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Seasonal changes in temperature and light drive photosynthetic acclimation in a lichen.

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Introduction

Lichens are unusual photosynthetic symbioses that persist through many seasonal cycles of environmental change, but are only intermittently active during that change because they are poikilohydric. Photosynthesis and growth of lichens are influenced by seasonal change in the ambient light and temperature environment. Lichens could either acclimate to seasonal environmental changes to maintain carbon reduction or wait to exploit only those interludes of conditions which match a more fixed physiological acclimation state.

Lobaria pulmonaria is an epiphyte of northern temperate, mainly deciduous woodlands which contains a primary green-algal photobiont. Recent studies have shown this lichen to be sensitive to changing light exposure in the field (Gauslaa and Solhaug 1999). In deciduous woods there is a seasonal change in light associated with canopy closure in spring and defoliation in autumn, which subjects lichens to highest light in cold, open-canopy winter months, while those in evergreen forests experience a lower and relatively consistent light. We tracked physiological and molecular parameters to assess the light and temperature acclimation status of *L. pulmonaria* from both deciduous and evergreen forests throughout seasonal environmental change.

Materials and Methods

Populations of *L. pulmonaria* were collected from three deciduous forest sites (Walker Road, N.B., Fenwick Park, N.S., and Sugarloaf Brook, N.S.) and one evergreen forest site (Economy Mountain, N.S.) in southeastern Canada (c. 45_N, 64_W) throughout one full year.

Chlorophyll fluorescence and CO_2 gas exchange measurements were conducted on hydrated lichen tissue disks in a controlled-environment cuvette. Cuvette temperature was either set to the field-ambient temperature at the time of sample collection, or fixed at 20_C to distinguish between effects of temperature and light intensity on PSII function. We calculated PSII electron transport rate (PSII ETR) and NPQ to track seasonal acclimation of PSII. The PSII ETR for the light intensity series were fitted with the model curve (modified from Ricker 1975):

$$\mathrm{ETR} = \alpha \, \mathrm{Ie}^{(-\beta \mathrm{I})}$$

where I is the PPFD. To assess realised field performance we calculated PSII ETR and NPQ at the field-ambient light level at the time of collection. We also estimated the maximum potential capacities of these parameters by measurements at a fixed 20 ºC.

Chlorophylls were extracted with $MgCO_3$-saturated 90% acetone (as in Barnes et al. 1992). Tissue remaining from the chlorophyll extractions were used for protein extraction and

RuBisCO immunodetection (as MacKenzie et al. 2001). Expression of *rbcL* was determined qualitatively using Reverse Transcriptase PCR amplication of sample RNA using DNA primers specific to conserved regions of chlorophyte *rbcL* (MacKenzie et al. 2001).

Results

At field-ambient temperatures, both $ETR_{0.5\ Max}$ and the PPFD to cause $ETR_{0.5\ Max}$ rose from low in winter to high in early summer in the deciduous forest samples, while the permanently closed canopy evergreen site showed less change. The non-photochemical dissipation of light energy (NPQ) at $ETR_{0.5\ Max}$ was low throughout the year except for two transient peaks in the spring and autumn in the deciduous forest samples, coinciding with periods when ambient light exceeded the PPFD required for $ETR_{0.5\ Max}$. At a fixed 20_C, the PPFD for ETR_{Max} and ETR_{Max} and NPQ were higher in the bright winter than in the dark summer in the deciduous forest samples, but the changes in the evergreen forest samples was lower (Fig. 1).

Maximum gross CO_2 uptake at field-ambient temperatures peaked in late spring, and was lowest in the winter. RuBisCO LSU was low from October through April and in the dark summer, but nearly doubled in May in the deciduous forest samples (Fig. 2a). The rates of CO_2 uptake per RuBisCO LSU were in a narrow range from April through November, but were depressed by low temperature in the winter samples (Fig. 2b). Our qualitative data for *rbcL* transcript levels (Fig. 2c) show a pattern closely matching that of RuBisCO LSU concentration. Chlorophyll content rose from basal levels of 85 to 130 μmol m^{-2} to a peak of about 180 μmol m^{-2} in the dark, late summer months, when the chlorophyll *a*/*b* ratio was minimum.

Discussion

In the deciduous forest populations of *L. pulmonaria*, the physiological and macromolecular data suggest that there was a strategy shift in light harvesting and electron transport from energy dissipation in the open canopy cold months to light-limitation in the closed canopy

