The Role of Anthocyanins for Photosynthesis of Alaskan Arctic Evergreens During Snowmelt

STEVEN F. OBERBAUER[1] and GREGORY STARR[1,2]

[1]*Department of Biological Sciences, Florida International University, Miami, FL 33199, USA*
[2]*School of Forestry Resources and Conservation, University of Florida, Gainesville, FL 32611, USA*

ABSTRACT

The period during spring snowmelt represents a challenging time for arctic tundra evergreens. As a result of relatively clear skies and increasing solar zeniths, radiation loads can be high, even under snow cover, while air and soil temperatures may be very low. Anthocyanin pigmentation is a prominent feature of most tundra evergreens during the spring melt. We suggest that these anthocyanins protect plants from photodamage at this critical period. The patterns of anthocyanin production and occurrence in tundra evergreen plants are compatible with light screening by these pigments. Anthocyanins are located in the outer palisade cells, consistent with protection of the photosynthetic tissues from radiation. Anthocyanin concentrations increase while plants remain under snow cover and attain their maximum shortly after snowmelt in the spring, when radiation levels are highest but temperatures remain low. Photosynthetic activity is present under the snow in tundra evergreen plants, and is similar in pattern to anthocyanin concentrations, increases as depth of snow cover declines. Anthocyanin content declines once temperatures increase during the growing season, but increase again with the occurrence of fall frost at the end of the growing season. Variation in anthocynanin pigmentation is related to plant microsite (exposure to light) and leaf cohort; leaves with high nitrogen (exposed or young leaves) have higher anthocyanin concentrations. We believe this light screening strategy is an important aspect of the acquisition of carbon reserves for many tundra evergreen plants during the short growing season.

Advances in Botanical Research Vol. 37
incorporating Advances in Plant Pathology
ISBN 0-12-005937-1

Copyright © 2002 Academic Press
All rights of reproduction in any form reserved

I. Introduction 130
A. Challenges to Plants During Snowmelt 130
B. Anthocyanins in Tundra Evergreen Plants 132
II. Evidence for Photoprotection by Anthocyanins 134
A. Subnivian Microclimate 134
B. Photosynthesis During Snowmelt 134
C. Seasonal Changes in Pigment Concentrations 137
D. Photosynthesis and Pigmentation 138
E. Importance of Microsite 139
F. Effects of Leaf Age 140
III. Conclusions and Perspectives 142
Acknowledgements 143
References 143

I. INTRODUCTION

A. CHALLENGES TO PLANTS DURING SNOWMELT

Until recently, research on the physiology of vascular plants in arctic tundra has focused on the short summer growing season on the assumption that conditions during the remainder of the year were too harsh to allow for any significant leaf physiological activity (Tieszen, 1981). However, striking changes in anthocyanin pigmentation in many arctic evergreens observed over winter and spring and evidence for a photoprotective function of these pigments (Gould *et al.*, 1995, 2000; Feild *et al.*, 2001; Hoch *et al.*, 2001) suggest that important physiological processes are taking place at these times (Grogan *et al.*, 2001; Starr and Oberbauer, unpublished data). Several recent studies have provided strong evidence that ecosystem level processes detected above snow cover during the winter and spring in the Arctic are a result of the active physiology of vascular plants under snow cover (Oechel *et al.*, 2000; Grogan *et al.*, 2001; Starr and Oberbauer, unpublished data). Anthocyanins may play an important role in these processes during this critical period of the year.

The growing season in the Alaskan Arctic is typically short, lasting only 8–12 weeks during which plants must accumulate sufficient carbon to provide for growth, reproduction, and maintenance. Any mechanisms that maximize carbon gain during this period are likely to be advantageous. Much of the year outside the growing season is characterized by little or no sunlight and temperatures substantially below freezing (Chapin and Shaver, 1985). However, for several weeks in the spring and fall, irradiance levels may be quite high, while temperatures are highly variable; moderate temperatures above freezing may be interspersed with hard freezes. In May and early June, snow cover often persists long after air temperatures and light levels would be suitable for photosynthesis of vascular plants without snow cover. Timing of snowmelt, however, is

