperature of the leaf chamber while also measuring their ambient levels. Photosynthetic measurements were taken simultaneously with the ARA incubation period. Six measurements were taken every 10 sec, and then averaged for each lichen sample. Gross photosynthetic measurements were found by calculating respiration levels using dark photosynthetic measurements under zero irradiance (Figure 5).

Figure 5. Net photosynthesis was found using ambient irradiance levels, while respiration was found using zero irradiance (i.e. dark photosynthesis).

$$Ps_{net} = Ps_{gross} + \text{Respiration}$$

Ambient temperatures, RH, and irradiance were used to measure net photosynthesis with the exception of the irradiance levels used for the May sampling. May 13, 2001 was an unusually cloudy day with lower irradiance levels than measured in earlier months, but also higher temperatures. To obtain positive net photosynthesis measurements the irradiance level used during the April sampling was repeated for the May measurements. Similarly lichen thalli sampled for photosynthesis and ARA during the May sampling were quickly rehydrated and dampened with a paper towel to activate their physiological mechanisms by increasing their water content.

Temperature & Water Content (%)

Temperature was recorded in the field during the one hr period when physiological measurements were taken (Table 3). Water content of lichen samples was determined for both nitrogenase and photosynthetic activity lichen samples. Water content of the lichen samples was estimated by dividing the recorded field weight by the dry weight. The subsequent value was multiplied by one hundred for a percentage. The mean water contents for each sampling date were not significantly different among lichen species with the exception of February (Table 4).

Table 3. Temperatures recorded in the field during one-hour period when ARA incubation and photosynthetic measurements were conducted. Temperatures shown represent the average temp. recorded for each sampling date.

Sample Date 2001	Temperature (°C)
1/20/01	8
2/18/01	14
4/22/01	15
5/13/01	21

Table 4. Mean percent water contents of lichen samples used for physiological measurements conducted at the Menagerie Wilderness. Significant differences among water contents of lichen samples only during the February sampling. All other sampling dates no significant difference among water contents of lichen samples ($p>0.10$, from single factor ANOVA)

Nitrogenase Activity			
	P. britannica 2	*P. britannica* 1	*P. neopolydactyla*
1/20/01	380	340	370
2/18/01	440	330	400
4/22/01	300	240	250
5/13/01	340	300	300
Photosynthetic Activity			
	P. britannica 2	*P. britannica* 1	*P. neopolydactyla*
1/20/01	300	340	230
2/18/01	440	330	400
4/22/01	340	240	260
5/13/01	340	300	310

Results

Nitrogenase Activity

Significant differences in mean nitrogenase activity were found among the three lichens studied ($f = 5.75$, $p < 0.01$, from single-factor ANOVA; Table 5). The secondary cyanolichen *P. britannica* 2° was found to have no significant difference in nitrogenase activity or nitrogen fixation rate compared to its primary photomorph (Figure 6). Of the primary cyanolichens, *P. britannica* 1° was observed to have lower nitrogenase activity than the much more developed *P. neopolydactyla*.

Figure 6. Mean nitrogenase activity of *Peltigera* sp. from acetylene reduction assay measurements taken during Jan. 20, Feb. 18, April 22, and May 13, 2001 at the Menagerie Wilderness. Bars represent standard error of means.

