

# Age Dependence of Photosynthesis in the Caribou Lichen *Cladina stellaris*<sup>1</sup>

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## ABSTRACT

The fruticose thallus of the lichen *Cladina stellaris* (Opiz.) Brodo can be subdivided into individual whorls of branches of known age. Photosynthesis declines steadily with age from a maximum rate of 0.76 milligram CO<sub>2</sub> per gram dry weight per hour in 1-year-old whorls to 0.02 milligram CO<sub>2</sub> per gram dry weight per hour after 15 years. Conversely, the dry biomass of the whorls increases up to age 9 years and then approximately levels off. Photosynthesis in whorls older than 15 years is less than 0.01 milligram per gram per hour. Progressive changes in thallus color with age are associated with the observed photosynthetic decline. Whorls aged 6 years and younger together account for 18% of thallus biomass but 50% of photosynthetic activity. The implications of these results for the idea that the lichen symbiosis results in truly integrated organisms with senescence phenomena akin to those in higher plants is discussed.

Many studies of vascular plants have documented physiological changes associated with the aging of both whole plants and individual organs (18, 19), but very few comparable data exist for nonvascular plants. The control mechanisms which coordinate developmental processes in higher plants (5) may not function effectively in taxa lacking vascular tissues and having only very simple apical organization. This may especially be the case in lichens which are a fungal-algal symbiosis whose status as integrated organisms has been generally recognized only relatively recently (17). Although the nutritional relationships between the symbionts remain in dispute (1), it now appears clear that the lichen symbiosis reflects a long history of coevolution resulting in stable lichen species.

Because of the difficulties in determination of the age of lichen tissue, previous reports of within-thallus variation in Chl concentrations (6, 14), photosynthesis and respiration (3, 13, 14), and growth rates (7) have not usually been directly related to tissue age differences. Only in the fruticose *Cladonia* and *Cladina* lichens which branch once each year can age be readily determined (2). In the abundant caribou lichen, *Cladina stellaris*, Chl concentration and relative growth rate both decline steadily with increasing thallus age (6, 7). The purpose of this paper is to document the related age dependence of photosynthesis, one of the important measures of plant senescence (15), in *C. stellaris* and briefly discuss its implications in lichen biology.

## MATERIALS AND METHODS

*Cladina stellaris* (Opiz.) Brodo was collected in a subarctic lichen woodland near Schefferville, Quebec (11) in late June 1981,

air-dried at room temperature, flown to Montreal, and stored dry at -20°C until assayed for photosynthetic activity. These collection and storage procedures do not effect subsequent determinations of lichen gas exchange (10). Fine forceps were used to dissect 10 individual thalli from the collected lichen mats with the lichen lightly wetted by a mist of distilled H<sub>2</sub>O to avoid loss of branch tips which are very brittle when dry. These 10 thalli were photographed, briefly labeled with <sup>14</sup>CO<sub>2</sub>, immediately killed, and then partitioned into annual branch whorls. Each whorl of known age was weighed, combusted to recover <sup>14</sup>CO<sub>2</sub> in a scintillation cocktail, and counted to determine its photosynthesis. The relevant details of these experimental procedures are provided below.

The sampled thallus was submerged 15 min in an artificial rain solution (11) to initiate recovery of its drought dormant metabolism, centrifuged to remove excess water that can impede gas exchange, and then sealed into a Plexiglas cuvette. Cooling water circulated through the double wall of the cuvette maintained tissue temperature at 20°C under a PAR flux density of 2,000 μE m<sup>-2</sup> s<sup>-1</sup>. The cuvette design assured high irradiance of the entire thallus, and a squirrel cage fan within the cuvette rapidly mixed the airstream. After 60 s, radioactive air with a CO<sub>2</sub> concentration of 362 μl l<sup>-1</sup> and a <sup>14</sup>CO<sub>2</sub> specific activity of 1.48 mCi mmol<sup>-1</sup> flowed through the cuvette at 0.34 l m<sup>-1</sup> for exactly 60 s. The cuvette was immediately flushed with normal air at 3.0 l m<sup>-1</sup>; after 60 s, the lichen was removed, immersed in liquid N<sub>2</sub> to stop metabolic activity, and then killed by rapid drying in a 105°C oven.