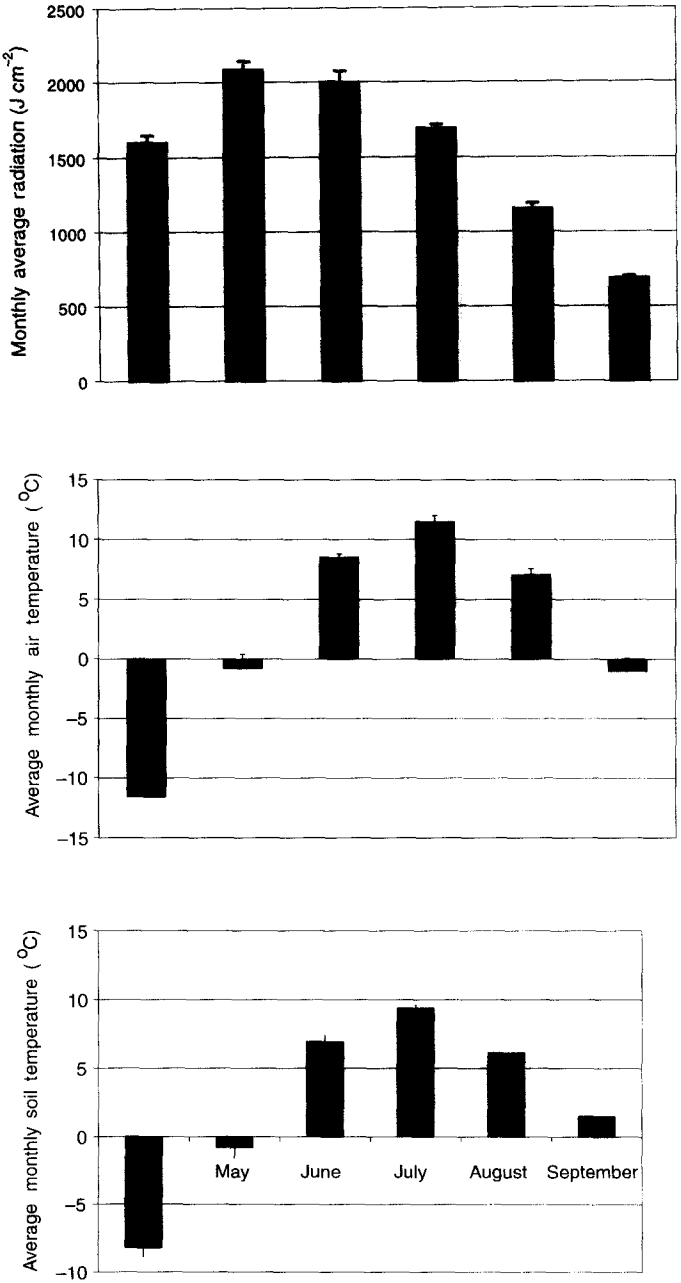

Fig. 1. Monthly mean global radiation (shortwave, J cm^{-2}) for 1990–2000 (upper) and mean monthly air (middle) and soil temperature at 5 cm depth (lower) at Toolik Field Station Long Term Ecological Research weather station.

highly variable and depends on both snow depth and density, arrival of warm air masses, and prevalence of clear skies. Snow 20 cm deep can melt in a few days or persist for weeks. Date of snowmelt at Toolik Lake, Alaska over the last 7 years has varied by 26 d with the earliest date on 13 May and the latest 9 June. At Imnavait Creek, a site close to Toolik, full snow melt ranged from 12 May to 11 June over a 17 year record (Kane *et al.*, 2000).

