A COMPARATIVE STUDY OF THE PHOTOSYNTHETIC ACTION SPECTRA FOR A DECIDUOUS AND FOUR CONIFEROUS TREE SPECIES

by

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A COMPARATIVE STUDY OF THE PHOTOSYNTHETIC ACTION SPECTRA FOR A DECIDUOUS AND FOUR CONIFEROUS TREE SPECIES.

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ABSTRACT

The isoenergetic action spectra of apparent photosynthesis (APS) for intact current-year foliage of five tree species were determined from 400 - 710 nm by CO_2 exchange analysis. The blue (400 - 500 nm) peak of APS activity for the green broad leaf of alder was reduced to a plateau for the green needle leaves of Douglas-fir and Sitka spruce, a shoulder for the blue-green needles of Colorado spruce, and a reduced shoulder for the blue-white needles of Blue spruce. These differences were due neither to a differential selective blue light stimulation of photorespiration nor to the differential presence of a non-plastid screening pigment. The conifers had very similar carotenoid/chlorophyll ratios with 40 - 50% more carotenoid relative to the chlorophyll as compared to alder. Blue light absorption and low efficiency of energy transfer by the carotenoids probably accounts for the low APS activity of the conifer group compared to alder. Reflectance spectrometry, scanning electron microscopy, and CO_2 exchange data showed that the blue colour and low APS activities of Colorado and Blue spruce results from the enhanced selective reflection of blue light which is a secondary "carry over" effect of the UV scattering properties of the needle bloom. From these results it is concluded that the basic photosynthetic apparatus of these broadleaf and conifer plants are the same.

DEDICATION

To my late father,

whose own work in the physiology of photosynthesis in conifers was an inspiration to me, and whose interest and encouragement enabled me to bring this endeavour to a rational conclusion.

ACKNOWLEDGMENTS

Without the assistance and interest of several individuals, this study would not have been completed. My thanks are due to my supervisor Dr. G. R. Lister, who initially suggested the project as "the action spectra of photosynthesis of several conifer species", for his assistance in pigment analyses and determination of reflectance spectra; to Drs. W. E. Vidaver, R. C. Brooke, and K. Loach for their helpful comments and criticism during revision of the manuscript; to Mr. J. Glasbergen of the Highway Nursery in Burnaby, who obtained the Colorado and Blue spruce seedlings; to Dorothy Turkowski for her enthusiasm during the several "planting and tagging" parties; to Henk Dykstra of the S. F. U. Electronics Shop who spent many hours with me diagnosing erratic IRGA's; to Mr. Geoff Hollingdale for the scanning electron microscopy; to my fellow graduate students Messrs. Tom Brown and Wayne Duval for the spectral intensity measurements of the xenon lamp and solar radiation and Alan Williams for his data on the specific leaf area of alder; to Mr. Peter Foreman for the photographs; and finally to Shirley Heap, for her competent typing.

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GENERAL INTRODUCTION

It has been shown for some conifers that blue light sustains relatively less photosynthesis than for broadleaf plants (Burns 1942, Gaudillere and Costes 1971, Linder 1971). It is not known whether this peculiarity is present only in the reported conifer species or if it indicates general group differences in photosynthesis between the conifers and the broadleaf plants. The less efficient utilization of blue light must be related to either (1) differences in the conifers' basic photosynthetic apparatus, or (2) species-dependent differences in the optical properties of the conifers' needle-type leaves.

The evidence favours the second possibility. Poskuta (1968a) reported that respiration during photosynthesis (photorespiration) was selectively stimulated by blue light in white spruce. However, Bulley, Nelson and Tregunna (1969) and Voskresenskaya et al. (1970) were unable to demonstrate a selective blue light effect on photorespiration in a variety of herbaceous broadleaf plants, therefore the net CO_2 uptake during photosynthesis for white spruce could possibly be reduced by photorespiration as compared to the uptake in such broadleaf plants. For two pine species, the reduced photosynthetic activity under blue light was attributed to absorp-

tion by photosynthetically inactive pigments (Burns 1942, Linder 1971).

These reports do not provide sufficient evidence however, to reject either one or the other possibility. Only Burns (1942) made a direct comparison of the two leaf types (pine and wheat) under the same experimental conditions. Secondly, any possible influence of selective light filtering occurring at the leaf surface was not considered.

The purpose of this study then is to first determine the relationship between photosynthesis and the wavelength of light (action spectrum) for several conifer and a broadleaf species on a consistent and comparable basis (Part I). Any relative differences noted will be tested to determine if they could have arisen from species-dependent differences in the following photosynthesis-screening mechanisms:

(a) metabolic screening by stimulation of respiration during photosynthesis (Part II),

(b) absorption screening by photosynthetically inactive pigments (Part III),

(c) physical screening by light filtering at the leaf surface (Part IV).

On the basis of these results, it can be determined whether or not the basic photosynthetic apparatus of conifers is the same as for broadleaf plants.

PART I

THE PHOTOSYNTHETIC ACTION SPECTRA FOR A DECIDUOUS AND FOUR CONIFEROUS TREE SPECIES

INTRODUCTION

For a light-dependent biological process such as photosynthesis, the action spectrum is a plot of the rate of photosynthesis as a function of wavelength for either a constant irradiant flux density (incident light intensity, Heath 1969) or quantum flux density (Fork and Amesz 1969). The most preferable basis for quantum yield studies is a constant number of quanta absorbed by the photoactive molecules (Rabinowitch and Govindjee 1969). By comparing the action spectrum with the absorption spectrum for the same cells or plant organ, conclusions as to which pigments are concerned and their relative efficiencies in the process, can be drawn. Whatever the type of action spectrum, it is essential that the quantity of light be the rate-limiting factor so that the variations in the photosynthetic rate are due specifically to the absorption characteristics of the pigments.

Since the absorption of light energy by molecules is a quantum process and because the energy per quantum varies with the wavelength, the photosynthetic rate depends on the total energy of a light beam only insofar as the energy content determines the number of quanta. The representation of an action

spectrum as a function of isoenergetic beams therefore does not take into consideration the mechanisms responsible for the absorption of light energy. The relative effectiveness of photons from the shorter wavelengths is reduced in an isoenergetic action spectrum because the quantum energy is inversely proportional to the wavelength of the light. Fortunately for comparative purposes, only the relative photosynthetic effectiveness of different wavelengths is required under standardized light conditions. The relative ease of measuring light in energy units and of obtaining isoenergetic light beams as compared to the difficulties involved in measuring the number of absorbed quanta at each wavelength (Heath 1969) justifies the expression of the photosynthetic action spectra in this study on an equal incident energy basis.

The experimental condition will be such that the only variable is the plant materials used. Any relative differences between the action spectra will therefore be due to either (1) species-dependent plant factors serving to mask the effects of different wavelengths of light before reaching the site of action within the photochemical apparatus, or (2) basic differences in the photochemical apparatus.

MATERIALS

TREES

Seedlings of the following types were obtained (Plate 1):

(a) Two-year old Douglas-fir (Pseudotsuga menziesii (Mirb.)

Plate 1. Typical examples of the tree seedlings used in this study.

Back row (l-r): Alder, Douglas-fir, Colorado spruce.

Front row (l-r): Blue spruce and Sitka spruce.

Franco) coastal, low-altitude type and Sitka spruce (Picea sitchensis (Bong.) Carr.), from the British Columbia Forest Service Green Timbers Nursery,

(b) Two "blue" forms of spruce, five-year old Colorado spruce (Picea pungens Engelm.) and a two-year old blue-white variety of Colorado spruce grafted stock, hereafter referred to as Blue spruce (Picea pungens var. hoopsii), both from a local ornamental tree nursery,

(c) Two-year old red alder (Alnus rubra Bong.) from Burnaby Mountain.

The particular tree materials were selected to provide comparisons between the effects of leaf form (broad vs needle) and colouration (green vs "blue") and their relationship to the respective photosynthetic action spectra. The local broadleaf deciduous tree, red alder, was chosen as being somewhat intermediate between the annual herbaceous broad leaf and the evergreen conifer needle leaf. Similarly, the foliage of Douglas-fir fills the "green, flat needle" gap between Sitka spruce and alder.

To ensure that the photosynthetic tissues were of comparable physiological ages, the experiments were conducted with current-year foliage from mid-summer onwards by which time the high rates of respiration associated with the spring flush of leaf expansion should have declined to relatively low and stable levels (Bourdeau 1959, Clark 1961, Ludlow and Jarvis 1971a).

The seedlings of all species were tagged and potted out

into field plots to be grown under natural conditions until brought into the laboratory.

MEASUREMENT OF PHOTOSYNTHESIS

Gas exchange measurements were made on attached current-year shoots and leaves in a closed circuit system (Figure 1.1) consisting of the photosynthesis chamber (Cuvette), a Beckman 215 infra-red (CO_2) gas analyser (IRGA), flowmeter (Matheson #303), O_2 monitor (Chemtronics), gas circulation pump (Neptune Dyna-pump) and interconnecting glass and tygon tubing. During any one series of gas exchange measurements made over several days, the plants were returned each evening to the field plots to maintain their normal photoperiodicity.

The IRGA, equipped with a Riken Denshi SP-J2 potentiometric (10 mv) recorder, was used on the 0 - 600 ppm (v/v) CO_2 scale. Standard gases, obtained from Matheson of Canada, were cross-checked against special gas mixing pumps from H. Wösthoff O. H. G. Germany (Series NA 18/2aF) before being used for IRGA calibration. The IRGA monitored the change of CO_2 concentration with time inside the closed system. Since respiratory processes are still in progress within the tissues even during photosynthesis, the rate of change of CO_2 concentration is a measure of the "apparent" rate of photosynthesis (APS). APS rates are positive only when the incident light intensity drives photosynthesis at a rate sufficient to counterbalance the respiratory processes, hence a decreasing CO_2 concentration with time indicates a net CO_2 uptake by the

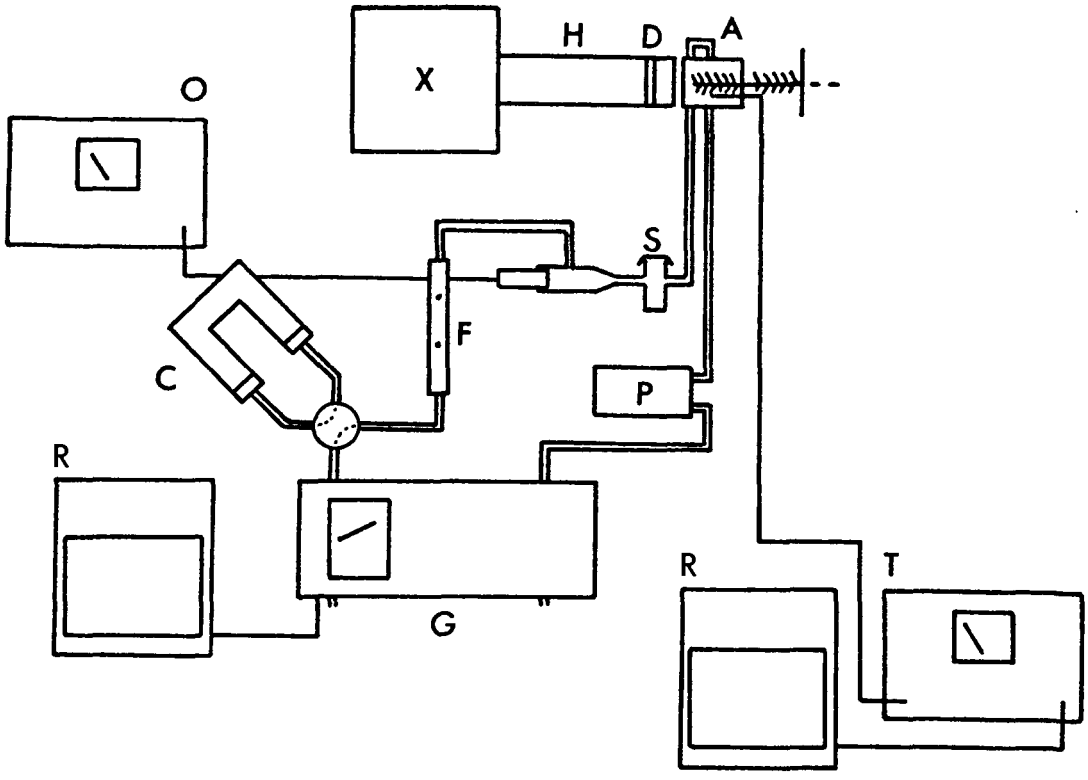


Figure 1.1 General diagram of the apparatus used for the measurement of changes in CO_2 concentration with time for the determination of the rates of photosynthesis and respiration (not to scale)

A	Cuvette	O	O_2 analyzer with probe
C	CO_2 absorber	P	Circulation pump
D	Dicrolite heat filter	R	Recorders
F	Flowmeter	S	Serum cap
G	Gas analyzer (IRGA)	T	Tele-thermometer with probe
H	Heat filter (15 cm H_2O)	X	Xenon lamp and housing

enclosed plant tissue; conversely, a rising CO_2 concentration indicates a net CO_2 evolution.

The rate of apparent photosynthesis in $\mu g\ CO_2\ min^{-1}$ unit plant tissue^{-1} was calculated from the following formula:

$$APS = \frac{\Delta\ CO_2\ (ppm)}{\Delta\ time\ (min)} \times \frac{10^{-6}}{ppm} \times \frac{44 \times 10^3\ \mu g\ CO_2}{22.4\ ml \times 1.095} \times \frac{V\ ml}{Z\ (dm^2\ or\ g)}$$

where:

APS = rate of apparent photosynthesis as net CO_2 exchange

$\frac{\Delta CO_2}{\Delta time}$ = rate of change of CO_2 concentration in ppm min^{-1},

$\frac{10^{-6}}{ppm}$ = conversion from ppm to whole units,

$\frac{44 \times 10^3\ \mu g\ CO_2}{22.4\ ml}$ = molar volume of pure CO_2 at 760 mm Hg and 0 C (STP),

1.095 = correction factor for molar volume of a gas from STP to the standard experimental conditions of 750 mm and 22 C,

V = volume of the closed system, 266 ml for alder and 305 ml for the conifers,

Z = amount of enclosed plant tissue (leaf area, one side only, in dm^2 for alder and branch ovendry weight, ODW-24 hours at 80 C, in g for the conifers).

Although the alder ODW was not determined, the specific leaf area ($cm^2\ g^{-1}$ ODW) of 177 (Krueger and Ruth 1970) or 220 - 240 (Williams, personal communication) provides an approximate conversion factor for the alder APS rates from a leaf area to a leaf weight basis.

Variations in the CO_2 concentration in the system during

photosynthesis measurements were held between 325.0 and 275.0 ppm. The relationship between photosynthesis and CO_2 supply was assumed to be linear over this range (Jackson and Volk 1970, Ludlow and Jarvis 1971a), hence all rates of photosynthesis are quoted at 300 ppm, the mean value. At lower light intensities, when the CO_2 compensation point (CCP; APS = zero) approached 300 ppm, a correspondingly smaller range of CO_2 concentration was used in the calculation. Other useful information not specifically required for the calculations, is the gas flow rate (approx. 2.5 l min^{-1}) indicating that the gas in the system was being recirculated several times a minute, ensuring adequate CO_2 supply and turbulence over the tissue.