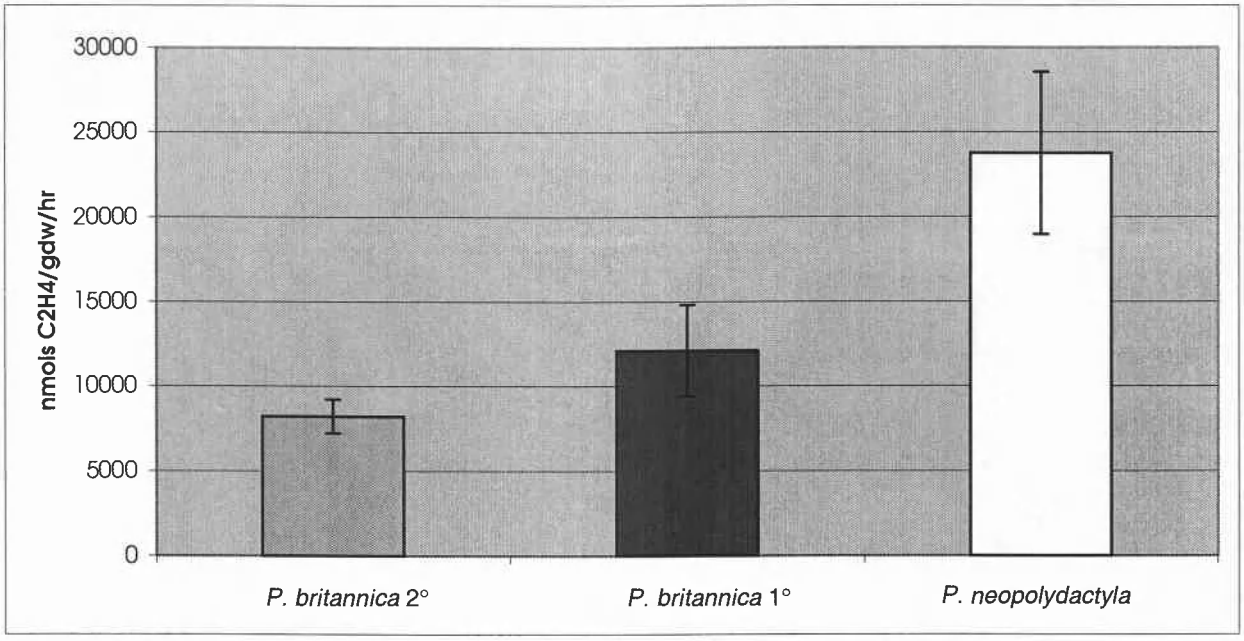


Table 5. Anova single factor analysis of acetylene reduction assay measurements of *Peltigera* species taken on Jan. 20th, Feb. 18th, April 22nd, and May 13th 2001 at the Menagerie Wilderness.

Peltigera species	***P. britannica 2***	***P. britannica 1***	***P. neopolydactyla***
mean nmolsC2H4/gdw/h	8237.45	12130.57	23780.59
Standard Error	2702.75	2553.86	4519.51

Anova Single Factor Analysis

Source of Variation	*SS*	*df*	*P-value*	*F*
Between Groups	3391652442	2	0.002023701	5.7453
Within Groups	18855579312	75		
Total	22247231754	77		

Analysis of nitrogenase activity for each sampling date

Distinctions between nitrogenase activity become more clear by comparing the nitrogenase activity among species during each separate sampling date (Figure 7). Single factor ANOVA analysis (α=0.05) was conducted on nitrogenase activity among all three lichens for each separate sampling date. The results show significant differences among all three lichens studied for each sampling date except for the January sampling (Table 6). Additional single factor anova analysis of only the *P. britannica* photomorphs revealed no significant difference between the photomorphs during the January and February sampling (Table 6).

Figure 7. Nitrogenase activity of *Peltigera* sp. as measured by the acetylene reduction assay during the 2001 study period at the Menagerie Wilderness. Bars represent standard error of means.