The period during snowmelt represents a challenging time for tundra evergreens. As a result of relative clear skies and increasing solar zeniths, radiation loads can be very high. At Toolik, the highest radiation loads of the year occur in May (Fig. 1). Because significant radiation penetrates the snow (Hamerlynck and Smith, 1994; Liston *et al.*, 1999; Starr and Oberbauer, unpublished data), evergreen plants are exposed to light levels that can be either potentially damaging or useful. As snow melts and plants project above the snow, radiation levels on leaves can be extreme due to reflectance off the surface of the snow (DeLucia *et al.*, 1991). At the same time, plants may experience relatively low air temperatures, and equally important, soil temperatures below freezing (Fig. 1). Low temperatures inhibit photosynthesis both by increasing the CO_2 diffusion resistances within leaves when solutes freeze (~ –5°C) and by directly affecting photosynthetic membrane and enzyme function (Kappen, 1993; Larcher, 1995). Low soil temperatures increase root resistance (Kramer, 1940) and water viscosity (Nobel, 1999). The combination of high irradiances and low temperatures are known to cause significant photoinhibition and photodamage to leaves exposed to the combination of these conditions (Hamerlynck and Smith, 1994; Manuel *et al.*, 1999; Germino and Smith, 2000; Gould *et al.*, 2000).

Evergreen plants under the snow and during snow melt have several alternatives for coping with the high radiation loads (Streb *et al.*, 1998; Manuel *et al.*, 1999; Germino and Smith, 2000): use the light for carbon fixation; minimize absorption by increased reflectance or minimizing cross-sectional area exposed; screen the high irradiance with photoprotective pigments such as anthocyanins; and dissipate the energy via photochemical and non-photochemical means. A combination of these processes is likely occurring in tundra evergreens during snowmelt.

B. ANTHOCYANINS IN TUNDRA EVERGREEN PLANTS

The presence of leaf anthocyanins is extremely widespread among tundra plants, with spectacular fall coloration in deciduous species the norm. However, anthocyanins are also widespread in evergreen tundra species in the Ericaceae or closely related families (Plate 5, Table I). Notable

TABLE I

Common tundra evergreens at Toolik Lake, Alaska showing strong seasonal variation in anthocynanin pigmentation. Nomenclature follows that of Hultén (1968)

Species	Family	Growth form
Andromeda polifolia L.	Ericaceae	Dwarf erect shrub
Cassiope tetragona (L). D. Don	Ericaceae	Dwarf erect shrub
Diapensia lapponicum L.	Ericaceae	Cushion plant
Empetrum nigrum L.	Ericaceae	Dwarf creeping shrub
Ledum palustre (L.)	Ericaceae	Dwarf erect shrub
Oxycoccus microcarpus Turcz.	Ericaceae	Filiform prostrate
Rhododendron lapponicum (L.) Wahlenb.	Ericaceae	Dwarf erect shrub
Vaccinium vitis-idaea L.	Ericaceae	Dwarf creeping shrub
Pyrola grandiflora Radius	Pyrolaceae	Rosette
Dryas integrifolia M. Vahl.	Rosaceae	Dwarf creeping shrub

exceptions are the wintergreen graminoids, including the dominant species in tussock tundra, *Eriophorum vaginatum*. In four species examined (*Cassiope tetragona*, *Empetrum nigrum*, *Ledum palustre*, and *Vaccinium vitis-idaea*), anthocyanins were found in palisade cells just beneath the outer epidermis (Plate 5).

The hypothesis underlying this study is that anthocyanins play an important role in photoprotection of photosynthetic apparatus in tundra evergreen species under the high irradiances and low temperatures during snow melt. This hypothesis, 'the light screen hypothesis', suggests that anthocyanins protect the photosynthetic apparatus from photoinhibition when high light is coupled with low air temperatures (Feild *et al.*, 2001; Hoch *et al.*, 2001). The light screen hypothesis engenders a number of testable predictions. These are that anthocyanins should be:

1. located in leaf tissues so as to encounter incoming radiation before it is intercepted by the bulk of the photosynthetic cells;
2. at their highest concentrations when radiation levels are highest and temperatures are low;
3. highest in tissues with the greatest investment in the photosynthetic apparatus.

Although this hypothesis has been evaluated for leaves of temperate species during autumn senescence, this is the first evaluation of the hypothesis for arctic evergreen species during the spring snowmelt period. The intent of this paper is to review recent findings on the role of anthocyanins for photosynthesis of arctic evergreens during the snow melt season. The majority of the findings come from studies conducted on evergreen plants near Toolik, Alaska (68° 38′N, 149° 34′W, elevation 730 m; Walker *et al.*, 1994) during May of 1997, 1998, 1999, 2000 and 2001.