THE LIGHT SOURCE

The plant material in the photosynthesis chamber was illuminated by a Hanovia 5,000 watt xenon arc lamp (Plate 2). The beam was focussed with a quartz lens through 15 cm of water and a Dicrolite heat filter before entering the photosynthesis chamber. The quality of the light was modified by introducing different narrow-bandpass interference filters (Schott-Depal and Balzer Filtraflex; average half band widths - 16 nm and 11 nm, respectively) into the beam before the light entered the chamber (Plate 3). The light intensity was controlled with Balzer neutral density filters and measured using a Yellow Springs Instrument Company (YSI) radiometer - model 65, with the probe housed in a brass heat sink to reduce baseline drift. The heat sink was designed to slip onto the filter

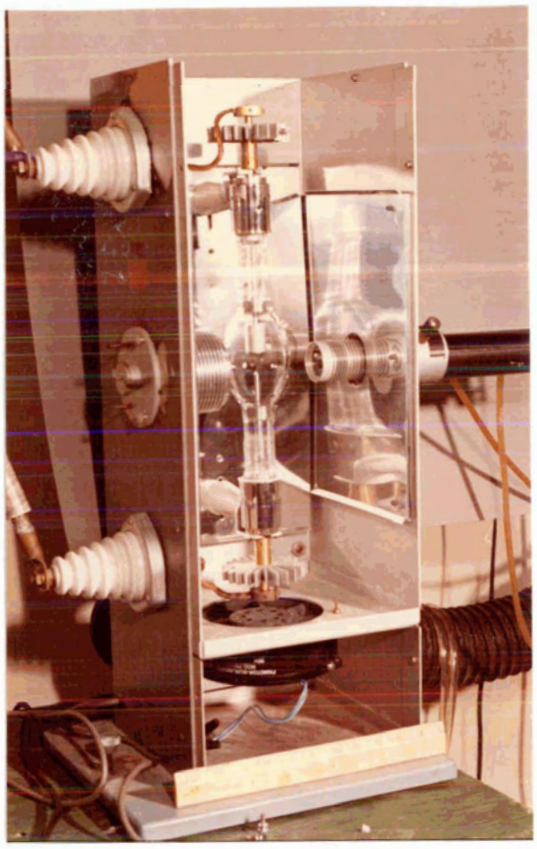


Plate 2. Hanovia 5,000 watt xenon arc lamp in Oriel Optics housing complete with focussing lens and spherical rear reflector.

Note: Adaptors and cooling fins on each bulb terminal, axial flow, high capacity fan in base of lamp housing (replacing squirrel-cage fan). These modifications increased ventilation and prolonged the bulb life.

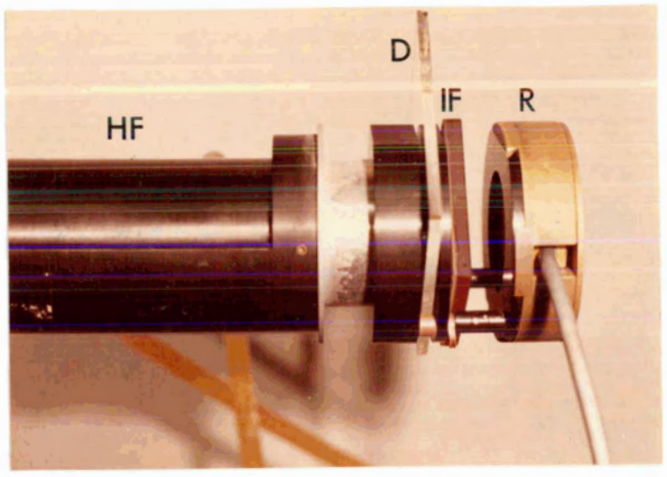


Plate 3. Details of light-train: (from l - r)

Continuous flow 15 cm water heat filter (HF)

Dicrolite heat filter (D)

Interference filter (IF). During actual measurement of light intensity and experiments, these filters are positioned as close as possible to the radiometer face and the tissue cuvette.

Brass heat sink with radiometer probe (R)

holder in a manner such that all light intensity measurements were consistently made at the centre of the light beam (Plate 3). Darkness in the chamber was achieved by placing a metal plate into the filter holder to block the light beam.

PLANT CUVETTES

Two cuvettes, each consisting of two main parts, were designed to hold the different plant materials. One, constructed of plexiglass, was used for the essentially two-dimensional, small alder leaves and the other, mostly of brass, was for use with the conifer twigs. Because the light beam illuminated a circular area of only 9.6 cm^2, both cuvettes were circular in face view with an internal diameter of 3.5 cm. They differed basically only in their internal depths, 4 to 5 mm for the leaf chamber versus 4.5 cm for the conifer chamber, and in the point of entry for the "attached" experimental material.

The alder leaf chamber (Plate 4A) was integrated into the main plexiglass body and after the leaf petiole had been sealed into its slot with Apiezon Q sealing compound, the transparent faceplate complete with O-ring was twist-locked into position simultaneously forming the front wall of the chamber and sealing it. The sealed-in leaf was maintained in the central plane of the chamber by fine support wires in the body and the face-plate, such that the three incoming air streams were split by the leaf edge to pass over both the front and back leaf surfaces (Plate 4B). To exclude room light all the cuvettes' surfaces

Plate 4A. Alder leaf cuvette.

Plate 4B. Composite cross section of Alder leaf cuvette.

(X - Z — detail construction of the gas inflow and outflow ports)

Y - Z — detail construction of temperature probe port)

CS - cuvette support

PS - petiole slot

OR - O-ring

FP - faceplate

TP - temperature probe port

I - gas inflow port (3 sub-ports)

O - gas outflow port (3 sub-ports)

W - leaf support wires

FA - faceplate anchor posts

L - leaf

← - gas flow pattern

TH - thermistor probe

Plate 4A

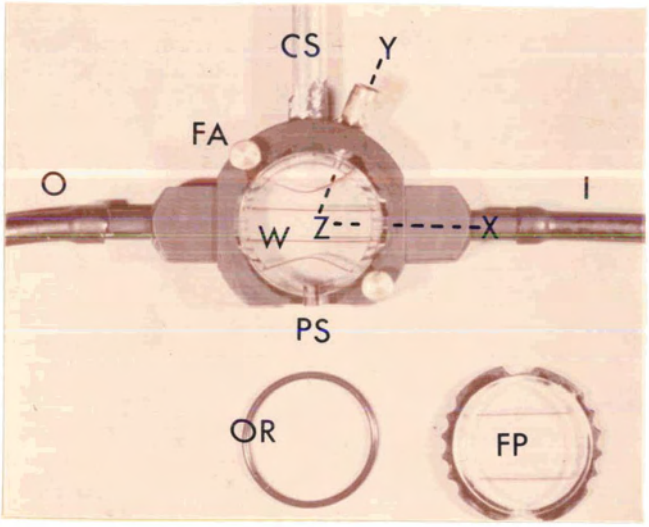
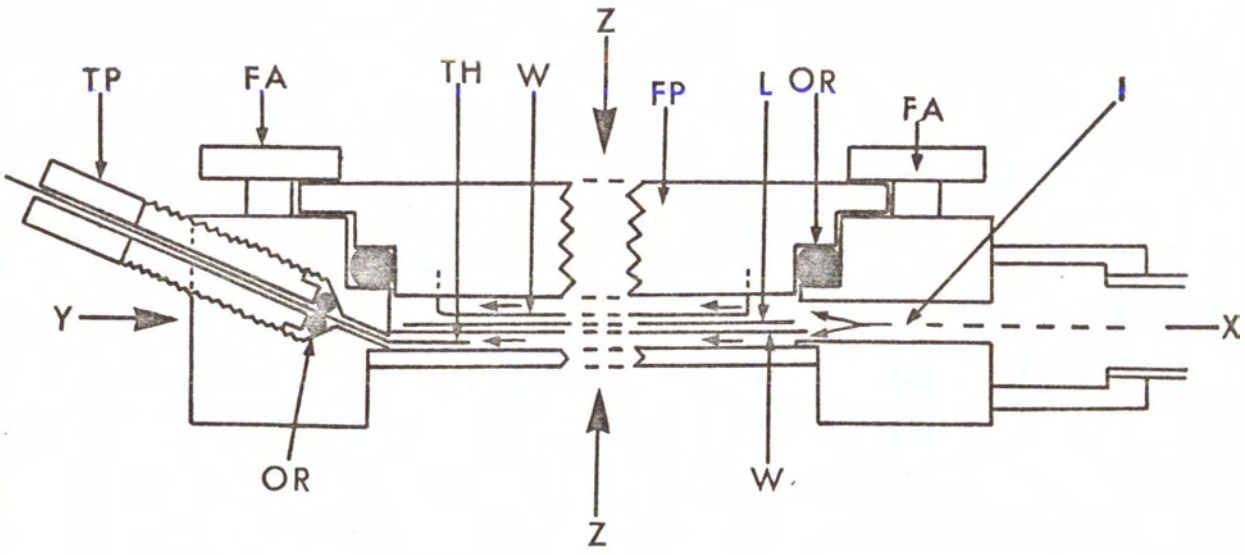


Plate 4B



were blackened except for the faceplate. This cuvette was not temperature controlled but provision was made to record leaf temperatures (Plate 4B).

The conifer cuvette consisted of a two-piece baseplate, one fixed to the support rod, the other free (Plate 5), and a brass barrel with a plexiglass face, gas stream leads, and temperature coil (Plate 6). After removal of a few needles, the attached twig was placed in the slot in the fixed portion of the baseplate and the other baseplate half was inserted and secured into position with screws. The twig was sealed into the baseplate and then the lightly greased O-ring was slid over the twig to its seat on the baseplate followed by the cuvette barrel, again sealed in a twist-lock manner (Plate 6). The whole assembly was then placed in front of the light beam in preparation for gas exchange measurements (Plate 7).

Because the conifer twig was illuminated along its apex-to-base axis, a fashion dictated primarily by the small diameter of the light beam, there is a distance along the twig beyond which successive needles receive very little or no direct light from the beam by virtue of the helical arrangement of the needles on the twig. After completion of 1 to 2 helices from the tip, most of the light has been removed from the beam by the preceding needles, leaving the successive foliage in shade. Therefore, for optimum light utilization and gas exchange only that length of twig subtending sufficient needles to simulate the plane light-absorbing surface of a broad leaf when

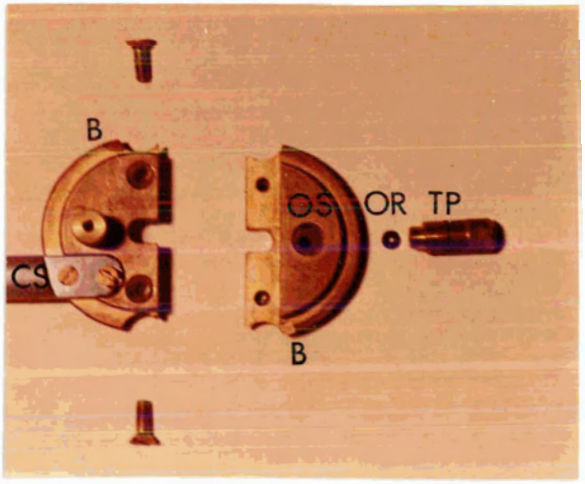
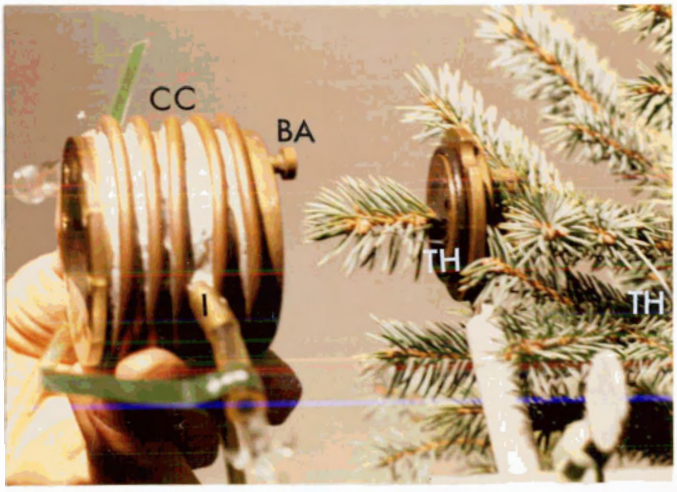


Plate 5. Conifer cuvette baseplate.

B - beveled edge to exert pressure and seal O-ring
TP - temperature probe port
OR - O-ring
OS - O-ring seat
CS - cuvette support rod

Plate 6. Baseplate with conifer twig sealed in and ready to couple to cuvette barrel.

TH - thermistor probe
I - gas inflow or outflow port
CC - copper cooling coil
BA - baseplate anchor posts



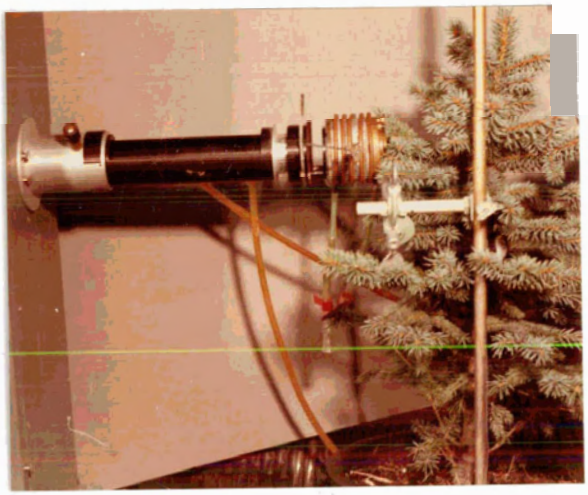


Plate 7. Complete cuvette and light-train assembly in operating position.

viewed from the direction of illumination, was enclosed in the cuvette. It was found that a chamber depth of 4.5 cm satisfied these conditions.

Recently, Ludlow and Jarvis (1971a) illuminated their Sitka spruce twigs in a similar fashion but to overcome some of the difficulties mentioned above, they lined the walls of their chamber with a highly-reflecting aluminized sheet. The machined-brass walls of my chamber, by contrast, are essentially non-reflecting.

The conifer cuvette baseplate also contained two position-adjustable temperature probe ports designed with twist-lock seals, consisting of a small O-ring on a conical seat compressed by the screw to seat against the conical base, the screw base and eventually, the thermistor probe (Plate 4B). In this manner, the probe is not only sealed, but is also fixed in position with a slight twist of the screw and is completely independent of the other seals around the petiole or twig and the O-ring seal between the two major parts of each cuvette. Similarly, the position of the probe is completely adjustable by a slight backing-off of the screw and retightening without affecting the main cuvette seal.

TEMPERATURE CONTROL AND MEASUREMENT

All experiments were carried out at an air temperature of 22 ± 0.5C with the temperature being continuously recorded from a YSI Model 47 Scanning Tele-thermometer. A YSI #520 Implantation thermistor probe was found to be most useful

because of its length, flexibility and small diameter. This probe could be used with either the leaf or the conifer cuvette. A #514 Hypodermic probe was used only with the conifer cuvette. Both #520 and #514 thermistor probes were detachable from their extension leads allowing the probe to remain sealed into the cuvette baseplate which remained attached when the plants were returned each evening to the field plots. Both probes had identical temperature responses, giving inverse linear relationships between temperature (0 - 50C) and millivolt (0 - 100 mv) output. A small refrigerator coil immersed in a Haake constant temperature bath provided adequate temperature control between 10C and 50C for the conifer chamber. Under most conditions, the temperature remained constant to ± 0.5C, the maximum observed temperature increase of 1.0C accompanied the change from darkness to white light when only the neutral density filters were used.

METHODS

The action spectra for the five species were determined from APS versus light intensity measurements for 19 narrow wavebands between 400 and 710 nm, with the rate in white light given to indicate the relative effectiveness of light energy from the various narrow wavebands to sustain photosynthesis as compared to the more natural conditions of illumination with white light.

The first step was to determine the relationship between

light intensity and the photosynthetic rate at each wavelength for each species at normal O_2 (21%) and CO_2 (300 ppm) concentrations as suggested by Brown (1968). In general, the rate of APS was measured at several intensities for each waveband from intensities above light saturation when possible and down through a range of lower intensities, where APS was proportional to light intensity, to darkness.