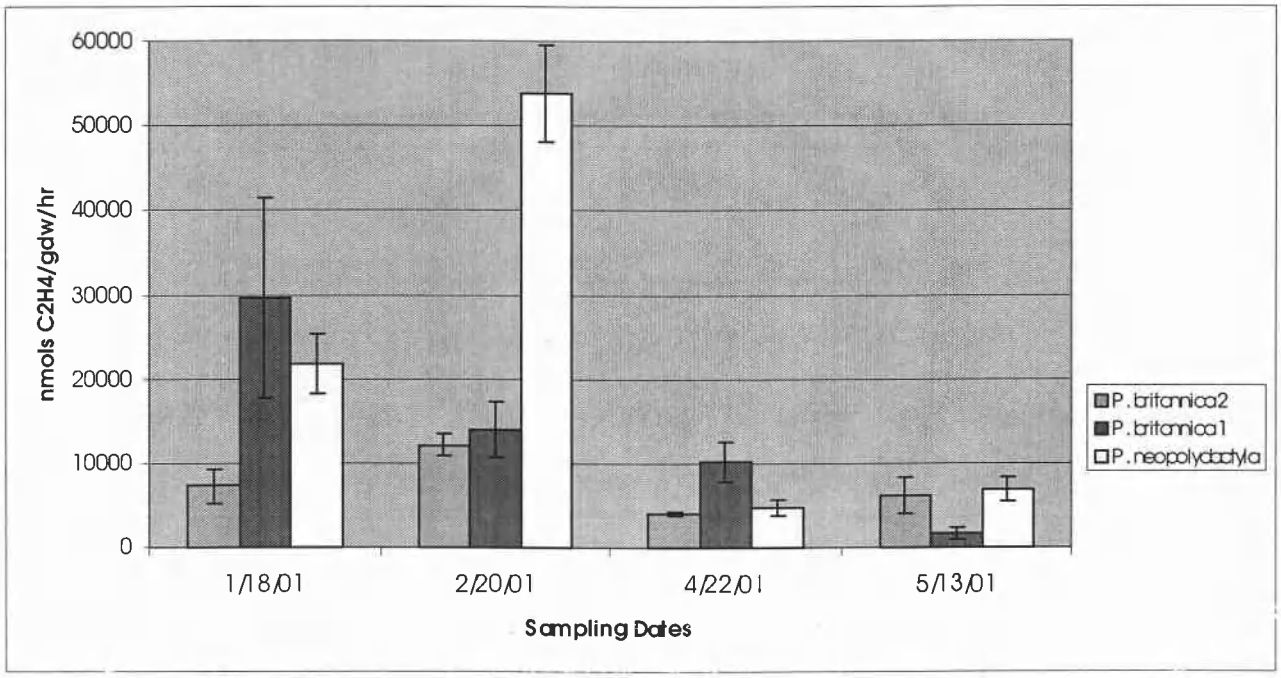


Table 6. Anova single factor analysis (α=0.05) between nitrogenase activity of *Peltigera britannica* photomorphs and *P. neopolydactyla* for each sampling date.

Anova Single Factor Analysis between *Peltigera* species				
	Source of variation			
Date	between sp.	within sp.	F-value	P-value
1/20/01	1.04E+09	1.98E+09	2.35	0.15
2/18/01	9.43E+09	2.58E+09	43.82	9.75E-09
4/22/01	1.33E+08	1.90E+08	5.27	0.018
5/13/01	1.14E+08	2.91E+08	3.54	0.051
Anova Single Factor Analysis between *Peltigera* photomorphs				
	Source of variation			
Date	between sp.	within sp.	F-value	P-value
1/20/01	1.01E+09	1.62E+09	3.72	0.1
2/18/01	1.65E+07	7.68E+08	0.37	0.55
4/22/01	1.12E+08	1.62E+08	6.89	0.025
5/13/01	7.44E+07	2.03E+08	4.4	0.058

During the beginning of the study period, nitrogenase activity of the primary cyanolichens far exceeded that of the secondary cyanolichen *P. britannica*. By the end of the study period *P. britannica*2° outperformed *P. britannica*1°, and was equivalent to *P. neopolydactyla* in terms of nitrogenase activity (f=0.059, p=0.810, from single-factor ANOVA).

Analysis of nitrogenase activity for each *Peltigera* species

No significant difference in nitrogenase activity of *P. britannica* 2° was found between the warmest and driest sampling date, 5/13/01, and the mean of the rest of the sampling period (ANOVA single factor, p-value=0.24, f=1.45, α=0.05). In contrast, both primary cyanolichens studied showed lower activity by the last sampling date of the study period compared with the mean of all other sampling dates (ANOVA single factor: *P. britannica* 1° f=7.55, p<0.05; *P. neopolydactyla*, f=5.90, p<0.05).

Photosynthetic Activity

In contrast to the nitrogenase activity results, *P. britannica* 2° was found to have the highest photosynthetic rates for the dates sampled (Figure 8). Significant differences in photosynthetic activity were found among all three lichens studied (f=6.01, p<0.01, from single-factor ANOVA;Table 7). The mean photosynthetic rates observed in *Peltigera britannica* 2° were twice as high as those of the primary photomorph, *P. britannica* 1°, during the periods sampled.

Figure 8. Mean photosynthetic activity of *Peltigera* sp from Jan. 20, Feb. 20, April 22, and May 13 2001 samplings. as measured by the Li cor LI-6400 photosynthesis system at the Menagerie Wilderness. Bars represent standard error of means.