II. EVIDENCE FOR PHOTOPROTECTION BY ANTHOCYANINS

A. SUBNIVIAN MICROCLIMATE

As melt-out approaches, springtime snow cover creates an environment that is favorable for photosynthesis of plants under the snow. The insulative properties of snow buffer plant temperatures so they usually remain above –5.0°C at this time of year despite air temperatures that may be substantially lower. Midday temperatures of monitored leaves approached or exceeded 0°C. Spot measurements of temperature in the air spaces and at the moss/soil surface within the depth hoar can be 5.0°C warmer than air temperatures above the snow (Starr and Oberbauer, unpublished data). Sufficient light penetrates beneath the snow to drive photosynthesis at depths less than 30 cm (Fig. 2). At snow depths of 28 cm, photosynthetic photon flux density (PPFD) can reach 300 μmol m^2 s^{-1} and increase to 800 μmol m^2 s^{-1} at solar noon when snow depths are below 20 cm. At the same time, build up of respiratory CO_2 under the snow may enhance CO_2 uptake (Starr and Oberbauer, unpublished data). CO_2 concentrations within the depth hoar layer are substantially elevated over ambient atmospheric levels with average values ranging from 405 to 675 and individual readings exceeding 1000 μmol mol^{-1} (Fahnestock *et al.*, 1998; Starr and Oberbauer, unpublished data).

B. PHOTOSYNTHESIS DURING SNOWMELT

That tundra evergreen plants are photosynthetically competent upon emergence from the snow has been known for some time (Oberbauer *et al.*, 1996), but clear evidence that photosynthetic activity under the snow occurs in tundra evergreens has only recently been reported (Starr, 2000; Starr and Oberbauer, unpublished data). Significant photosystem II (PSII) activity and carbon uptake capacity has been found in leaves of four tundra species, *Eriophorum vaginatum*, *Ledum palustre*, *Vaccinium vitis-idaea* and *Cassiope tetragona*. Although the averages across all plants for both parameters were low, some individuals of all species showed high photosynthetic capacity. Rates as high as 35% of the species physiological maximum were recorded: 3.98, 1.42, 1.60, and 2.51 μmol CO_2 m^{-2} s^{-1} for *E. vaginatum*, *L. palustre*, *V. vitis-idaea*, and *C. tetragona* respectively. Rates under snow measured at saturating light (A_{max}) in spring 1999 were elevated relative to A_{amb} rates in 1998 (Table II). The mean A_{max} of three of the species was well above the physiological compensation point (Table II) and for some plants exceeded 60% of A_{max} at mid-season (Oberbauer and Oechel, 1989). *Ledum palustre* showed less photosynthetic activity, averaging near the compensation point (Table II). Snow cover depth and A_{max} were not significantly correlated for the four

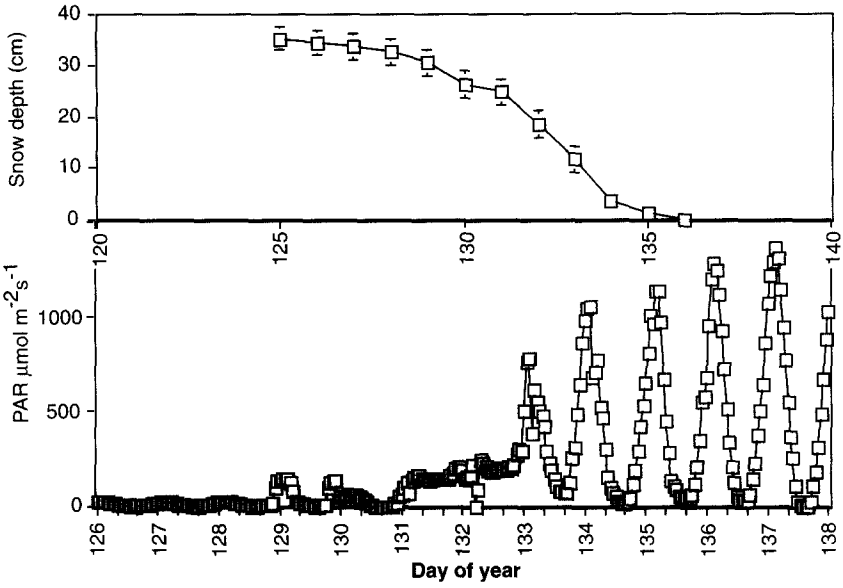

Fig. 2. Light penetration to the vegetation under the snow and snow depth during the spring of 1999, a year with very rapid snowmelt. Values are means of 19 sensors.

species (Table III), probably as a result of variation in temperature and CO_2 concentrations.