The dead, oven-dried lichen was partitioned into tissues of different ages based on the branching pattern of the thallus. The anisotomic, usually tetrachotomous branching of *C. stellaris* produces one set or whorl of four branches each year (2). One of the branches becomes larger and ascends vertically to extend the main axis of the thallus. Thus, counting back from the apex, the thallus can be neatly subdivided into each successive year's whorl of branches. Because of intercalary growth in *C. stellaris* (7), the individual whorls are, however, comprised of mixed age tissues. In this paper, age therefore refers to the number of years since a branch whorl was initiated. Dry biomass of each annual whorl was measured on a Cahn model G-2 electrobalance to the nearest 0.01 mg. For five of the 10 replicate thalli, the colors present on each annual whorl when wetted were recorded under standardized lighting conditions by comparison to the ISCC-NBS centroid color charts (12). The lichens were only lightly wetted by a mist of distilled H<sub>2</sub>O to avoid leaching of any labeled solutes.

The annual whorls of each thallus were individually combusted in an Intertechnique Oxymat and assayed for radioactivity on a Beckmann LS-200 Scintillation Counter. Quench correction was by the internal standard channels-ratio method (21) and photosynthetic rates were calculated as mg CO<sub>2</sub> g<sup>-1</sup> dry weight h<sup>-1</sup> (20).

## RESULTS

Figure 1 summarizes both the mean biomass and the photosynthetic rates of different age branch whorls within the *C. stellaris*

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thallus. Photosynthesis declines steadily with age from a maximum rate of  $0.76 \text{ mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$  in 1-year-old branch whorls to only  $0.02 \text{ mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$  after 15 years. Conversely, whorl biomass increases up to about age 9 and then approximately levels off. The 1.2-mg biomass of a 1-year-old branch whorl increases to an asymptote some 20 times greater. The peak biomass at 9 years in this population is associated with the frequent appearance of a secondary branch system at this age which can develop into an essentially independent thallus.

For a number of reasons, the photosynthesis and biomass results are reported only through 15 years even though individual samples included branch whorls up to 26 years old. First, beyond 15 years the thalli begin to disintegrate making accurate biomass estimates increasingly difficult. Second, photosynthetic rates after 15 years are all below  $0.01 \text{ mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$  and the contribution of these older tissues to thallus photosynthesis is therefore negligible. Finally, the analysis of thallus color (Fig. 2) provides some ancillary evidence that the primary activities of fungal as well as algal metabolism are localized in this younger portion of the thallus. The youngest, most photosynthetically active tissues are mainly pale yellowish green and become increasingly yellowish white with age. By age 12, the brownish pink associated with senescent tissues appears and is followed by increasing proportions of light grayish reddish brown and light grayish yellowish brown. The

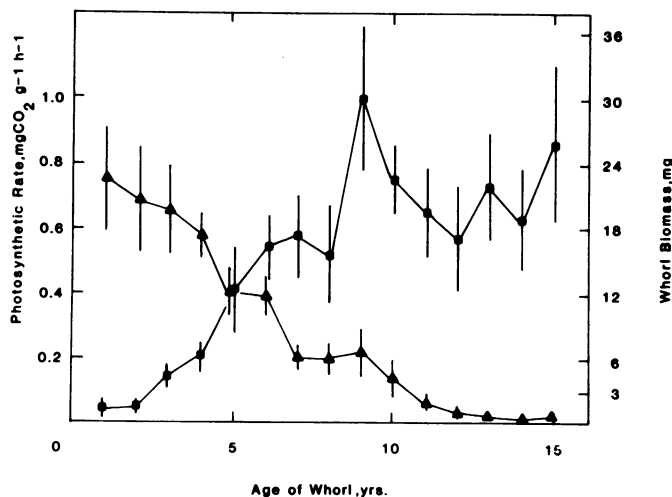


FIG. 1. The age dependence of dry biomass (■) and photosynthetic rate (▲) in individual branch whorls within a thallus of *C. stellaris*. The values are the mean  $\pm$  SE based on 10 replicates.

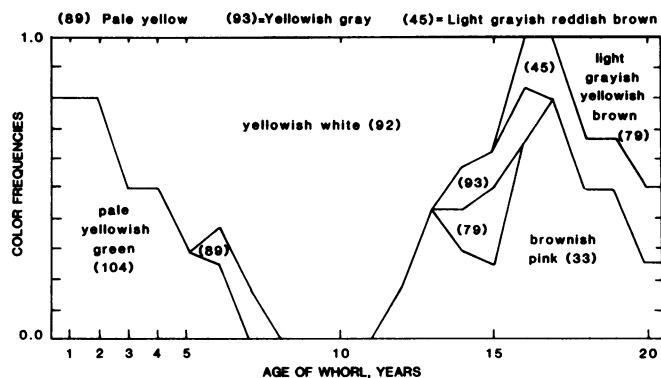


FIG. 2. The age dependence of surface color in individual branch whorls within a thallus of *C. stellaris*. The colors of five thalli wetted by distilled  $\text{H}_2\text{O}$  were determined by comparison to the ISCC-NBS centroid color chart (12); numbers and descriptive names in the figure directly follow this standard color reference system.

yellowish white typical of photosynthetically active tissues does not however completely fade until a branch whorl reaches age 16. This regular pattern of color change with age which depends in part on fungal secondary products (4) further supports the decision to limit the present discussion to whorls 15 years old or younger.