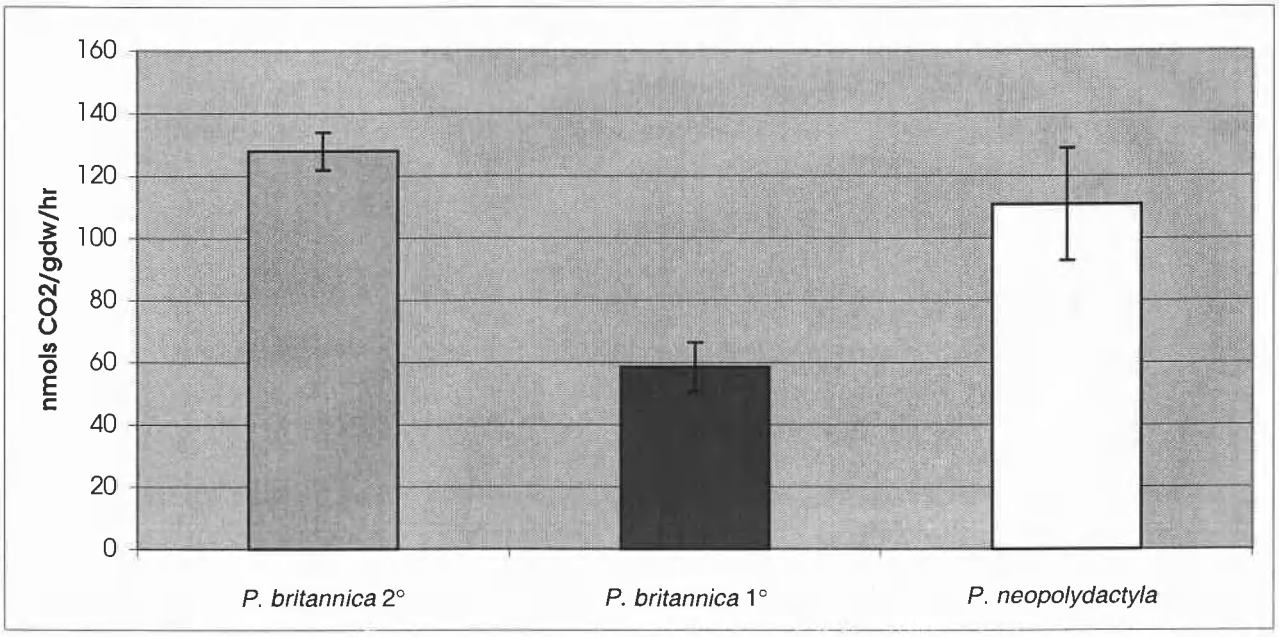


Table 7. Anova single factor analysis of Li cor LI-6400 photosynthesis measurements of *Peltigera* species taken on Jan. 20th, Feb. 18th, April 22nd, and May 13th 2001 at the Menagerie Wilderness.

Species	***P. britannica 2***	***P. britannica 1***	***P. neopolydactyla***		
mean nmols CO2/gdv	127.93	58.55	110.89		
S.E.	16.29	8.31	17.84		
ANOVA single factor					
Source of Variation	*SS*	*df*	*MS*	*P-value*	*F*
Between Groups	49676.0836	2	24838.0418	0.00440688	6.0079
Within Groups	223247.6984	54	4134.216637		
Total	272923.782	56			

Analysis of photosynthetic activity for each sampling date

Photosynthetic activity among cyanolichen taxa was also compared separately for each sampling date (Figure 9). Significant differences in photosynthetic activity among the three

cyanolichens were found only for the January and May sampling dates (Table 8). Comparison between only *P. britannica* photomorphs produced the same result (Table 8). Photosynthetic activity of the photomorphs were virtually equivalent during February and April sampling dates.

Figure 9. Photosynthetic activity of *Peltigera* sp. as measured by the Li cor LI-6400 Photosynthesis system during the 2001 study period at the Menagerie Wilderness. Bars represent standard error of means.