The average F_v/F_m of these four species was approximately half of their summer maximum (Table II, Figure 3). Similar to A_{max}, the variance in F_v/F_m was high: some individuals exceeded 90% of summer maximum. Unlike A_{max}, F_v/F_m increased as depth of snow cover decreased. This relationship was statistically significant for *E. vaginatum*, *L. palustre*, *V. vitis-idaea*, but not for *C. tetragona* (Table III).

All four species do not show equivalent photosynthetic activity under the snow. The lower photosynthetic capacity of *L. palustre* may be attributed in part to their leaf orientation. During winter the leaves of this species are strongly deflexed against the stem, reducing light absorption and possibly hindering the diffusion of CO_2 to the stomata (Smith *et al.*, 1997). One would also expect the CO_2 exchange of the other three species to be higher than measured because they maintained a relatively high level of PSII activity. Low soil temperatures under these conditions likely limit water uptake and induce stomatal closure (Delucia, 1986; Day *et al.*, 1989, 1990). Laboratory studies of *E. vaginatum* report stomatal closure in response to low soil temperatures independent of air temperature (Starr, Neuman and Oberbauer, unpublished data). Manuel *et al.* (1999) reported that the wintergreen alpine plant, *Geum montanum*, is not subject to photodamage at high light, low temperature conditions,

TABLE II

Mean photosynthesis (1 ± SE) under ambient light (A_{amb}, 1998) and saturating light (A_{max}, 1999) and F_v/F_m for evergreens under the snow. n = 50 measured in May of 1998 or 1999

	Ledum palustre	*Vaccinium vitis-idaea*	*Eriophorum vaginatum*	*Cassiope tetragona*
1998				
A_{amb} (μmol m^{-2} s^{-1})	–0.364 ± 0.157	0.032 ± 0.119	–0.066 ± 0.500	0.089 ± 0.411
F_v/F_m	0.294 ± 0.009	0.332 ± 0.007	0.448 ± 0.011	0.339 ± 0.012
1999				
A_{max} (μmol m^{-2} s^{-1})	–0.06 ± 0.16	1.05 ± 0.16	1.31 ± 0.65	1.11 ± 0.31
F_v/F_m	0.33 ± 0.02	0.39 ± 0.01	0.46 ± 0.02	0.40 ± 0.04

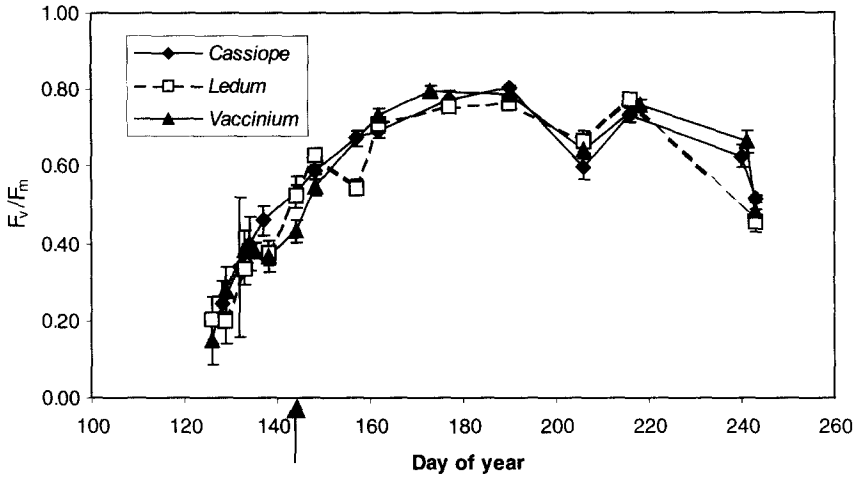

Fig. 3. Seasonal patterns of chlorophyll fluorescence (F_v/F_m, mean ± 1 SE) of three arctic evergreens in Alaskan tundra during the 1999 growing season at Toolik Lake, Alaska, n = 10. Arrow at day of year 142 indicates completion of snowmelt.