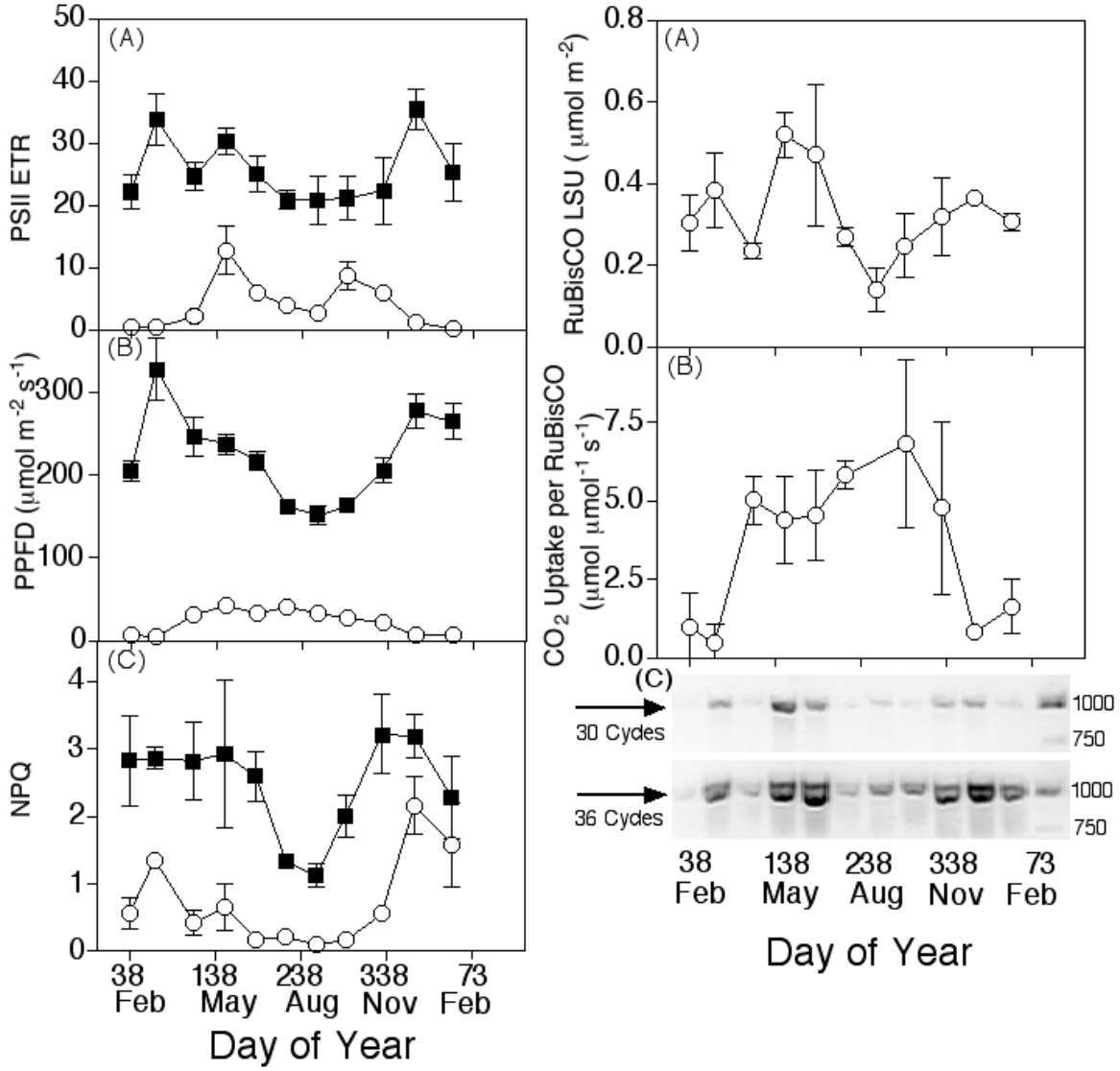

Fig. 1. (A) PSII ETR and (B) NPQ at field-ambient temperature and light conditions (open circles) and at maximum PSII ETR at a fixed 20 ºC (closed squares).

Fig. 2. (A) RuBisCO LSU content, and (B) RT-PCR product density after 30 and 36 amplification cycles. Arrows indicate RT-PCR product density, with lanes aligned by date; lane at extreme right is a DNA size standard with sizes in kilobases as indicated.

summer months. In the open canopy months, chlorophylls and PSII ETR at ambient temperatures were low, despite abundant light. PSII ETR peaked in spring and autumn when high light and moderate temperatures coincided. In the closed canopy warm months, when light was minimal, chlorophylls rose and PSII ETR again fell. Ambient light was in excess of the PPFD to drive $ETR_{0.5\ Max}$ for most of the year, except in the dark summer months.

The performance of the photosynthetic apparatus is sensitive to its redox status, which in turn responds to the integrated light and temperature environment (Huner et al. 1998). We separated seasonal light and temperature effects on PSII ETR and NPQ by fixing measurement temperature at 20 ºC, and showed that the potential for both were high in the bright winter months in the deciduous forest populations, and lower in the darker summer

months. Furthermore, the seasonal patterns of ambient PPFD, PPFD for potential ETR_{Max} and potential ETR_{Max} at 20 ºC were all similar, showing that the lichens actively tracked ambient PPFD by acclimating their potential for electron transport, even though low temperature depressed the realised PSII ETR in winter. The dependence of these seasonal patterns on the light environment was clear because only small seasonal changes were observed in samples from the permanently closed canopy site. The reversal of the seasonal patterns of realised PSII ETR measured at the field-ambient temperature, however, showed the overwhelming importance of temperature in determining the realised performance. RuBisCO LSU content was dynamically modulated in response seasonal light and temperature changes, that maintained and maximised the rate of CO_2 fixed per RuBisCO LSU between April and November.

The large seasonal physiological and molecular changes in *L. pulmonaria* occurred despite the discontinuous nature of lichen physiology, which relies only on infrequent hydration episodes. Temperature caused great physilological change, but changes in ambient light also drove striking physilological changes throughout the seasons in these lichens, allowing them to maintain photosynthetic performance under a wide range of conditions.

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