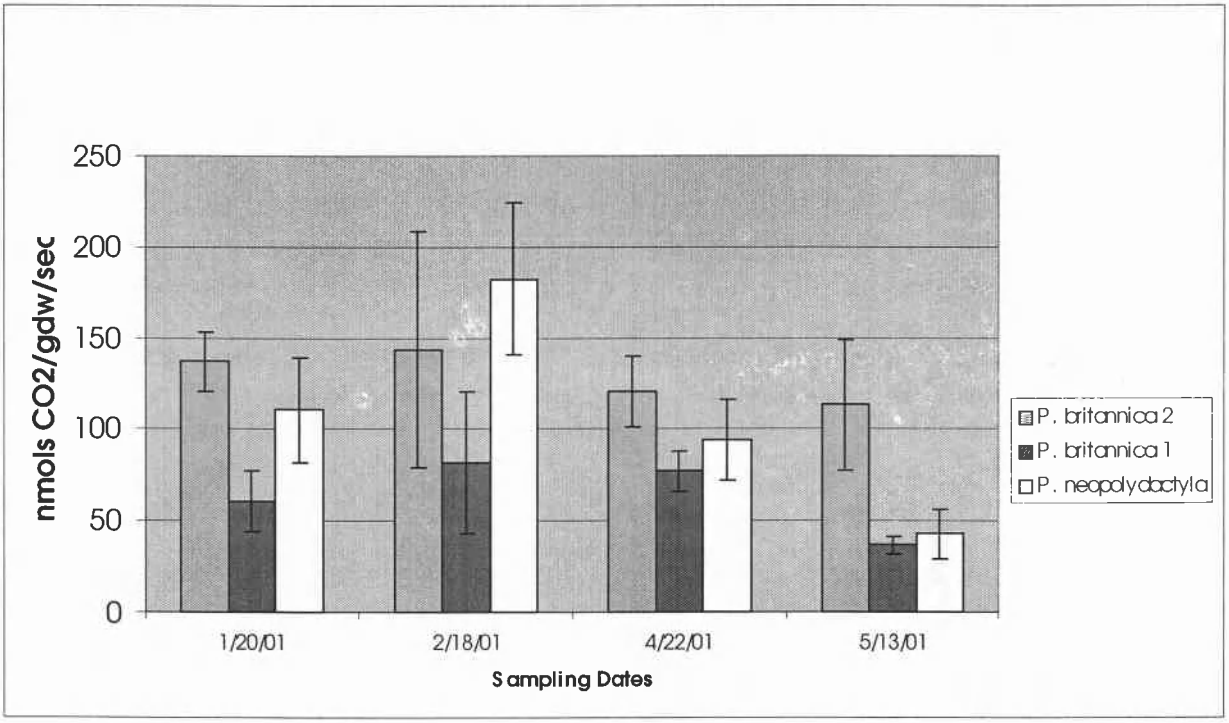


Table 8. Anova single factor analysis (α=0.05) between photosynthetic activity, as measured by the Li cor LI-6400, of *Peltigera britannica* photomorphs and *P. neopolydactyla* on each separate sampling date.

Anova Single Factor Analysis between		***Peltigera***	**species**	
	Source of variation			
Date	between sp.	within sp.	F-value	P-value
1/20/01	1.51E+04	2.74E+04	3.31	0.072
2/18/01	1.91E+04	9.41E+04	0.91	0.44
4/22/01	4.40E+03	1.86E+04	1.3	0.31
5/13/01	1.91E+04	2.86E+04	4.33	0.036
Anova Single Factor Analysis between		***Peltigera***	**photomorphs**	
	Source of variation			
Date	between sp.	within sp.	F-value	P-value
1/20/01	1.46E+04	1.10E+04	10.64	0.012
2/18/01	6.64E+03	5.90E+04	0.56	0.49
4/22/01	4.22E+03	8.80E+03	3.36	0.11
5/13/01	1.71E+04	2.64E+04	6.49	0.029

Throughout the study period, *P. britannica* 1° consistently exhibited the lowest photosynthetic activity, although differences among photosynthetic activity of the three cyanolichens were not significant for the February and April sampling (Table 8).

Analysis of photosynthetic activity for each *Peltigera* species

Photosynthetic activity of *P. britannica* 2° showed little variance throughout the sample period and there was no significant difference in activity among all dates sampled (f=0.16, p=0.92, from single-factor ANOVA; Figure 9). In contrast, the primary cyanolichen *P. neopolydactyla* showed a significant difference in photosynthetic activity among all sampling dates (f=0.16, p=0.039, from single-factor ANOVA). The other primary cyanolichen studied, *P. britannica* 1°, showed no significant difference in photosynthetic activity among all sampling dates (f=1.85, p>0.10, from single-factor ANOVA).

Significant difference in photosynthetic activity of *P. britannica*1° was only observed when comparing the last sampling date of May, with the mean photosynthetic activity for all other sampling dates ($f=5.00$, $p<0.05$, from single-factor ANOVA). In contrast, no significant difference in photosynthetic activity of the secondary cyanolichen *P. britannica* 2° was found between sampling date of May and the mean of all other sampling dates ($f=0.28$, $p>0.50$, from single-factor ANOVA).