even under desiccation stress. This tolerance apparently results from an interplay between photorespiration and photoassimilation by a combination of mechanisms including shifting excitation energy from PSII to photosystem I (PSI), cyclic electron transport, and the xanthophyll cycle. Similar mechanisms are likely interacting in the arctic evergreens along with light screening by anthocyanins.

For tundra plants, the requirement for carbon acquisition is great after a long winter during which light input is effectively zero and foliar respiration accounts for large carbon losses. With these four species able to activate their photosynthesis under the snow during the spring, they partially compensate for their winter losses. This activity also enables the plants to reach their maximum photosynthetic capacity quickly once melt has occurred (Defoliart *et al.*, 1988; Kudo, 1999). Consequently subnivean photosynthesis appears to be an adaptive trait for survival of the species at high latitudes, where the growing season is limited (Huner *et al.*, 1998).

C. SEASONAL CHANGES IN PIGMENT CONCENTRATIONS

Of the four species examined, the sedge, *E. vaginatum*, did not contain any anthocyanins. The three species of ericaceae (*Cassiope*, *Vaccinium*, and *Ledum*) emerge from the snow in the spring with intense anthocyanin pigmentation (Starr and Oberbauer, unpublished data). Excavation of plants under the snow revealed that anthocynanin concentrations, while

already elevated under deep snow, significantly increased as snow depth decreased in *L. palustre* and *V. vitis-idaea* (Table III). The same tendency was found for *C. tetragona*. In all three species, anthocyanin concentrations reach their maximum immediately following snowmelt, then rapidly decline during the short growing season (Fig. 4), a finding consistent with the predictions of the light screen hypothesis. In the early fall anthocyanin production increases again following the onset of fall frosts. Fall concentrations were much lower than those encountered during the spring season as snow melts. The limited production of pigments during the fall can be attributed to the relatively lower light levels at that time of the year: at Toolik, day length in August decreases approximately 18 min/d and cloud cover is typically high (Fig. 1).

Total carotenoid concentrations also increased with decreasing snow depth in three of the four species, but the increase was significant only for *C. tetragona* (Table III). In the exception, *V. vitis-idaea*, carotenoids significantly decreased as snowmelt proceeded in the spring. Most study species tended to increase both their chlorophyll a and chlorophyll b concentrations as snow depth declined, however *C. tetragona* was the only one to show significant changes during this period (Table III).

D. PHOTOSYNTHESIS AND PIGMENTATION

Based on the light screening hypothesis, a relationship between pigment concentrations and CO_2 exchange might be expected. Correlations between pigments concentrations and A_{max} were not significant, again probably because of variation in subnivean CO_2 concentrations and temperatures. However, significant correlations were found between potential quantum yield, F_v/F_m, and anthocyanin concentrations for *L. palustre* and *V. vitis-idaea* ($P<0.001$ for all cases), but not for *C. tetragona* (Figs 3 and 4). The same trend was also seen between total carotenoids

TABLE III

*Pearson correlation analyses of photosynthetic characteristics and pigment concentrations with snow depth. r-values are presented. * indicates significance at P<0.05 and ** at P<0.001*

	Ledum palustre	*Vaccinium vitis-idaea*	*Eriophorum vaginatum*	*Cassiope tetragona*
A_{max}	0.1726	0.0418	0.0327	0.2557
F_v/F_m	–0.4255**	–0.3937**	–0.4255**	–0.2504
Chlorophyll a	0.0730	–0.2161	–0.2013	–0.2162
Chlorophyll b	–0.0132	0.1679	–0.1286	–0.2868*
Total carotenoids	–0.0462	0.3110*	–0.2527	–0.3000*