The results were graphed so that the appropriate light intensity could be chosen as described below. The rate of APS at this intensity, plotted against the corresponding wavelength produces an isoenergetic action spectrum. In choosing an appropriate light intensity, it is important that the rate of APS be nearly proportional to the light intensity for each wavelength used so that the shape of the action spectrum does not change appreciably with the value of the chosen intensity (Fork and Amesz 1969).

Because each APS versus light intensity curve was slightly curvilinear in the region between the light compensation and light saturation intensities, the constant intensity was chosen which sustained a standardized rate of APS approximately $\frac{2}{3}$ of the light saturated rate in the waveband with the greatest light-limited APS rates. This was done to use the greatest absolute values possible for APS and light intensity, while simultaneously maintaining the effective proportional relationship between them. For the alder, Douglas-fir and Sitka spruce, the intensity fitting this criterion was 0.4×10^5 ergs cm^{-2}

sec^{-1} while for the blue spruces the intensity was 0.8×10^5 ergs cm^{-2} sec^{-1}.

The results presented here were obtained from one continuous series of determinations on one sample of tissue for each species. Due to the complexity of the total system, biological and mechanical, this proved to be the exception rather than the rule during the three seasons over which the investigation was conducted. Replicate sub-sections of data over the whole light spectrum obtained from determinations on different tissues from the same or different trees have yielded results which can be superimposed on the detailed action spectra curves presented here, reinforcing the confidence in the validity and reproducibility of the demonstrated relationships between light quality and APS rates.

In order to approximate the action spectrum of a given species as quickly as possible, the filters were divided into three equal groups each spanning most of the visible spectrum, to approximate a very full day's measurements if no technical or other troubles developed during that time. The results from the successive day's measurements using the next group of filters could be back-checked and compared to the first day's results by repeating determinations at two or three of the wavebands used the previous day. Any changes in the plant response were therefore detected and corrected for, provided the change was relatively small ($\underline{<15\%}$).

RESULTS AND DISCUSSION

The action spectrum of net CO_2 uptake (APS) by alder leaves at 300 ppm CO_2, 21% O_2 and a constant incident energy of 0.4×10^5 ergs cm^{-2} sec^{-1} is given in Figure 1.2. The rate of APS shows a peak of activity in the blue region at about 455 nm. Between 455 nm and 550 nm there is a 23.5% reduction in activity at 500 nm to 520 nm compared to the peak rate at 455 nm. The rate of APS then increases with increasing wavelength, to a broad peak in the red from 620 nm to 665 nm and then decreases rapidly at wavelengths above 680 nm. The ratio, red/blue, of peak rates at equal incident energies of red and blue light was 1.00/0.71. For comparison to other units of light measurement, "white light" (400 - 700 nm) with an intensity of 0.42×10^5 ergs cm^{-2} sec^{-1} is approximately equivalent to 1,000 foot-candles (Zelitch 1971).

The action spectra for the four conifers are given in Figure 1.3. Intergeneric and interspecific comparison of the action spectra are facilitated by "normalizing" the curves. This is accomplished by assuming the APS rate at 619 nm from the smoothed action spectrum curve to be 100 units for each species and then expressing the remainder of the data as a percentage of the rate at 619 nm (Figure 1.4).

The most striking feature of Figure 1.4 is the differing contributions by blue (400 - 500 nm) light to APS activity (Figure 1.4A) attended by a parallel decrease in relative APS activity in white light (Figure 1.4B). The blue peak of APS

Figure 1.2 A. The action spectrum of apparent photosynthesis for single attached alder leaves at a constant incident light energy of 0.4×10^5 ergs cm^{-2} sec^{-1} at 22C, 300 ppm CO_2 and 21% O_2.

B. The rate of apparent photosynthesis in white light of 0.4×10^5 ergs cm^{-2} sec^{-1} intensity at 22C, 300 ppm CO_2 and 21% O_2.

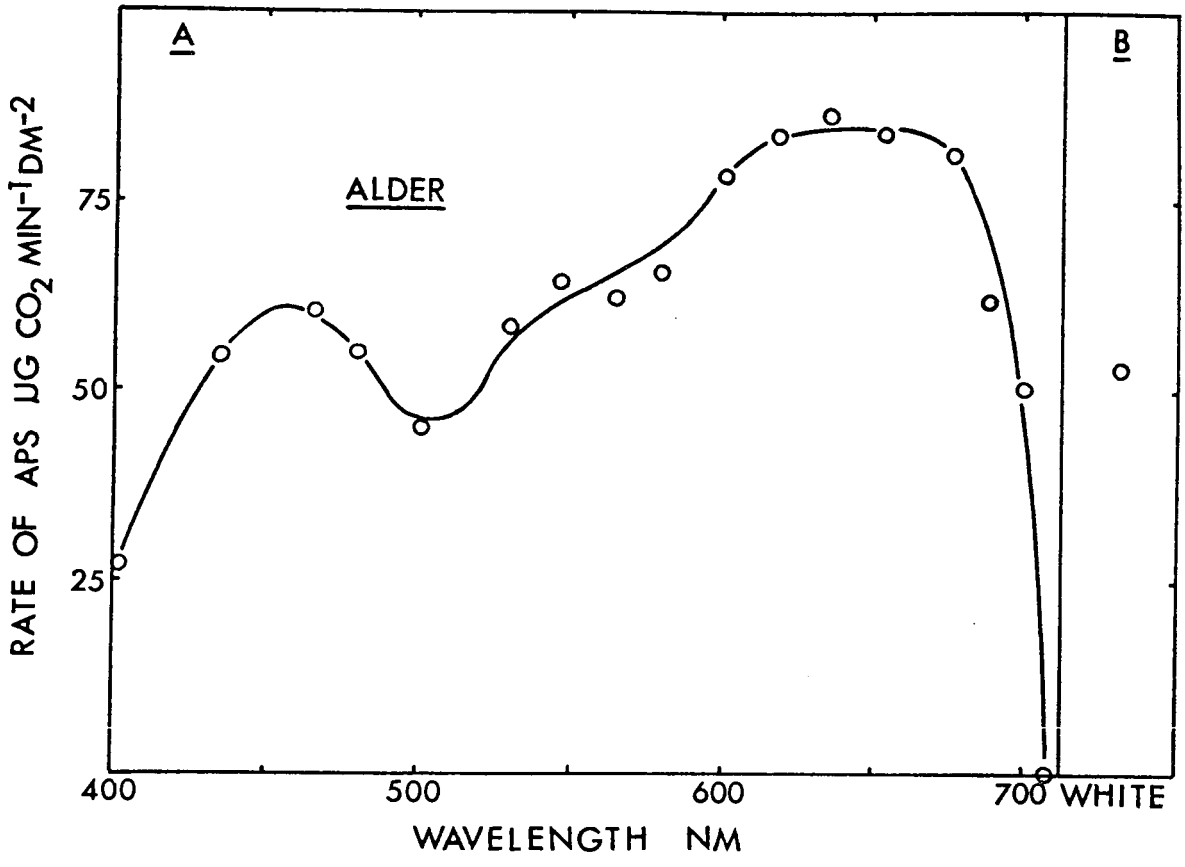

A
B
ALDER
RATE OF APS UG CO_2 MIN^{-1} DM^{-2}
75
50
25
400
500
600
700
WHITE
WAVELENGTH NM

Figure 1.3 A. The action spectra of apparent photosynthesis for attached branches of four conifer species at 22C, 300 ppm CO_2, 21% O_2 and a constant light incident energy of 0.4×10^5 ergs cm^{-2} sec^{-1} for Douglas-fir and Sitka spruce, and 0.8×10^5 ergs cm^{-2} sec^{-1} for Colorado and Blue spruce.

B. The rate of apparent photosynthesis for each species in white light of equivalent intensity under the same experimental conditions.

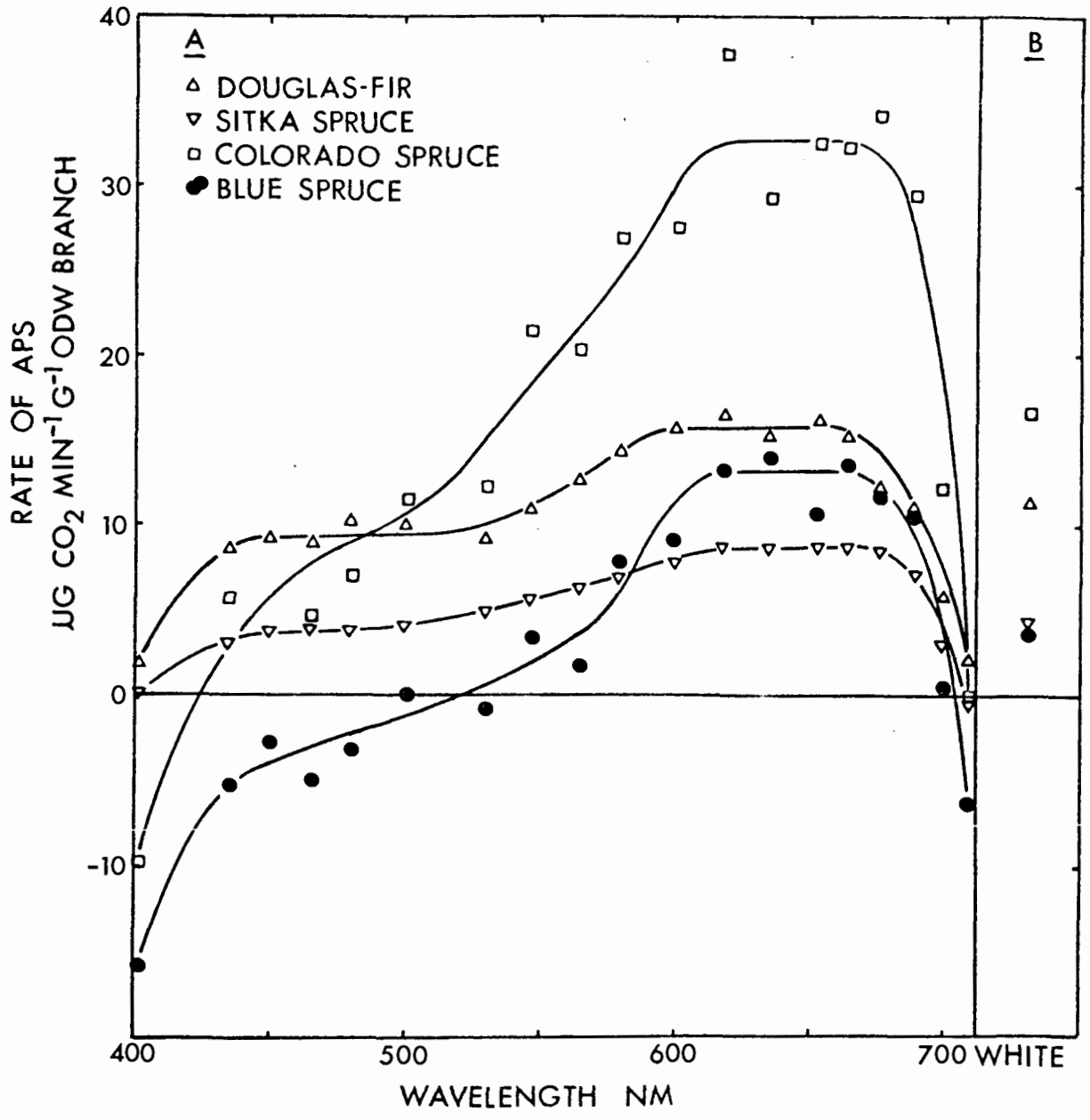
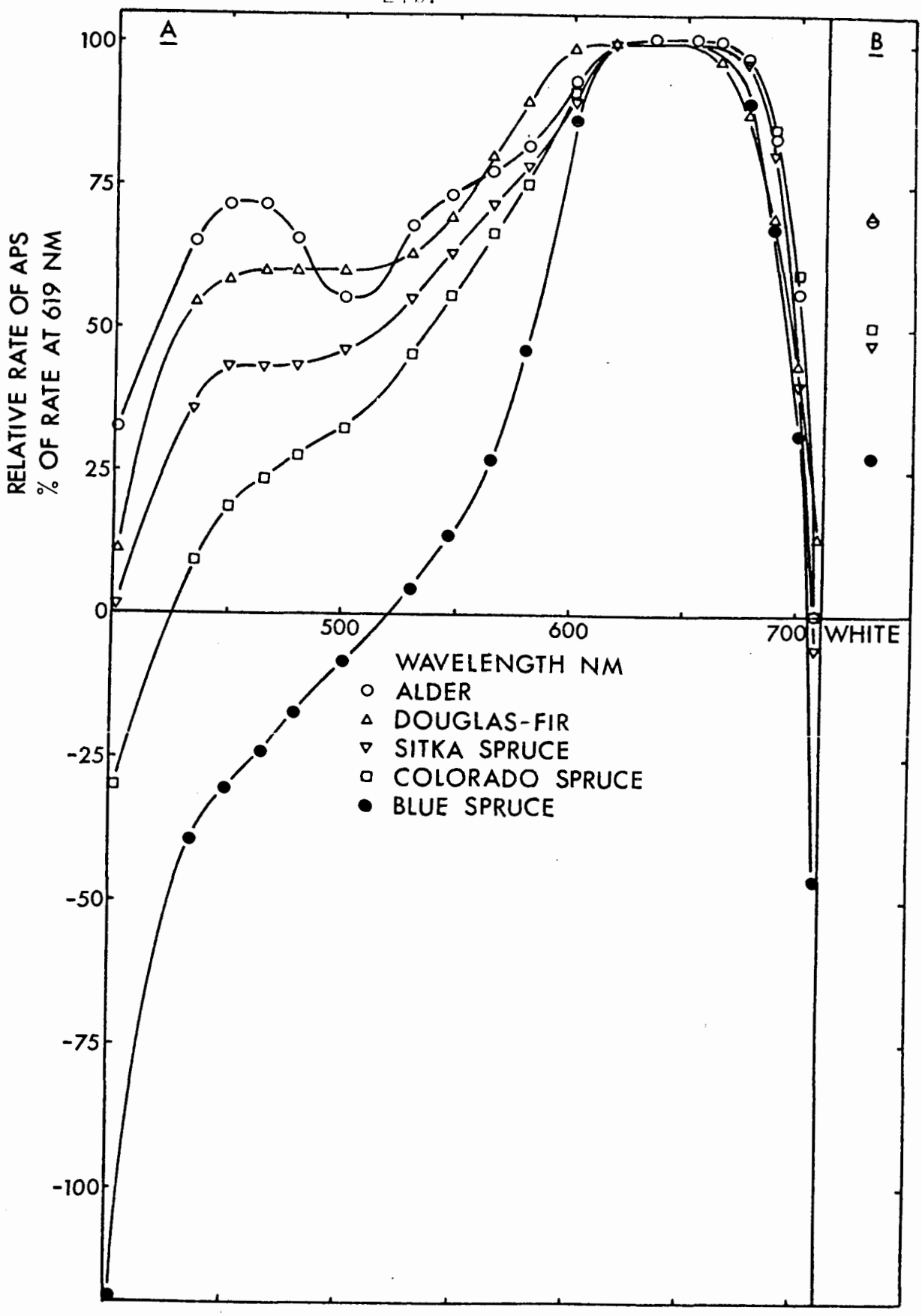

A
B
DOUGLAS-FIR
SITKA SPRUCE
COLORADO SPRUCE
BLUE SPRUCE
RATE OF APS
JUG CO_2 MIN^{-1} G^{-1} ODW BRANCH
40
30
20
10
0
-10
400
500
600
700
WHITE
WAVELENGTH NM

Figure 1.4 A. The normalized action spectra of apparent photosynthesis of five tree species. The APS rate at 619 nm is made equal to 100%. Other experimental conditions: 300 ppm CO_2, 21% O_2 and an incident light intensity of 0.4 x 10^5 ergs cm^{-2} sec^{-1} for alder, Douglas-fir and Sitka spruce, and 0.8 x 10^5 ergs cm^{-2} sec^{-1} for Colorado and Blue spruce.