Discussion

The secondary cyanolichen *P. britannica* 2° was found to have lower nitrogenase activity but higher photosynthetic activity than both primary cyanolichens studied. Of the primary cyanolichens studied, higher physiological rates (photosynthesis and nitrogenase activity) were consistently observed in *Peltigera neopolydactyla* compared to the less developed (i.e. smaller thalli) *P. britannica* 1°. However, *P. britannica* 2° (with algae and cyanobacteria) showed higher photosynthetic activity than *P. neopolydactyla* (Figure 8). The increased photosynthetic activity observed in the secondary photomorph of *P. britannica* was anticipated, and is most likely due to the chlorophyll provided by the additional photosynthetic partner, *Coccomyxa* species. The markedly decreased photosynthetic activity observed in *P. britannica* 1° with respect to the other cyanolichen studied, *P. neopolydactyla* is notable.

It was unclear what effect the addition of a photosynthetic partner to the *P. britannica* symbiosis would have on nitrogenase activity. Cyanobacteria in secondary cyanolichens have been shown to have higher heterocyst frequency than primary cyanolichens, allowing secondary cyanolichens to fix more nitrogen per cyanobacterium chain (Englund 1977, Hitch & Millbank 1975, Hitch & Millbank 1974). Comparing the heterocyst frequencies of secondary and primary cyanobacterial associations e.g. photomorphs of the same lichen species may reveal the effects of an additional photosynthetic partner more accurately than comparing the activity of distinct secondary and primary cyanolichen species.

Increased heterocyst frequencies in secondary cyanolichens may be due to the additional photosynthetic partner providing photosynthates for the nitrogen metabolism (Hitch & Millbank 1975). Increased heterocyst frequency in secondary cyanolichens may also be due to the

additional photosynthetic partner providing all the photosynthates for the symbiotic organism. The cyanobacterium partner may only fix nitrogen, and the algal partner may only fix carbon in secondary cyanolichens, while both nitrogen and carbon must be fixed by the cyanobacteria in primary cyanolichen symbiosis (Stewart & Rowell 1977).

Though heterocyst frequencies have been studied in both secondary and primary cyanolichens, the data has not been scaled to lichen biomass for ecological relevance. While primary cyanolichens contain their cyanobacteria in the photobiont layer, secondary cyanolichens compartmentalize their cyanobacteria in cephalodia, and the green algae makes up the photobiont layer (see Nash 1996). The primary cyanolichens may be assumed to contain a higher frequency of cyanobacteria cells by lichen mass than secondary cyanolichens. Increasing the heterocyst frequency in *Nostoc* chains increases their nitrogen-fixing efficiency, but the amount of nitrogen fixed in lichen thalli depends upon the total amount of cyanobacteria present, and its heterocyst frequency.

The results show that the secondary cyanolichen studied (*P. britannica* 2°) did not have higher nitrogenase activity, regardless of increased heterocyst frequencies, than the primary photomorph, *P. britannica* 1°. Rather, lower nitrogenase activity was observed in the secondary photomorph. Thus, *P. britannica* 2° may contain less cyanobacteria by lichen biomass, but higher heterocyst frequencies accounting for the observed lower nitrogenase activities. Lab experiments analyzing the total amount of cyanobacteria and heterocysts per gram lichen thallus may confirm the results presented here.

As mentioned earlier, it is unclear whether both *Peltigera* species studied (*P. britannica* and *P. neopolydactyla*) have the same species of *Nostoc* as their photosynthetic partner. Culturing photosynthetic partners is possible and recommended for identification and distinction

of cyanobacteria species, and would allow more extensive studies of physiological responses to different photosynthetic taxa. The observed differences in physiological activity between *P. neopolydactyla* and *P. britannica* 1° may have been affected by unequal lichen thalli size and development. The confounding nature of this factor can be limited by selecting thalli of similar size for physiological measurements. Finding thalli of equal development would require a study site location with an abundance of appropriate samples of all taxa. Small thalli of *P. neopolydactyla* were rarely observed and difficult to distinguish from *P. britannica* 1°. Culturing of *P. britannica* 1° lichen thalli would provide the appropriate sized thalli, but may not accurately reflect ecological significance. Alternately the cultured thalli could be transported and 'transplanted' into the field, allowed to acclimate, and then measured for physiological activity.