Considering trends in biomass distribution and photosynthetic rates in branch whorls up to 15 years old, the contribution of each age of whorl to photosynthesis by the entire thallus can be determined (Fig. 3). The high photosynthetic rates of the youngest whorl is offset by its low biomass so that it contributes only  $0.0003 \text{ mg CO}_2 \text{ h}^{-1}$  to thallus photosynthesis (Fig. 3A), 0.8% of the total photosynthetic activity (Fig. 3B). The contribution of the young whorls increases rapidly to a peak of  $0.0067 \text{ mg CO}_2 \text{ h}^{-1}$  at age 6. The whorls 6 years old and younger together account for only 18% of the thallus biomass but 50% of its photosynthetic activity. In this population of *C. stellaris*, a secondary peak of  $0.0052 \text{ mg CO}_2 \text{ h}^{-1}$  occurs in 9-year-old whorls after which the photosynthetic contribution of older whorls drops steadily. Whorls of age 9 or younger represent 45% of the thallus biomass but provide 82% of its photosynthetic capacity.

## DISCUSSION

After their initiation, the photosynthetic capacity of branch whorls on the fruticose lichen *C. stellaris* gradually declines reaching essentially zero after 15 years, but the growth of these branch whorls stops after only 9 years. This developmental pattern differs from that in most plant leaves where maximal photosynthetic rates occur at the time of full leaf expansion which is achieved in weeks rather than years (15, 19); this photosynthetic pattern is maintained even in evergreen leaves which, like the lichen branch whorls considered here, can persist for many years (15). Despite such differences in temporal dynamics, the consistent age dependence of these phenomena in *C. stellaris* suggests some similarities to the closely integrated syndrome of events associated with senescence in higher plants (19).

Competition for light among spatially removed portions of the thallus, a correlative factor known to influence senescence in vascular plants (19), may be especially important in controlling lichen senescence. Vertical light extinction within the thallus of *C. stellaris* is very rapid with only about 10% of the surface irradiance penetrating below the upper 40% of the thallus (9). This is likely to result in the reduction of the algal symbiont in older tissues deep in the mat with consequent loss of photosynthetic capacity; Chl concentrations do decline rapidly with depth in the *C. stellaris* mat (14). Such light competition or some similar factor must be

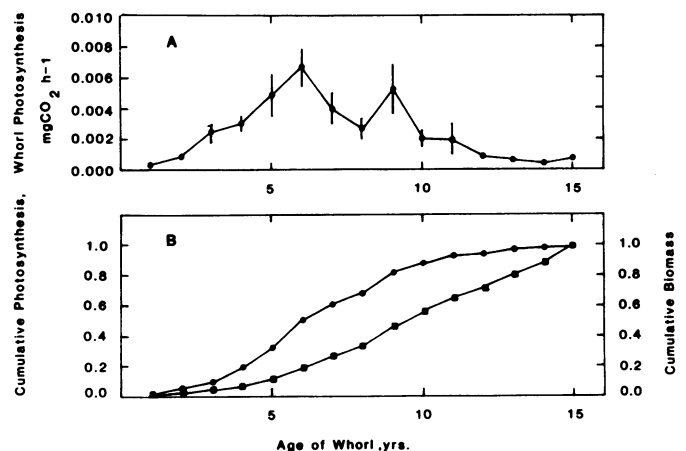


FIG. 3. The photosynthetic contribution of each branch whorl in relation to age (A) and the cumulative distribution with age of photosynthesis (●) and biomass (■) within the thallus of *C. stellaris* (B).

influencing metabolic activity within the lichen because isolated fragments of even older lichen tissue can regenerate vital new thalli (3, 8).

Whether or not further work on lichen physiology supports such analogies to senescence phenomena in higher plants, it remains clear that the growth and production of *C. stellaris* is heavily dependent on the youngest tissues within the thallus. Fully 82% of the photosynthetic capacity of the thallus resides in tissues 9 years old or younger which comprise only 45% of the thallus biomass. The remaining 55% of the thallus may be marginally sustained by its relatively meager photosynthetic activity or may depend at least in part on photosynthate translocated from the more active, younger tissues. Despite our considerable knowledge of translocation between the symbionts (16), we know virtually nothing of carbohydrate translocation within the thallus. The age dependence of photosynthesis observed here emphasizes the need for additional studies of the integrated physiology of the whole lichen thallus.

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