B. The normalized APS rate in white light for each species under the same experimental conditions as in A.


A
B
100
75
50
25
0
-25
-50
-75
-100
RELATIVE RATE OF APS
% OF RATE AT 619 NM
500
600
700
WHITE
WAVELENGTH NM
ALDER
DOUGLAS-FIR
SITKA SPRUCE
COLORADO SPRUCE
BLUE SPRUCE

activity for alder is reduced to a plateau for Douglas-fir and Sitka spruce, a shoulder for Colorado spruce and a reduced shoulder for Blue spruce. The following table summarizes the relationship between APS activity in the blue waveband and the visual leaf differences with as few leaf factors as possible changing between the various levels of comparison.

Level of Comparison	Example Species	APS activity in blue light	Visual leaf characteristics
	Alder	"Peak"	Dark green broad-leaf
Inter-division	Douglas-fir	"Plateau I"	Bright green flat needle
Inter-generic	Sitka spruce	"Plateau II"	Yellow-green flat needle
Inter-specific	Colorado spruce	"Shoulder I"	Blue-green 4-sided needle
Intra-specific	Blue spruce	"Shoulder II"	Blue-white 4-sided needle

The action spectra for Colorado and Blue spruce have values of zero APS at wavelengths above 400 nm, meaning that the light compensation point has been reached in these species simply by changing the wavelength of the incident light. For a constant incident light energy of 0.8×10^5 ergs cm^{-2} sec^{-1}, Colorado spruce shows positive APS rates only at wavelengths greater than 425 nm, while in Blue spruce, positive APS rates are restricted to wavelengths greater than 520 nm. Below these wavelengths, both species have negative APS rates (CO_2 evolution) in the light at 0.8×10^5 ergs cm^{-2} sec^{-1}.

In conclusion, there are obvious disparities between the action spectra at all levels of comparison from inter-division to intra-specific, even under standardized illumination conditions. The subsequent sections will therefore determine if any of the differences in APS activity at the different levels can be related to specific leaf characteristics by testing the three photosynthesis-screening mechanisms outlined earlier.

PART II

THE EFFECTS OF LIGHT QUALITY AND OXYGEN CONCENTRATION ON PHOTOSYNTHESIS AND PHOTORESPIRATION IN ATTACHED SHOOTS OF SITKA AND COLORADO SPRUCE.

INTRODUCTION

Many recent studies have demonstrated that dark respiration is curtailed in photosynthesizing leaves and is replaced in some species by photorespiration, a biochemically different CO_2 evolution process specifically associated with substrates produced during current photosynthesis (see review by Jackson and Volk 1970). A brief history of the work which lead to the recognition of photorespiration has been given by Decker (1970), a pioneer in this area. The immediate re-oxidation of some photosynthetic products (up to 50% of gross CO_2 assimilation, Zelitch 1971) seems to confer little or no benefit to the plants. How such an apparently wasteful process could evolve in a highly competitive world has been discussed by Goldworthy (1969).

The photosynthetic action spectrum for each species (Part I), determined under conditions where the CO_2 uptake in the light is partially counterbalanced by the simultaneous CO_2 evolution due to photorespiration, is potentially a composite action spectrum for two opposing processes, each of which could have its own photoactive pigments (Bulley et al. 1969). Thus, the lower relative rate of APS for Sitka spruce in blue light

compared to that for alder could result from a greater blue light stimulation of photorespiratory CO_2 evolution in Sitka spruce. Similarly, photorespiratory activity in blue light would be greater in Colorado spruce and greatest in Blue spruce if this mechanism were to account for the differences in the action spectra for the five species in Part I.

The object of this study was to determine whether photorespiration is selectively stimulated by blue light in conifer needles, particularly in spruce species (Poskuta 1968a), to a greater degree than in broadleaf species where no blue light effect has been demonstrated (Bulley et al. 1969, Voskresenskaya et al. 1970). Poskuta's (1968a) basic experimental regime of light-dark cycles was followed. In addition to CO_2 compensation point (CCP) data, the post-illumination burst of CO_2 (PIB) measured here provided an independent and simultaneous estimate of the rate of photorespiration (Ludlow and Jarvis 1971b). The post-illumination burst of CO_2, now widely accepted as representing the momentary continuation of the high rate of CO_2 evolution in the preceding light period ie. photorespiration (Decker 1955, 1957, Tregunna, Nelson and Krotkov 1961, 1964, 1966, Semenenko 1964, Moss 1966, Egle and Fock 1967) was the only other index of photorespiratory activity compatible with Poskuta's (1968a) regime.

It has been shown that the rate of photorespiration in white spruce is strongly dependent on the O_2 concentration while the rate of dark respiration (DR) remains essentially unaffected

from 1% to 100% O_2 (Poskuta 1968b). Dark respiration in Sitka spruce is inhibited only by very low (ca. 0%) O_2 concentrations (Ludlow and Jarvis 1971a).

This investigation will therefore determine the relative effects of white, red and blue light on the rates of CO_2 exchange before and after "light-off" in a series of light-dark cycles with foliage of Sitka and Colorado spruce under photorespiration-permitting (21% O_2) and inhibiting (2 - 4% O_2) conditions using the PIB rather than the CCP as a measure of the relative rates of photorespiration.

MATERIALS AND METHODS

Using the closed circuit measuring and control system described in Part I, the rate of apparent photosynthesis (APS), the CO_2 compensation point (CCP) and the instantaneous rate of CO_2 evolution in the dark for 5 to 10 min after "light-off" were determined at normal (21%) and low (2 - 4%) O_2 concentrations. The experimental series were designed to consist of two parts:

Section I. Isoenergetic irradiation (approx. 0.8×10^5 ergs cm^{-2} sec^{-1}) from white, red (619 nm) and blue (450 nm) wavebands at both 21% and 2 - 4% O_2 for both species was used to determine the relative rates of photorespiration under the same conditions as the photosynthetic action spectra were measured,

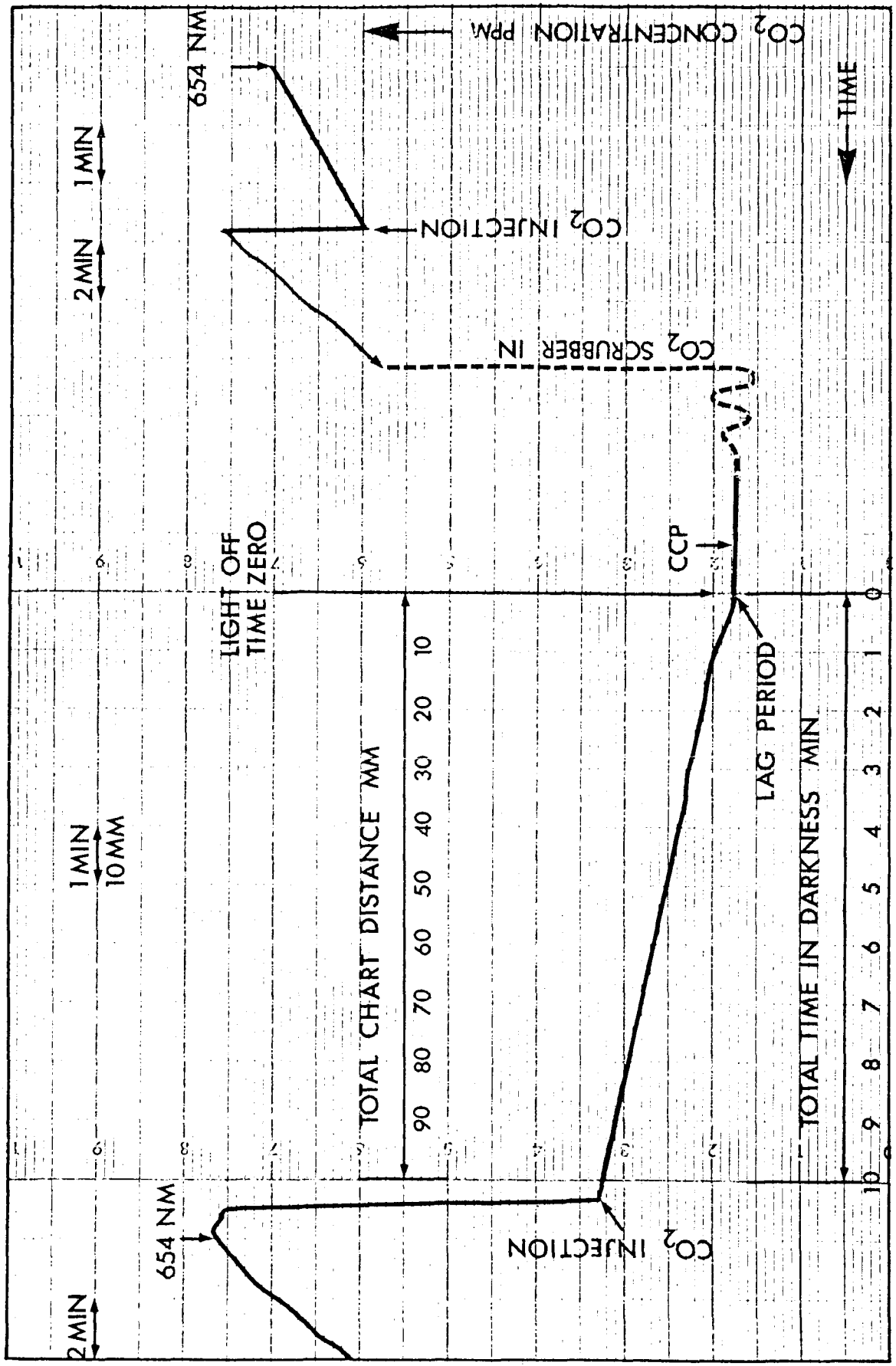
Section II. The relative light intensities were adjusted to produce equal APS rates in each waveband for Sitka spruce

under photorespiration-permitting conditions (21% O_2) (Poskuta 1968a) before the O_2 concentration was lowered. If blue light selectively stimulated photorespiration, the resulting inhibition of photorespiration should selectively enhance the APS rate in blue light as compared to those in white and red light.

Except for the illumination conditions, the light-dark cycles followed identical protocols. The decrease in CO_2 concentration in the closed circuit was recorded down to the CO_2 compensation point, then the foliage was darkened for 5 to 10 minutes, after which the system was opened, flushed with the appropriate gas mixture (either room air or a 2% O_2 in N_2 with 0.03% CO_2 mixture) then closed again and the cycle repeated. An example of the data available for analysis is shown in Figure 2.1. While under low O_2 conditions the CO_2 concentration in the circuit could be raised by hypodermic injection through a serum stopper of CO_2 generated in a flask from $NaHCO_3$ and dilute HNO_3 or lowered by means of a CO_2 scrubber (Ascarite absorber) which could be dialed into and out of the system by means of a 4-way stopcock. An O_2 electrode was used as a monitor to ensure that constant O_2 levels prevailed during the "low O_2" experiments. The addition or removal of CO_2 gave rise to a pressure and O_2 concentration change of less than 0.03% which was considered negligible.

The instantaneous rate of CO_2 evolution at different times during the dark period was calculated from the average rate of

Figure 2.1 An example of the carbon dioxide exchange data available for analysis.


654 NM
CO2 CONCENTRATION PPM
TIME
1 MIN
2 MIN
CO2 INJECTION
CO2 SCRUBBER IN
LIGHT OFF
TIME ZERO
CCP
LAG PERIOD
TOTAL CHART DISTANCE MM
TOTAL TIME IN DARKNESS MIN
1 MIN
10 MM
654 NM
CO2 INJECTION
2 MIN

change of CO_2 concentration with time within each CO_2 interval as delineated by the ruled chart lines (Figure 2.1). It was assumed that the rate of CO_2 evolution was constant within each CO_2 interval, thus the calculated rates of CO_2 evolution were plotted against the time corresponding to the mid-point of each CO_2 interval after the lag period. The data from Figure 2.1 is plotted in Figure 2.2, curve #1.

RESULTS AND DISCUSSION

SECTION I

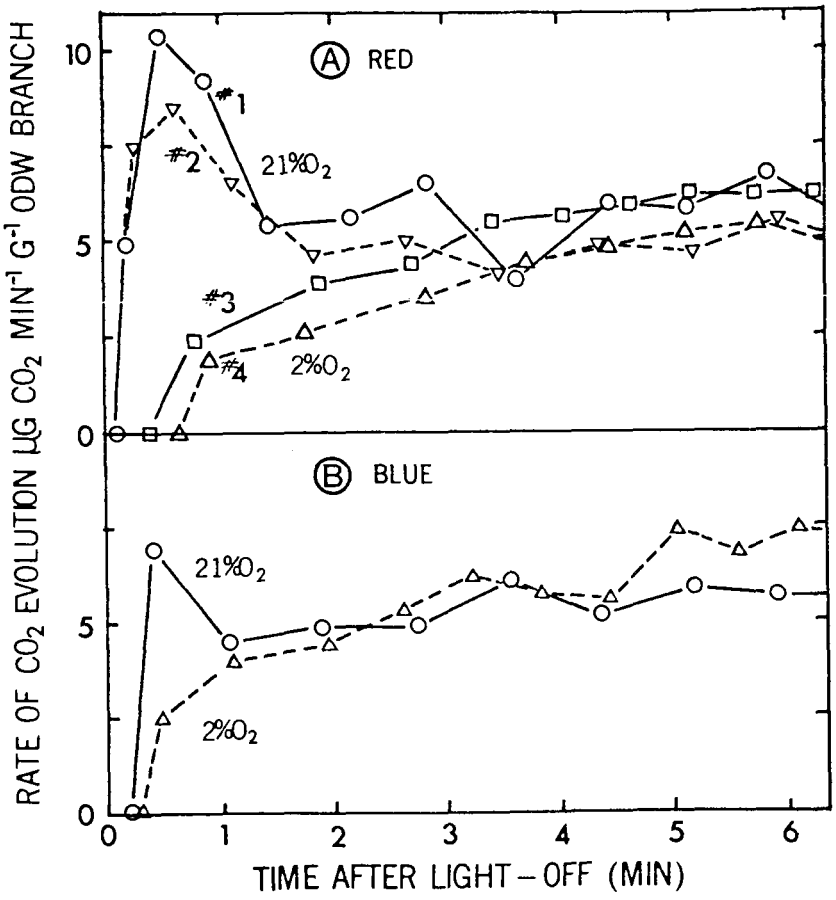
The results described here were obtained from Colorado and Sitka spruce under conditions similar to those for the action spectra determinations, that is, the intensities of the red and blue light were adjusted to approximately 0.8×10^5 ergs cm^{-2} sec^{-1} before the rate of APS was measured.

The effect of light quality and oxygen concentration on the magnitude of the PIB in Colorado spruce is shown in Table 2.1 and Figure 2.2 (A and B). Under normal O_2 concentrations and following "light-off" there is a lag period of about 6 sec (maximum time for the gas in the chamber to be carried to the IRGA is 2 to 3 sec) after which the rate of CO_2 evolution increases rapidly to a maximum at 30 to 35 sec from the onset of darkness, then decreases to the relatively steady rate observed from 1.5 to 2.0 min onwards. The transient rate of CO_2 evolution corresponds to the first post-illumination burst of Tregunna et al. (1961, 1964), while the steady rate

Table 2.1 The rate of apparent photosynthesis and the CO_2 concentration at the carbon dioxide compensation point for Colorado spruce branches at 21% and 2% oxygen under red or blue light.