P. britannica 2° was found to be more physiologically active (photosynthetic and nitrogenase activity) than it's primary photomorph, *P. britannica* 1°, during the last sampling date of May (Figure 7 & Figure 9). This indicates that secondary cyanolichens may remain physiologically active at higher temperatures and similar hydration levels. However, the results presented here represent only a small window in time, e.g. approximately once a month, and include sampling of the photosynthetic and nitrogen fixing activity of a small number of samples ($n<30$) of two selected cyanolichen species. To confirm these initial observations, a much more extensive study could be implemented.

Using the same techniques to measure photosynthesis and nitrogen fixation with a larger sampling size may conclude the general trends observed in this study. Population sizes of the selected species should also be large enough to provide primary photomorphs for the sample size needed without drastically reducing the photomorph population. Photomorphs provide a unique

opportunity to observe the differences in physiological activity that occur when the green algae is present or absent in the symbiotic cyanolichen relationship. Other photomorphs that may be chosen for study include *P. apthosa* (L.) Willd. and *P. leucophlebia* (Nyl). Gyelnik. Terrestrial photomorphs are easily accessible and may be preferred over the sampling of epiphytic photomorphs.

The results presented here include only four observations recorded during the 'moist season' (i.e. January through May 2001). The number of observations were limited by the resources available to the researcher, including time, equipment, and additional labor. Thus, a much larger number of observations is possible by addressing the availability of the needed resources, possibly on a graduate research level. Increasing the number of observations or samplings (e.g. once a week/day), as well as the number of research sites, would provide a more complete picture of the changes in the physiological activity of cyanolichens over a period of time.

The results presented here show that both the primary cyanolichens studied *P. britannica* 1° and *P. neopolydactyla*, regardless of their presumed lower heterocyst frequencies, have higher nitrogenase activity than the secondary cyanolichen *P. britannica* 2° on a lichen biomass scale during wetter and cooler periods (e.g. January, February & April sampling dates; Figure 7). However, the secondary cyanolichen *P. britannica* 2° was observed to have higher nitrogenase activity than either of the primary cyanolichens studied during the last date sampled of May (Figure 7), when temperatures were warmer (Table 4). The changes in nitrogenase activity observed for the lichens studied during the May sampling suggest the ability of secondary photomorphs/cyanolichens, with their additional photosynthetic partner, to withstand warmer temperatures. In addition, photosynthetic activity for *P. britannica* 2° was higher than either of

the primary cyanolichens sampled, with exception for the February sampling date, when again conditions were cooler and no significant differences were found among the three cyanolichens sampled (Figure 9 & Table 6). In contrast, by the last sampling date of May, the secondary cyanolichen *P. britannica* 2° clearly had higher photosynthetic activity than either of the primary cyanolichens studied (Figure 9), again suggesting the ability of secondary photomorphs/cyanolichens, with their additional photosynthetic partner, to withstand greater temperatures with respect to physiological activity.

Nitrogenase and photosynthetic activity have long been studied and recorded in cyanolichens (Hitch & Millbank 1975, Millbank 1981, Kershaw 1985). This study is, to the knowledge of the author, the first of its kind to study the physiological differences between photosynthetic partners. The differences in activity between the primary *P. britannica* 1° and *P. neopolydactyla* indicate that primary photomorphs may have physiological differences not seen in a closely related primary cyanolichen. Whether these differences are due to fungal and cyanobacterial species distinctions or thalli size is yet to be determined.

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Data from Acetylene Reduction Assay
Menagerie Wilderness 2001

Combining values from entire study period
nmols C2H4/gdw/hr

P. britannica 2	P. britannica 1	P. neopolydactyla
14545.8916	18869.2719	28236.5057
10173.7095	26001.2042	25020.0071
3636.0325	62115.5571	28510.7788
668.177	11740.7187	5627.2262
9569.3944	19199.9865	78648.7934
9863.3733	9735.258	69691.8743
12345.963	33040.671	32672.1755
19115.2317	4132.1234	64160.8179
10820.2194	12829.0864	47966.3104
16645.7973	19343.0362	56069.9128
12452.1238	7935.0051	38167.1965
7378.8937	6325.2588	43496.4679
7315.3658	4447.2068	2547.6941
14648.3526	12625.0961	3377.894
13796.7459	11925.9036	5593.9438
3853.1958	4970.2951	2732.1424
4385.2317	7664.8542	5687.8789
2942.459	19343.0362	8715.3606
3795.0952	369.4575	10268.6885
4369.9145	448.3786	11503.0282
5041.2008	173.8943	3924.6095
17604.3742	1659.0317	10175.9652
5229.9583	2945.637	6228.9747
7002.2036	5355.0917	2092.4016
5899.1916	69.0974	3398.1948
4317.7326		
565.3103		
2667.503		