Figure 2.2 The rate of CO_2 evolution in the dark by Colorado spruce branches at 21% and 2% oxygen following red (A) or blue (B) light of approximately equal intensity.

Light Quality	Intensity ergs cm^{-2} sec^{-1}	Oxygen %	$\mu g\ CO_2\ min^{-1}\ g^{-1}$ ODW branch		CCP ppm
			APS ± S.D.	PIB and DR	
Red	0.82×10^5	21	26.3 ± 1.3	Curve 1, Fig. 2.2A	61.0
			32.5 ± 0.6	Curve 2, Fig. 2.2A	52.0
		2	48.7 ± 0.9	Curve 3, Fig. 2.2A	24.0
			45.7 ± 2.5	Curve 4, Fig. 2.2A	19.0
Blue	0.73×10^5	21	8.6 ± 0.8	Figure 2.2B	83.0
		2	20.5 ± 1.2	Figure 2.2B	35.9



of CO_2 evolution is classical dark (mitochondrial) respiration (DR).

Comparing these results to those of a similar experiment conducted under low (2 - 4%) O_2 concentrations (Figure 2.2 A and B), the most obvious difference is that the PIB seen at 21% O_2 is not present under the low oxygen conditions. Similar results were obtained independent of light quality. The low O_2 concentrations thus had several effects on the gas exchange characteristics of Colorado spruce as compared to normal O_2 concentrations: (a) the APS rate was enhanced in both red and blue light, (b) the CCP was significantly lowered in all cases, (c) the PIB was inhibited, and (d) the subsequent average rate of dark respiration was essentially unaffected by O_2 concentration for periods of up to 10 min after illumination. The reduced PIB at 21% O_2 following blue light illumination could be due to an increased attenuation of blue light compared to red light before reaching the chloroplasts resulting in a lower rate of APS and a reduced pool of substrate for photorespiration.

The measurements were repeated with Sitka spruce, illuminated with approximately equal intensities of red and blue light at 21% and 2 - 4% O_2 concentrations. These measurements, made near the end of June, are reported for approximately 0.8×10^5 ergs cm^{-2} sec^{-1} illumination to give appreciable APS rates and substrate levels for photorespiration in the young foliage. In contrast, the action spectra measurements and all other PIB measurements were made after the high rate of respiration associated with the growth and expansion had declined ie.

from July to November (Bourdeau 1959, Clark 1961, Ludlow and Jarvis 1971a).

The results of the PIB measurements for Sitka spruce (Table 2.2 and Figure 2.3) agree with those presented for Colorado spruce. The major observable difference is that the rate of CO_2 evolution at 21% O_2 in the first 1.5 min of darkness increases rapidly to a more or less constant value for both red and blue light as compared to the distinct burst for Colorado spruce especially after red light. The effect of low O_2 concentrations is similar in both species for both red and blue light.

In conclusion, it appears that the ratio of photorespiration/dark respiration rates is greater in Colorado spruce than in Sitka spruce. This may be due to the younger foliage used for Sitka spruce. There is no evidence however, of a selective stimulation of photorespiration by blue light compared to red light for either species. An alternative procedure to determine light quality effects on photorespiration is to adjust the light intensities to achieve equal APS rates underred and blue illumination thus obviating the problem of differing light absorption and reflection characteristics of the different species. The results from these experiments are reported in Section II.

SECTION II

Table 2.3 and Figure 2.4 (C, D and E) present the results for the APS rates, CCP's and PIB's of Sitka spruce tissue at

Table 2.2 The rate of apparent photosynthesis and the CO_2 concentration at the carbon dioxide compensation point for Sitka spruce branches at 21% and 2% oxygen under red or blue light.

Figure 2.3 The rate of CO_2 evolution in the dark by Sitka spruce branches at 21% and 2% oxygen following red (A) or blue (B) light of equal intensity.

Light Quality	Intensity ergs cm^{-2} sec^{-1}	Oxygen %	μg CO_2 min^{-1} g^{-1} ODW branch		CCP ppm
			APS ±S.D.	PIB and DR	
Red	0.82×10^5	21	23.9±0.8	Figure 2.3A	98.3
		2	48.1±4.2	Figure 2.3A	31.2
Blue	0.84×10^5	21	9.5±0.8	Figure 2.3B	173.3
		2	23.8±1.5	Figure 2.3B	63.0

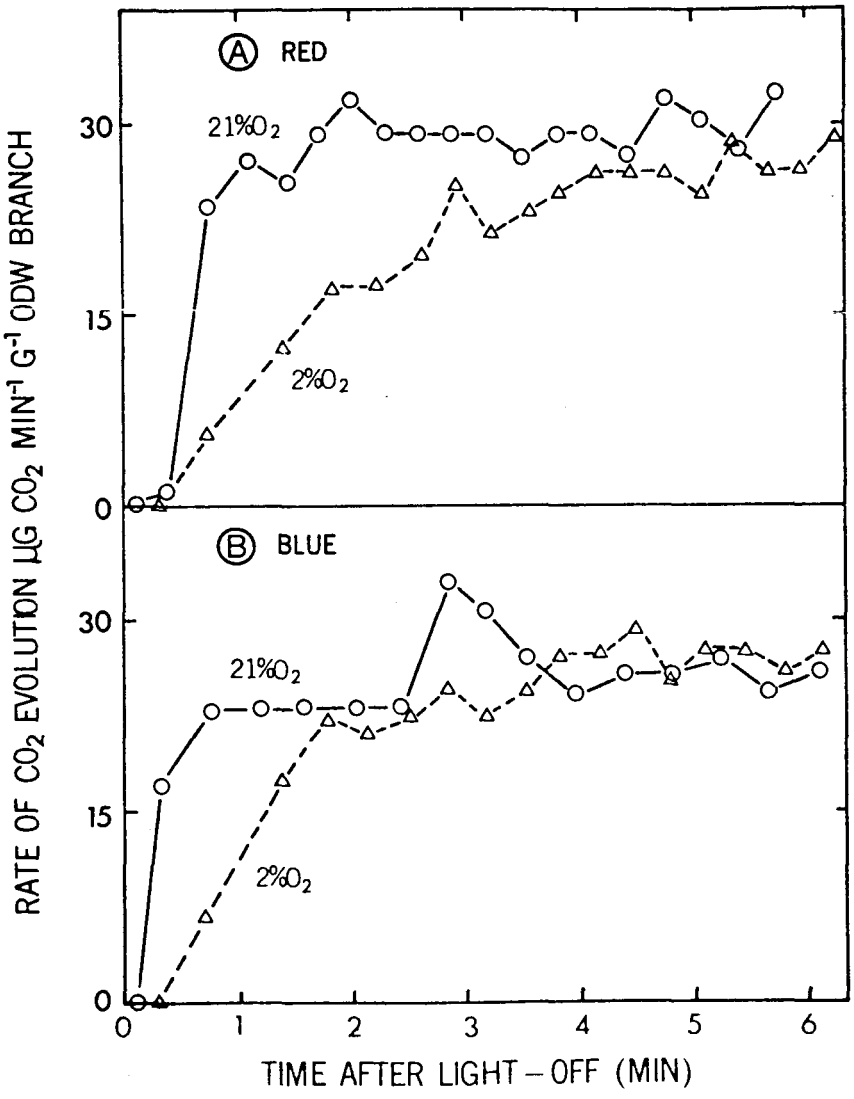
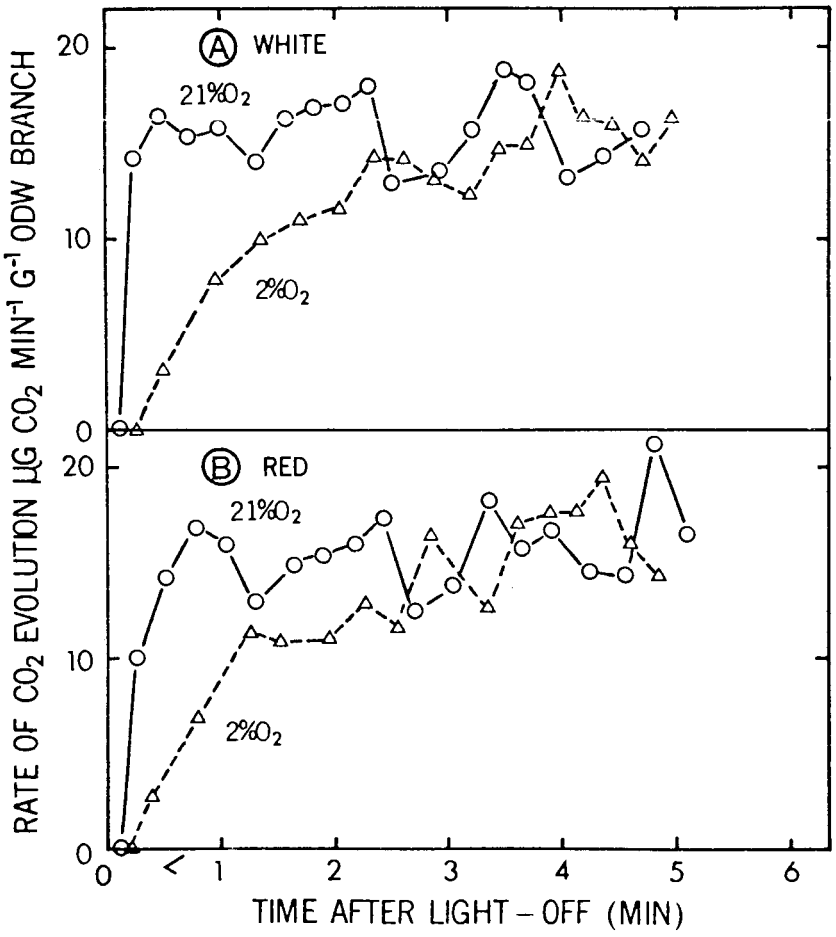
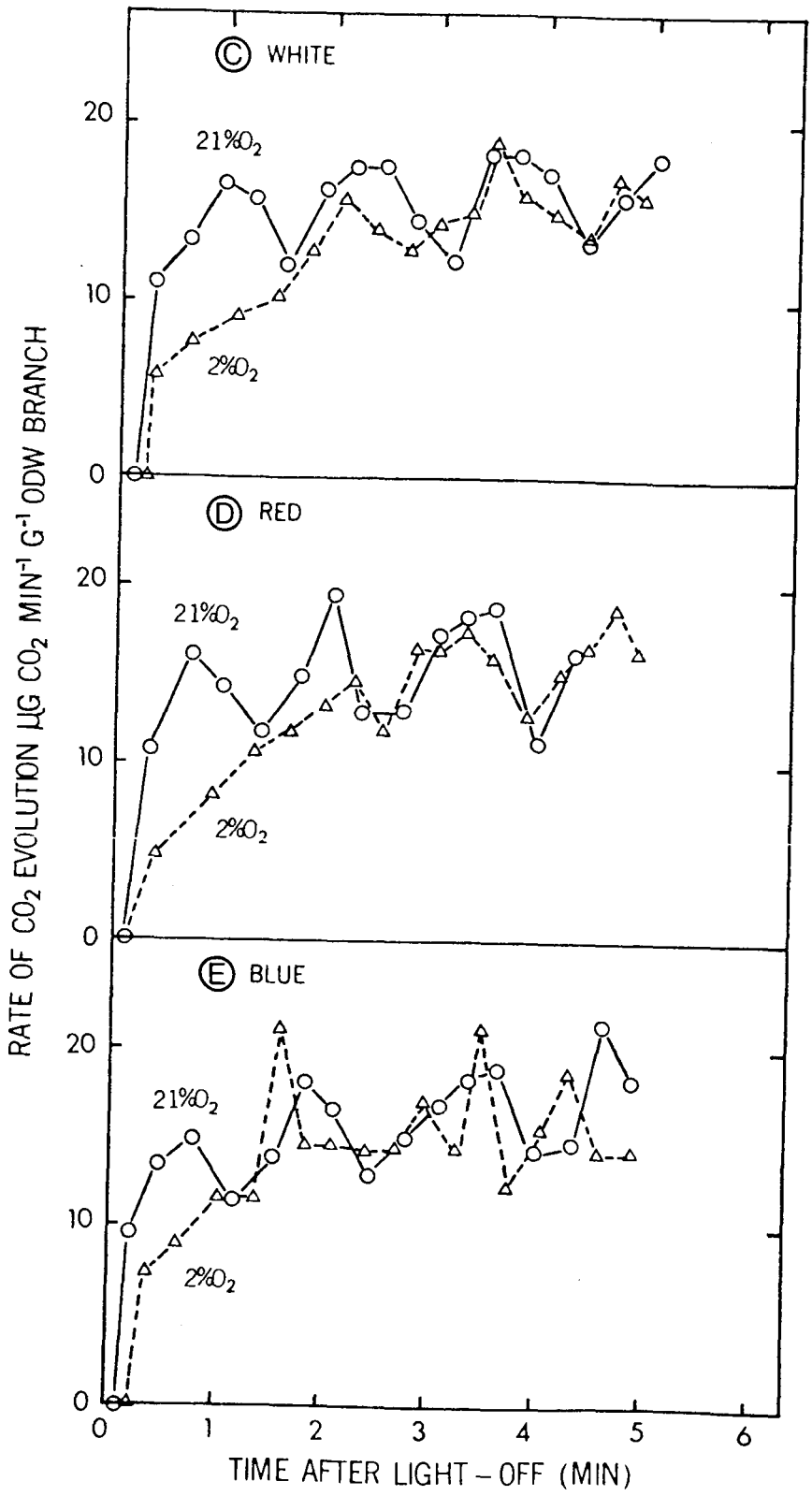


Table 2.3 The rate of apparent photosynthesis and the CO_2 concentration at the carbon dioxide compensation point for Sitka spruce branches at 21% and 2% oxygen under light of different qualities and intensities.

Figure 2.4 The rate of CO_2 evolution in the dark by Sitka spruce branches at 21% and 2% oxygen following white (A) and red (B) light of maximum available intensities. The rate of CO_2 evolution in the dark by Sitka spruce branches at 21% and 2% oxygen following white (C), red (D), or blue (E) light of intensities adjusted to attain equal APS rates (page 37c).

Light Quality	Intensity ergs cm^{-2} sec^{-1}	Oxygen %	µg CO_2 min^{-1} g^{-1} ODW branch APS ± SD.	PIB and DR	CCP ppm
White	4.70×10^5	21	25.2±1.3	Figure 2.4A	104.5
		2	33.2±3.4	Figure 2.4A	52.8
Red	1.25×10^5	21	18.7±0.4	Figure 2.4B	109.8
		2	27.9±2.9	Figure 2.4B	57.1
White	0.87×10^5	21	10.8±0.4	Figure 2.4C	148.5
		2	17.0±0.9	Figure 2.4C	77.0
Red	0.52×10^5	21	9.1±0.6	Figure 2.4D	157.0
		2	18.4±1.5	Figure 2.4D	67.0
Blue	1.70×10^5	21	9.6±0.8	Figure 2.4E	160.3
		2	19.2±1.9	Figure 2.4E	80.0




Ⓒ WHITE
21%O2
2%O2
Ⓓ RED
21%O2
2%O2
Ⓔ BLUE
21%O2
2%O2
RATE OF CO2 EVOLUTION µG CO2 MIN-1 G-1 ODW BRANCH
TIME AFTER LIGHT – OFF (MIN)
0
10
20
0
1
2
3
4
5
6

21% O_2 under or following illumination with white, red or blue light whose intensity was adjusted to result in approximately equal APS rates. In addition, the results from illumination with maximum available intensities of red and white light are given in Figure 2.4 (A and B) to check the effect of light intensity on the size of the PIB.

There does not appear to be a detectable enhancement of photorespiration in Sitka spruce at high light intensities (Figure 2.4, compare A and B with C, D and E), nor is there any evidence of a selective stimulation of photorespiration by blue light as compared to red or white light. In all cases, the PIB of Sitka spruce under these conditions agrees very well with those in Section I. Low O_2 concentrations again inhibited the PIB while having no effect on the rate of dark respiration. The regular periodicity of the variation in the rate of dark respiration for this Sitka spruce tissue, as contrasted to the nearly constant rates of dark respiration of both species in Section I, suggested that the effect was instrumental in nature rather than a physiological response of the experimental tissue.

When the light intensities were adjusted to give equal APS rates in each waveband it was found that all the subsequent gas exchange characteristics were essentially identical ie. beginning with equal APS rates, the CCP's were the same (Table 2.3) as opposed to the elevated CCP reported by Poskuta (1968a) for blue light. Moreover, when measurements were made under low oxygen conditions, no adjustment of the relative light intensi-

ties in the different wavebands was required to maintain equal APS rates. The APS rates increased the same amount for each waveband when the change from 21% to 2 - 4% O_2 was made (Table 2.3). This would not have been expected in blue light stimulated photorespiration. If it did, as Poskuta (1968a) reported, the switch to low O_2 should have resulted in a higher APS rate under blue light by the inhibition of photorespiration as compared to red or white light.

The conclusion from these results is that photorespiration in these two spruce species is not selectively stimulated by blue light which agrees with the results for a variety of broadleaf herbaceous species (Bulley et al. 1969, Voskresenskaya et al. 1970) and a conifer species (Linder 1971). The differences between the relative APS activities reported in Part I, therefore are not due to a differential blue light stimulation of photorespiration in the various tree species studied.

PART III

THE ABSORPTION SPECTRA OF PIGMENT EXTRACTS FROM ALDER, DOUGLAS-FIR AND SITKA, COLORADO AND BLUE SPRUCE PHOTOSYNTHETIC TISSUE

INTRODUCTION

Three possible mechanisms which could account for the relatively low APS rates in blue light observed for the conifer species as compared to alder, were outlined earlier. In Part II, the first of these hypotheses was investigated and shown to be invalid.

Attention was then directed to the second hypothesis, the presence of an internal wavelength-selective screening system. Either a non-chloroplast pigment as reported by Burns (1942) in white pine or a refined chlorophyll protection system involving photosynthetically inactive carotenoids as proposed by Linder (1971) for Scots pine, could explain the low photosynthetic efficiency of blue light for conifers.

The differences between the action spectra of the various tree species investigated would then be attributable to differential amounts of a screening pigment being present. One would predict that the two blue spruce types (Colorado and Blue spruce) would contain either (1) relatively more of Burns' type pigment than Douglas-fir and Sitka spruce which in turn would

contain more than alder, or (2) differing carotenoid complements.

Needle or leaf pigments of each species were therefore extracted, resolved into several components and their absorption spectra determined.

MATERIALS AND METHODS

Ten needles were chosen at random from each of two randomly selected examples of each conifer species and weighed. In the case of alder an equivalent weight of leaf tissue, including some midrib and secondary veins, was cut from the midsection of two randomly selected leaves. All tissues were from the current year's growth and were at the approximately 90% fully expanded stage in development as was the tissue used throughout this study. The pigments were extracted in 80% aqueous acetone during a 15 second homogenization at 0 C in a Willems Polytron homogenizer. Filtering and washing to remove the solid residue yielded a clear 80% aqueous acetone pigment solution. This extract was then partitioned three times against hexane, which in turn was partitioned three times against 80% aqueous methanol.

The continuous absorption spectra between 350 and 710 nm were determined by means of a Cary 14 double-beam recording spectrophotometer on the total 80% aqueous acetone extract, the acetone extract after partitioning against hexane, the hexane fraction before and after partitioning against 80%

aqueous methanol and the 80% aqueous methanol fraction. From these continuous spectra, the absorbance (in arbitrary units) at 10 nm intervals was abstracted and corrected for volume changes and weight of the original sample.

Similar results were obtained from tissues investigated over two consecutive seasons.

RESULTS AND DISCUSSION

The absorption spectra of the total acetone extracts for all species are very similar and for wavelengths greater than 500 nm absorption is due only to chlorophyll pigments (Figure 3.1). The action spectra of apparent photosynthesis for all species investigated also showed good agreement at wavelengths greater than 500 nm (Figure 1.4, Part I, p. 24b), thus the 500 to 710 nm region can be eliminated from further consideration. The alder tissue contained approximately three times the chlorophyll content of the conifer tissue on a per unit fresh weight basis, thus explaining the relative dark green colour of the alder foliage.

The absorption in the remaining 350 to 500 nm region (Figure 3.2) can be conveniently subdivided into two portions, the 350 to 400 nm region comprising the hexane washed acetone fraction (Figure 3.3) and the methanol washed hexane and methanol extracts (Figure 3.4 and 3.5 respectively). This effectively separates the remaining chlorophyll and carotenoid

Figure 3.1 The absorption spectra, between 500 and 710 nm, of the total 80% aqueous acetone pigment extract of:

A. Alder (□ - □), Douglas-fir (○ - ○) and Sitka spruce (△ - △).

B. Colorado spruce (○ - ○) and Blue spruce (□ - □)

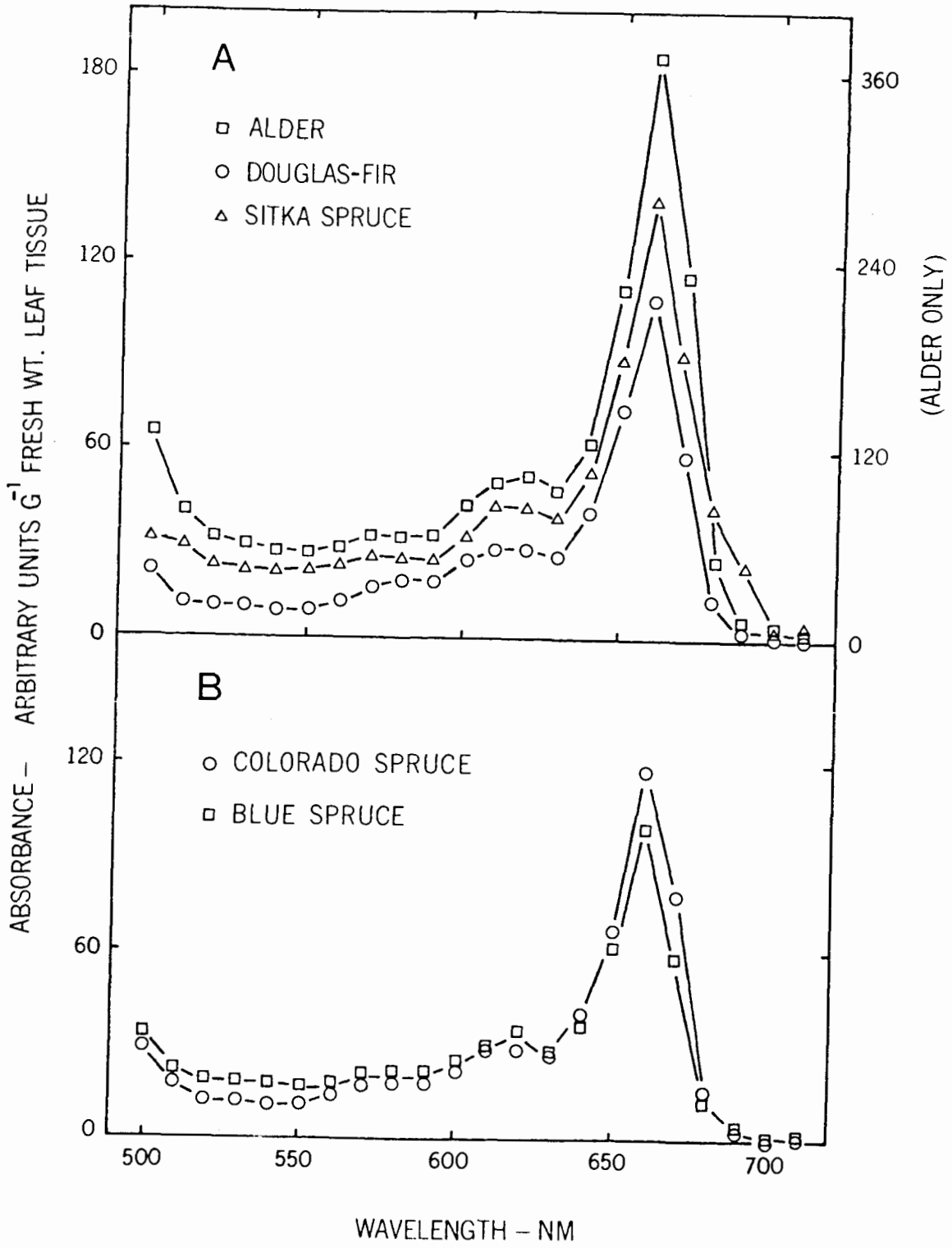

A
ALDER
DOUGLAS-FIR
SITKA SPRUCE
B
COLORADO SPRUCE
BLUE SPRUCE
180
120
60
0
360
240
120
0
(ALDER ONLY)
ABSORBANCE – ARBITRARY UNITS G^{-1} FRESH WT. LEAF TISSUE
500
550
600
650
700
WAVELENGTH – NM

Figure 3.2 The absorption spectra, between 350 and 500 nm, of the total 80% aqueous acetone pigment extract of:

A. Alder (□ - ◨), Douglas-fir (o - o) and Sitka spruce (△ - △).

B. Colorado spruce (o - o) and Blue spruce (□ - □).

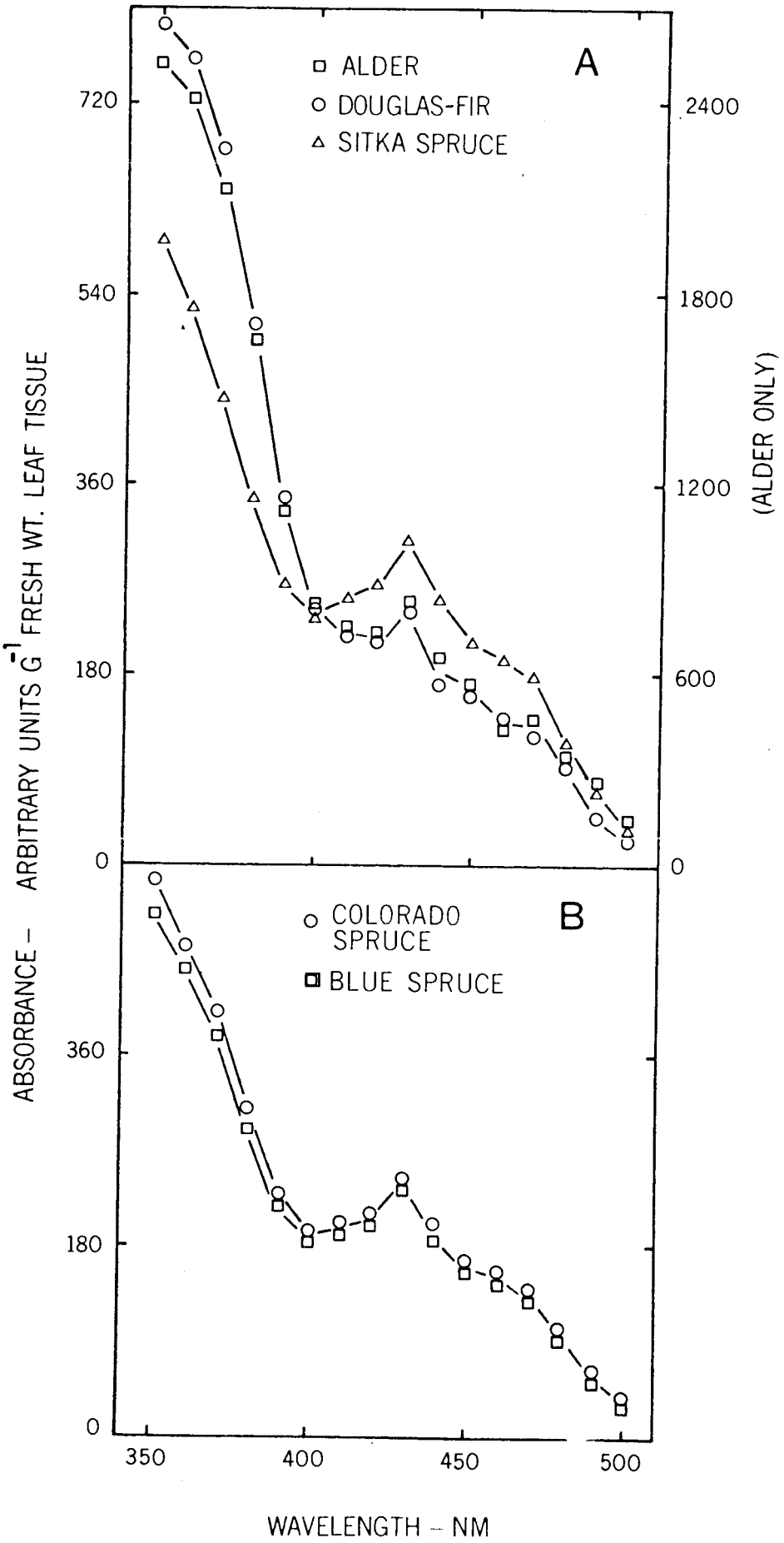
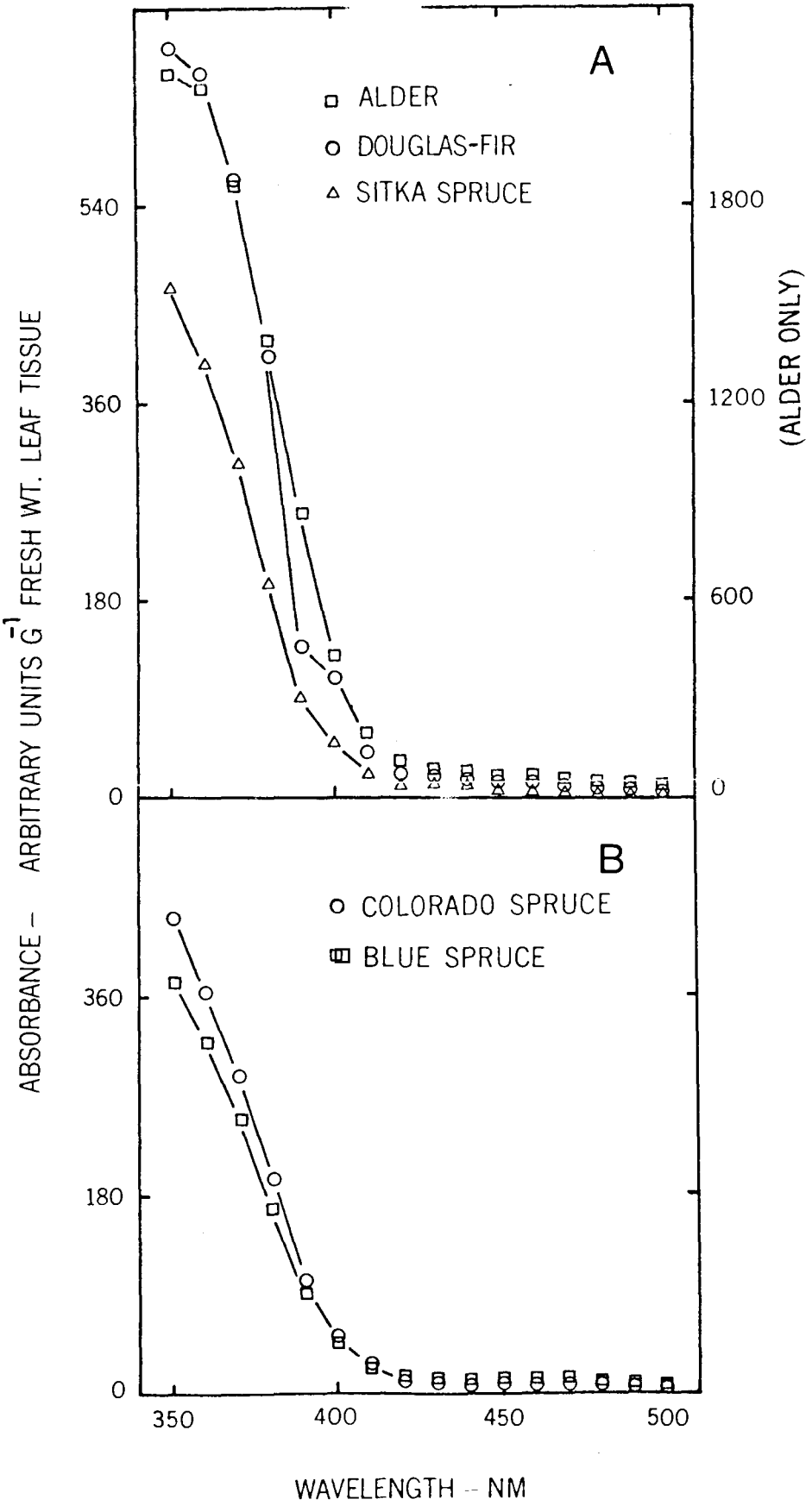