Data from Acetylene Reduction Assay
Menagerie Wilderness 2001

nmols C2H4/gdw/hr

5/13/01		
P. britannica 2	P. britannica 1	P. neopolydactyla
17604.3742	369.4575	10268.6885
5229.9583	448.3786	11503.0282
7002.2036	173.8943	3924.6095
5899.1916	1659.0317	10175.9652
4317.7326	2945.637	6228.9747
565.3103	5355.0917	2092.4016
2667.503	69.0974	3398.1948

4/22/01		
P. britannica2	P. britannica 1	P. neopolydactyla
3853.1958	4447.2068	2547.6941
4385.2317	12625.0961	3377.894
2942.459	11925.9036	5593.9438
3795.0952	4970.2951	2732.1424
4369.9145	7664.8542	5687.8789
5041.2008	19343.0362	8715.3606

2/18/01		
P. britannica 2	P. britannica1	P. neopolydactyla
9569.3944	19199.9865	78648.7934
9863.3733	9735.258	69691.8743
12345.963	33040.671	32672.1755
19115.2317	4132.1234	64160.8179
10820.2194	12829.0864	47966.3104
16645.7973	19343.0362	56069.9128
12452.1238	7935.0051	38167.1965
7378.8937	6325.2588	43496.4679
7315.3658		
14648.3526		
13796.7459		

1/20/01		
P. britannica 2	P. britannica 1	P. neopolydactyla
14545.8916	18869.2719	28236.5057
10173.7095	26001.2042	25020.0071
3636.0325	62115.5571	28510.7788
668.177	11740.7187	5627.2262

Rooster Rock - Photosynthesis
Photosynthesis as measured by Li Cor 6400

Combining values from entire study period
nmols CO2/gdw/hr

P. brittanica 2	P. brittanica 1	P. neopolydactyla
129.6416	29.1965	101.3854
198.7618	66.0158	54.0029
126.3344	79.5066	201.3521
128.0707	17.6474	145.5524
103.0188	111.1769	50.5981
268.274	45.5749	127.9527
240.03305	159.1792	109.2077
11.9514	40.1278	201.1688
55.2811	83.15	338.0413
64.4194	72.7619	135.78
154.5441	102.1801	39.4172
164.8051	50.1497	141.4461
130.8436	53.748	145.8239
88.6096	14.2242	50.6825
202.0035	38.5863	92.2023
45.6733	44.7344	12.8344
119.0957	42.9554	43.3198
178.9448	27.1277	37.3537
20.4124	34.4473	78.8545

Data from Photosynthetic Measurements
Menagerie Wilderness 2001

nmols CO2/gdw/hr

5/13/01		
P. britannica 2	*P. britannica 1*	*P. neopolydactyla*
202.0035	53.748	12.8344
45.6733	14.2242	43.3198
119.0957	38.5863	37.3537
178.9448	44.7344	78.8545
20.4124	42.9554	
	27.1277	
	34.4473	

4/22/01		
P. brittanica 2	*P. brittanica 1*	*P. neopolydactyla*
64.4194	83.15	39.4172
154.5441	72.7619	141.4461
164.8051	102.1801	145.8239
130.8436	50.1497	50.6825
88.6096		92.2023

2/18/01		
P. brittanica 2	*P. brittanica 1*	*P. neopolydactyla*
268.274	45.5749	127.9527
240.03305	159.1792	109.2077
11.9514	40.1278	201.1688
55.2811		338.0413
		135.78

1/20/01		
P. brittanica 2	*P. brittanica 1*	*P. neopolydactyla*
129.6416	29.1965	101.3854
198.7618	66.0158	54.0029
126.3344	79.5066	201.3521
128.0707	17.6474	145.5524
103.0188	111.1769	50.5981