ALDER
DOUGLAS-FIR
SITKA SPRUCE
A
720
540
360
180
0
2400
1800
1200
600
0
(ALDER ONLY)
ABSORBANCE – ARBITRARY UNITS G^{-1} FRESH WT. LEAF TISSUE
COLORADO SPRUCE
BLUE SPRUCE
B
360
180
0
350
400
450
500
WAVELENGTH – NM

Figure 3.3 The absorption spectra, between 350 and 500 nm, of the 80% aqueous acetone pigment extract after partitioning against hexane

A. Alder (▣ - ▣), Douglas-fir (o - o) and Sitka spruce (△ - △)

B. Colorado spruce (o - o) and Blue spruce (□ - □)


A
ALDER
DOUGLAS-FIR
SITKA SPRUCE
B
COLORADO SPRUCE
BLUE SPRUCE
ABSORBANCE - ARBITRARY UNITS G^{-1} FRESH WT. LEAF TISSUE
(ALDER ONLY)
0
180
360
540
600
1200
1800
350
400
450
500
WAVELENGTH -- NM

absorption region (400 - 500 nm) from the absorption band below 400 nm due to other, non-chlorophyll-carotenoid cellular components.

Since the photosynthetic action spectra were determined only down to 400 nm and the fact that the hexane washed acetone fraction essentially does not absorb at wavelengths greater than 400 nm (Figure 3.3), one must conclude that the differences in the action spectra of photosynthesis, if due to pigment of the Burns' type, must be due to a component in the chlorophyll-carotenoid fractions. This conclusion is supported by the data in Figure 3.3 which shows not only that at wavelengths greater than 400 nm is the absorbance zero, but also that at any given wavelength below 400 nm, there is increasing absorbance by the extracts in the ascending order of Blue spruce - Colorado spruce - Sitka spruce - Douglas-fir and alder. This is exactly the opposite order to that predicted by the screening hypothesis based on the action spectra observations. Therefore, there is no "unusual" pigment present in the hexane washed acetone fraction similar to that reported by Burns (1942).

The form of the chlorophyll and carotenoid curves for all species are essentially identical (Figures 3.4 and 3.5), thus completely ruling out the presence of differential amounts of a Burns' component as proposed by the general screening hypothesis.

There remain however, two possible and interrelated explanations which would support a pigment-screening hypothesis. First,

Figure 3.4 The absorption spectra, between 350 and 500 nm, of the pigments in the methanol washed hexane fraction from an 80% aqueous acetone extract of:

A. Alder (□ - ■), Douglas-fir (o - o) and Sitka spruce (△ - △).

B. Colorado spruce (o - o) and Blue spruce (□ - □).

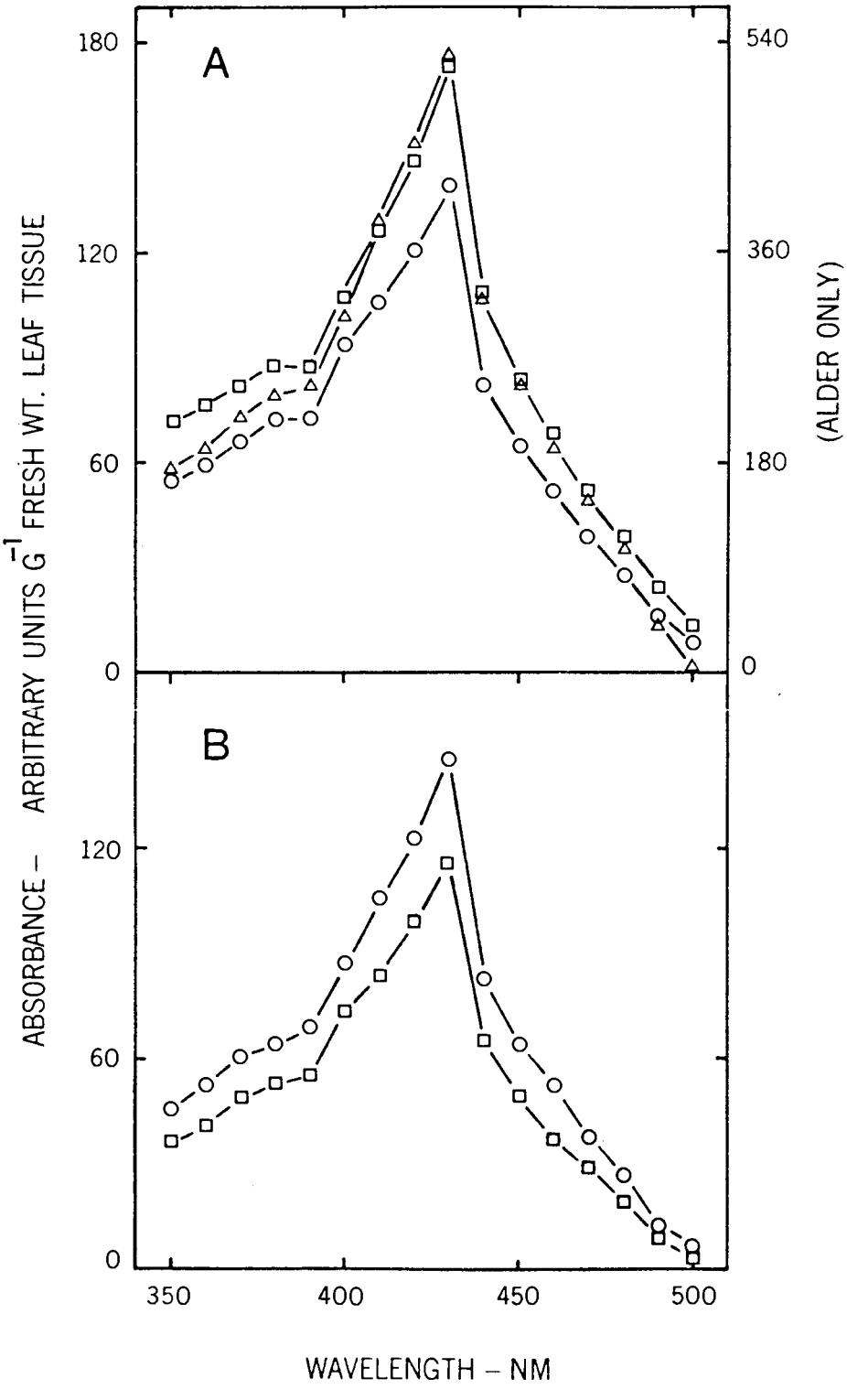
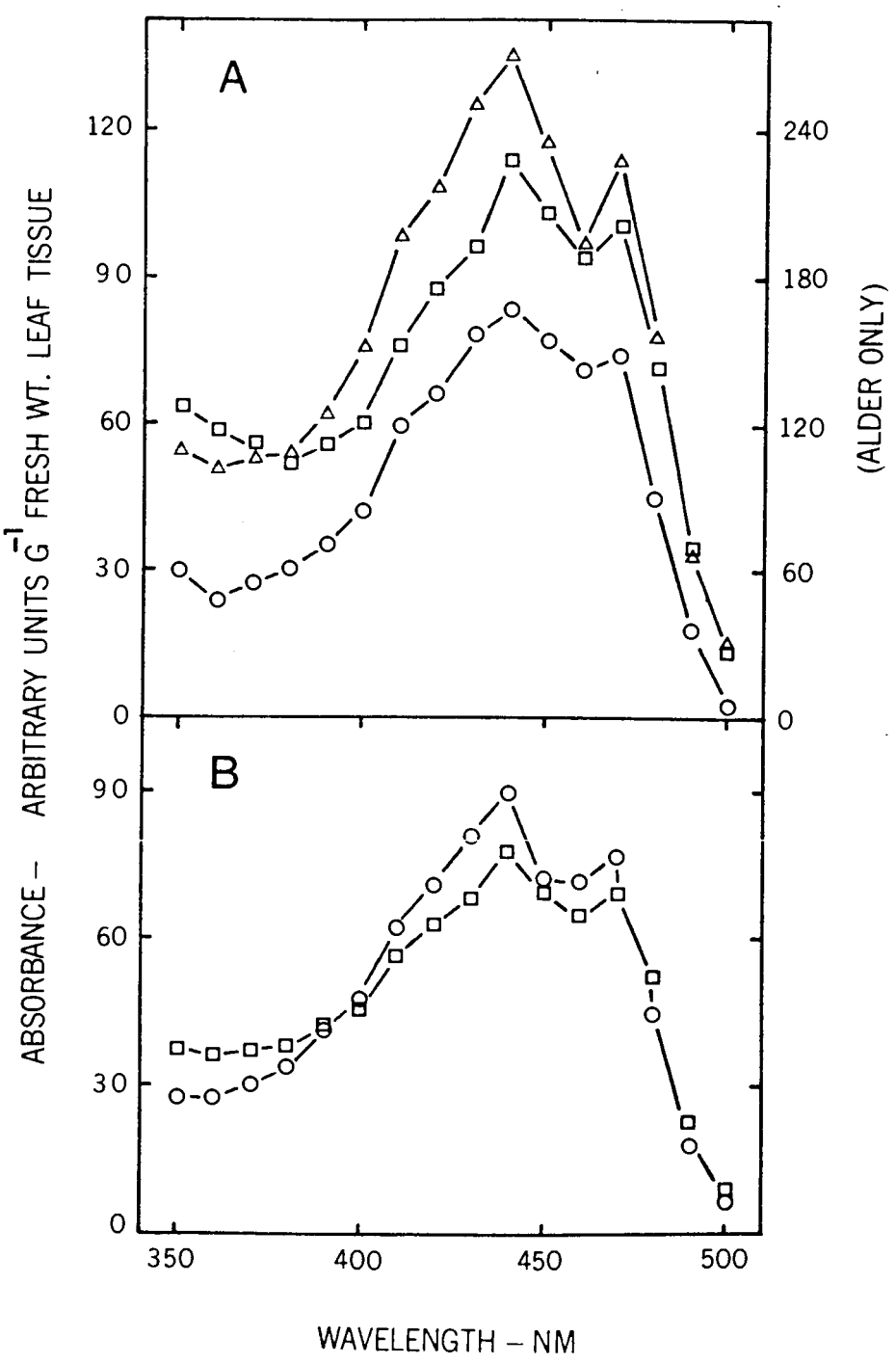

A
B
180
120
60
0
540
360
180
0
120
60
0
350
400
450
500
ABSORBANCE – ARBITRARY UNITS G^{-1} FRESH WT. LEAF TISSUE
(ALDER ONLY)
WAVELENGTH – NM

Figure 3.5 The absorption spectra, between 350 and 500 nm, of the pigments partitioned into 80% aqueous methanol from the hexane partition fraction of the 80% aqueous acetone extract of:

A. Alder (■ - ■), Douglas-fir (o - o) and Sitka spruce (△ - △).

B. Colorado spruce (o - o) and Blue spruce (□ - □)


A
B
120
90
60
30
0
240
180
120
60
0
(ALDER ONLY)
ABSORBANCE – ARBITRARY UNITS G^{-1} FRESH WT. LEAF TISSUE
350
400
450
500
WAVELENGTH – NM

the presence of different amounts of the carotenoids and second, the actual disposition of the carotenoids, influencing their relative efficiency in energy transfer. Rabinowitch and Govindjee (1969, p. 146-7) discussed the problem as follows:

> "To estimate more precisely the relative efficiency" (to sustain photosynthesis) "of the several pigments, one has to calculate the relative number of quanta absorbed by different pigments out of a monochromatic beam traversing a mixture of several of them. This is an awkward problem. In the first place we are not sure whether the several pigments form a uniform mixture, so that none has a first crack at absorption, shading the others" ... "The allocation of absorbed quanta to different pigments is therefore fraught with considerable uncertainties" ... However, ... "one can definitely assert from the analysis of the action spectra, that differences between the several pigments are more subtle than simple 'effective' or 'not effective'" ... "if the best sensitizing efficiency of chlorophyll a is set at 100%, that of the carotenoids varies, in different plants, between 20% and 50%".

First then, are there differences in the amounts of carotenoids, with their relatively low photosynthetic efficiencies, present in the various species? The data in Figure 3.5 and the calculated carotenoid$_{470}$/chlorophyll$_{430}$ ratios (alder - 0.38, Douglas-fir - 0.54, Sitka spruce - 0.67, Colorado spruce - 0.54 and Blue spruce - 0.59) show that while there were relatively minor differences amongst the chlorophyll contents of the conifers, their collective carotenoid content relative to the chlorophyll content was 40 - 50% higher than that of alder. Assuming that the energy transfer efficiency of the carotenoids is the same for all species, it appears therefore that the presence of relatively less caroten-

oid in alder allows more light to reach the chlorophylls to be utilized directly in photosynthesis. Thus the observed differences in pigment complements could at least partially account for the differences between the action spectrum of photosynthesis of alder and those of the conifers, but not for the differences between the action spectra of the various coniferous species.

There are presently no definitive answers to the second problem, that of the possibility of a varied disposition of the carotenoids, relative to the chlorophylls, resulting in a change of the photosynthetically active/inactive ratio for carotenoids. Thus there remains the possibility that in the series, alder - Douglas-fir - Sitka spruce - Colorado spruce - and Blue spruce, there is an increasing subcellular spatial/structural separation of the chlorophyll and carotenoid molecules, the latter shading the former or as Rabinowitch and Govindjee put it, allowing the carotenoids to have "first crack at absorbing" the light. The observed low efficiency of utilization in photosynthesis of light energy absorbed by carotenoids may be due to particular characteristics of the carotenoid molecule and/or a varying spatial separation between them and the chlorophyll molecules in the chloroplast lamellae membranes which dictates the efficiency of energy transfer.

It is generally accepted that the carotenoids have a protective function against the photodestruction of chlorophyll. Linder (1971) proposed that the lower carotenoid to chlorophyll

ratio observed in his greenhouse grown Scots pine compared to field grown plants is an adaptation to different environmental conditions of light and temperature. It could be that the carotenoid to chlorophyll content of conifers is generally higher than in many other groups of plants, but after reaching a specific and relatively high ratio, above which it cannot be increased for structural or whatever reasons, further protection from photodestruction of chlorophyll is achieved by increasing the relative amounts of photosynthetically inactive to active carotenoids by specific spatial separation and orientation of the carotenoid and chlorophyll molecules. Such a hypothetical strategy could have been adopted by the conifer types studied and especially the various spruce species. In spite of the very limited variety of material investigated, there does appear to be a correlation between their decreasing ability to utilize blue light in photosynthesis and their habitat range with respect to both higher altitudes and lower latitudes. The exposure to increased blue light (410 - 480 nm) intensities, at either high altitudes or lower latitudes may result in the photodestruction of chlorophylls if the intensities are not screened to physiologically tolerable levels.

SUMMARY AND CONCLUSIONS

The absorption spectra of the total and specific sub-fractions of the pigment complements of one deciduous broadleaf

and four coniferous tree species were determined during two successive growth seasons. There were no significant differences either between the data for comparable fractions from the two seasons within a species or between the relative absorption for equivalent fractions of different species. The conifer absorption spectra in general, and their carotenoid/chlorophyll ratios in particular, were all very similar, but there was 40 - 50% more carotenoid relative to chlorophyll in the conifers as compared to alder on a unit chlorophyll per unit fresh weight of material basis.

In conclusion, the hypothesis of different amounts of a Burns' type pigment being present as an explanation for the differences between the alder and conifer photosynthetic action spectra is incorrect for the species studied. Although the less efficient utilization of blue light in photosynthesis by Douglas-fir and Sitka spruce as compared to alder can be attributed to screening losses due to the greater presence of the carotenoids coupled with their low efficiency in energy transfer, this mechanism does not satisfactorily explain the results for the Colorado and Blue spruce. There is the rather remote possibility that a differential screening mechanism is present within the conifer group being dependent on the spatial disposition of the carotenoids. Changes in the spectral properties of the various conifer needles caused by differences in their cuticle structure appears to be a more plausible explanation. The results of the investigation into cuticle properties are presented in Part IV.

PART IV

THE RELATIONSHIP OF CUTICLE STRUCTURE TO THE VISIBLE AND UV SPECTRAL PROPERTIES OF NEEDLES FROM FOUR CONIFEROUS SPECIES

INTRODUCTION

The higher efficiency for blue light utilization in photosynthesis in the broad leaf of alder compared to the needle leaves of the conifer group was attributed to the lower carotenoid/chlorophyll ratio in alder (Part III) permitting more blue light to be directly absorbed by the main photoactive pigments. The four conifer species were essentially identical with respect to their carotenoid/chlorophyll ratios and in the inability of blue light to stimulate photorespiration. The differences in the conifer photosynthetic action spectra thus appear to be related to the foliage colour, "green" vs "blue". This seems reasonable because the colour of thick leaves such as spruce or pine needles is due wholly to reflected light, their transmittance being essentially zero (Burns 1942, Gates et al. 1965). Consequently, in the absence of any other differential screening mechanism, the needle spectral reflectance and absorbance curves (hence photosynthetic action spectra) must bear an inverse relationship to one another. The low APS rates in blue light for the two blue spruces would therefore result from a relatively greater reflection from the blue

waveband from the needle surface (hence the bluish colour) compared to the green foliage. Support for a physical selective light filtering mechanism being located at the needle surface (cuticular screening) is found in a review of plant cuticles by Martin and Juniper (1970, p. 6, 109):

> "Some plants exhibit a prominent waxy bloom. This is due to the reflection and scattering of light on the surface by waxy deposits whose dimensions are close to or only slightly above the wavelength of light ... A few wax patterns can be seen under the light microscope. The majority of species with glaucous leaves possess a superficial structure which is resolvable at best at the limit of resolution of a light microscope or only under the electron microscope. Whenever a plant surface possesses a visible bloom the electron microscope has revealed, without exception, a pattern of acicular projections from the surface. For a bloom to be formed, ie. for the incident light to be scattered in all directions from the surface, it seems that one or more of the dimensions of the projections must be of the same order of size or only slight larger than the wavelength of light."

The "blue colour", once removed by gentle stroking or wiping and revealing that these needles per se are as green as those of Douglas-fir and Sitka spruce, seems to be due to the extensive development in these species of a waxy epicuticular sculpturing or bloom.

The object of this study was to determine the effect of the bloom on the optical properties of the "green" and "blue" needles. As suggested by the cuticular screening hypothesis, control and treated needles were compared from each conifer species in three types of experiments. Firstly, is there a difference between the relative reflectance spectra for a Colorado or Blue spruce needle before and after removal of the

bloom (control vs treated foliage), and how does the result compare to the reflectance spectra of naturally green needles ie. Douglas-fir and Sitka spruce? Secondly, does the removal of the bloom from the two "blue" spruce needles change the relative photosynthetic action spectra so that the unscreened green needles show results more similar to Douglas-fir and/or Sitka spruce? Thirdly, does scanning electron microscope (SEM) examination of the needles' surfaces before and after treatment reveal the presence of a structure in the surface architecture which could be responsible for a selective filtering of the incident light?

MATERIALS AND METHODS

Control needles were unchanged from the natural condition, other than being detached for reflectance measurements and SEM examination. In order to leave the treated needles otherwise unaltered allowing "before and after" photosynthetic action spectra to be measured, the bloom removal was effected by gentle wiping with a cotton-tipped swab (Q-tip), a physiologically much milder treatment than solvent removal.

The relative reflectance at a given wavelength of control and treated needles was determined by measuring the intensity of the scattered radiation perpendicular to the incident beam (Slayter 1970) using a Farrand Optical Company Spectroflourimeter Mk II which has two monochromators at right angles. The

needle was placed in a special holder in the sample chamber and rotated until the maximum signal was obtained (light incident at 45°) with the two monochromators set at 540 nm (± 2 nm). The reflectance spectrum was then determined by resetting the excitation monochromator at 10 or 20 nm intervals from 220 to 700 nm and scanning with the analyzer monochromator to achieve the maximum signal. Since the characteristics of the entire light train were not uniform with respect to wavelength, the instrument was standardized with a "white" needle, i.e. one coated with magnesium oxide. The spectral reflectivity of MgO is known to be essentially uniform and very high (>90%) from 200 to 700 nm (Parker 1968).

The use of additional optics to focus both the incident and reflected light beams to cover a circle of only 2 mm diameter still did not allow the generation of absolute reflectance data due to the variability in both the needle width between species, from 2 mm to less than 1 mm (ie. less than the total beam diameter), and the irregularity of the needles' surfaces. The amounts of light lost due to non-interception and diffuse scattering from the needle surface cannot be measured by this method. Therefore, the reflectance data were expressed as a percentage of the reflectance at 540 nm, the wavelength of maximum reflectance for the majority of samples in the region of photosynthetically active light. In addition, it was found that an exact and reproduceable achievement of a 90° reflectance angle was not possible. However, normalization

of these data to a percentage of the reflectance at 540 nm yielded consistent and comparable results of spectral reflectance.

To determine the possible influence of the bloom on photosynthetic CO_2 uptake, the action spectrum of APS was determined for control foliage of Blue spruce using the same methods described in Part I. The rate of APS was measured at several intensities in each waveband after which the foliage was treated, resealed into the photosynthesis chamber and then APS vs light intensity measurements repeated on the treated foliage. The action spectra of APS for control and treated Blue spruce foliage were expressed at a constant incident light intensity of 0.8×10^5 ergs cm^{-2} sec^{-1} as in Part I.

For SEM examination of the needle surfaces, a 5 mm length from the central portion of each of the control and treated needles was mounted on individual pegs, dried, gold shadowed under vacuum, and viewed in a Cambridge Stereoscan microscope operated at 20 KV. Intermediate (x2,400) and high (x12,000) power magnifications clearly showed the nature of the surface sculpturing.

RESULTS AND DISCUSSION

The relative reflectance spectra from 220 nm to 700 nm for control and treated conifer needles from four species are presented in Figure 4.1 A-E. Measurements from a greenish and blue-green coloured Colorado spruce foliage are also given.

Figure 4.1 The relative reflectance spectra between 220 nm and 700 nm, normalized at 540 nm, for control (O - O) and treated (bloom removed, Δ - Δ) needles from current year foliage of:

A. Douglas-fir (cf. Douglas-fir data of Woolley, 1971, □ - ■)

B. Sitka spruce

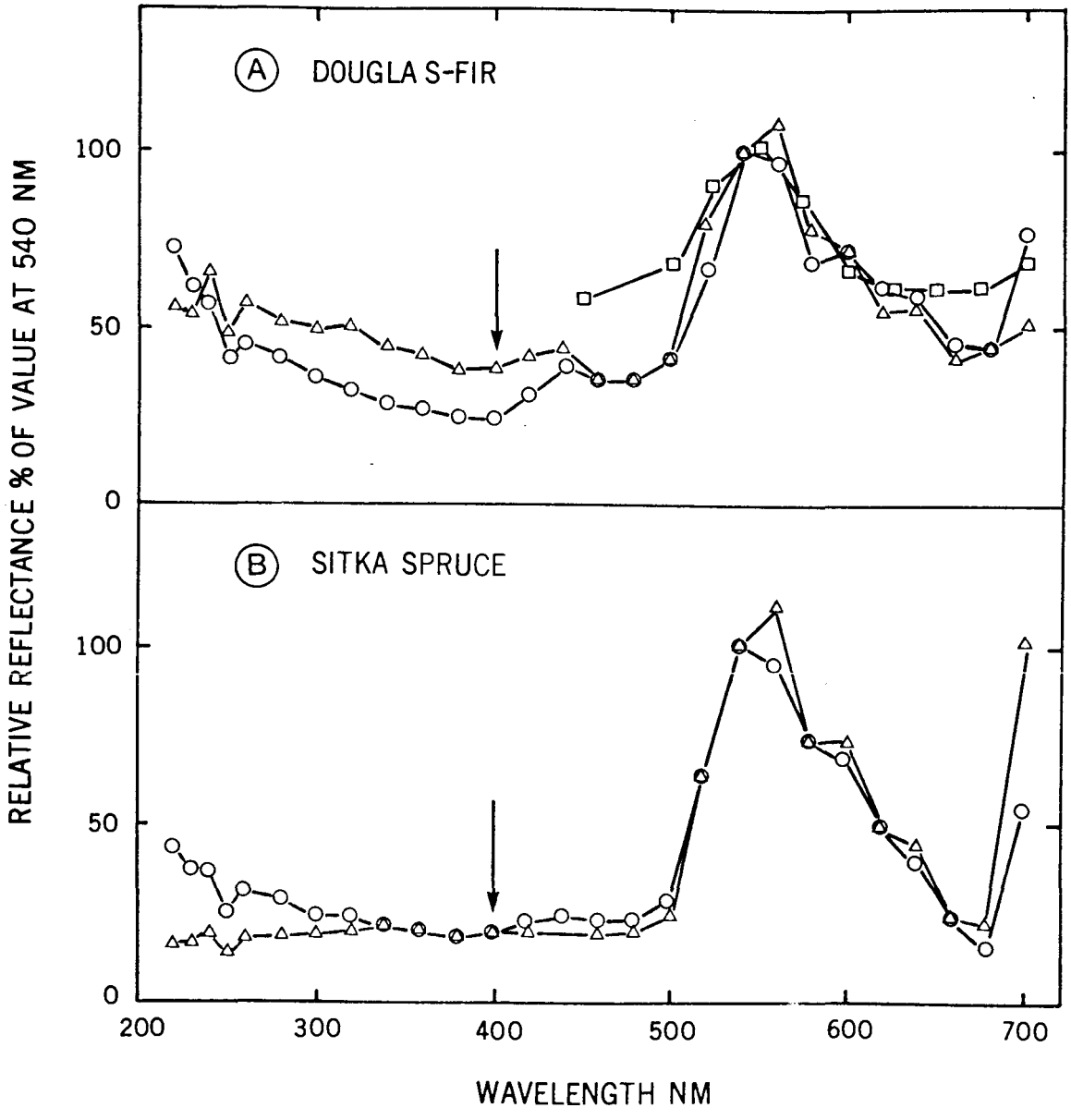
C. Colorado spruce - greenish (page 58c)

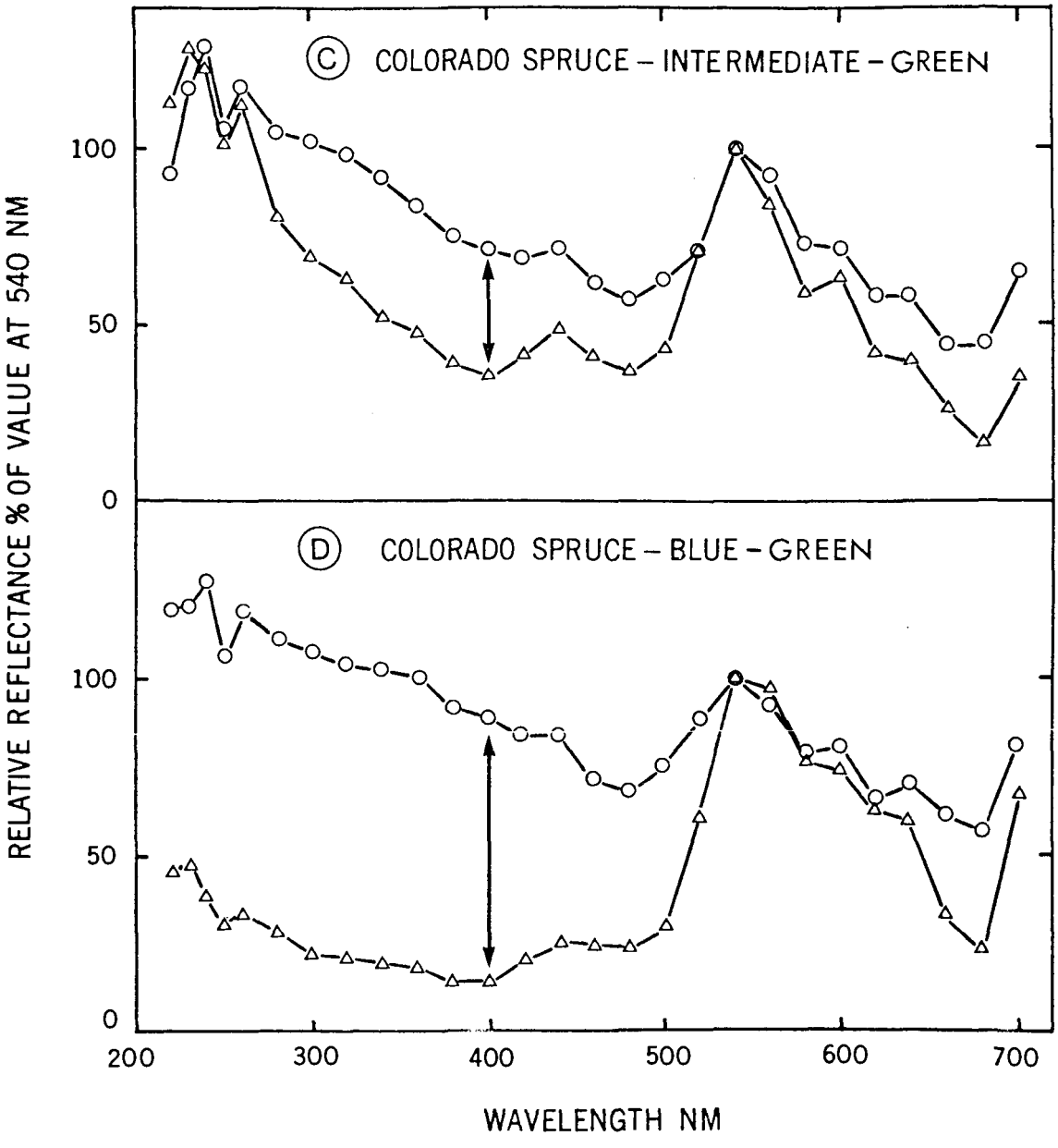
D. Colorado spruce - blue-green (page 58c)

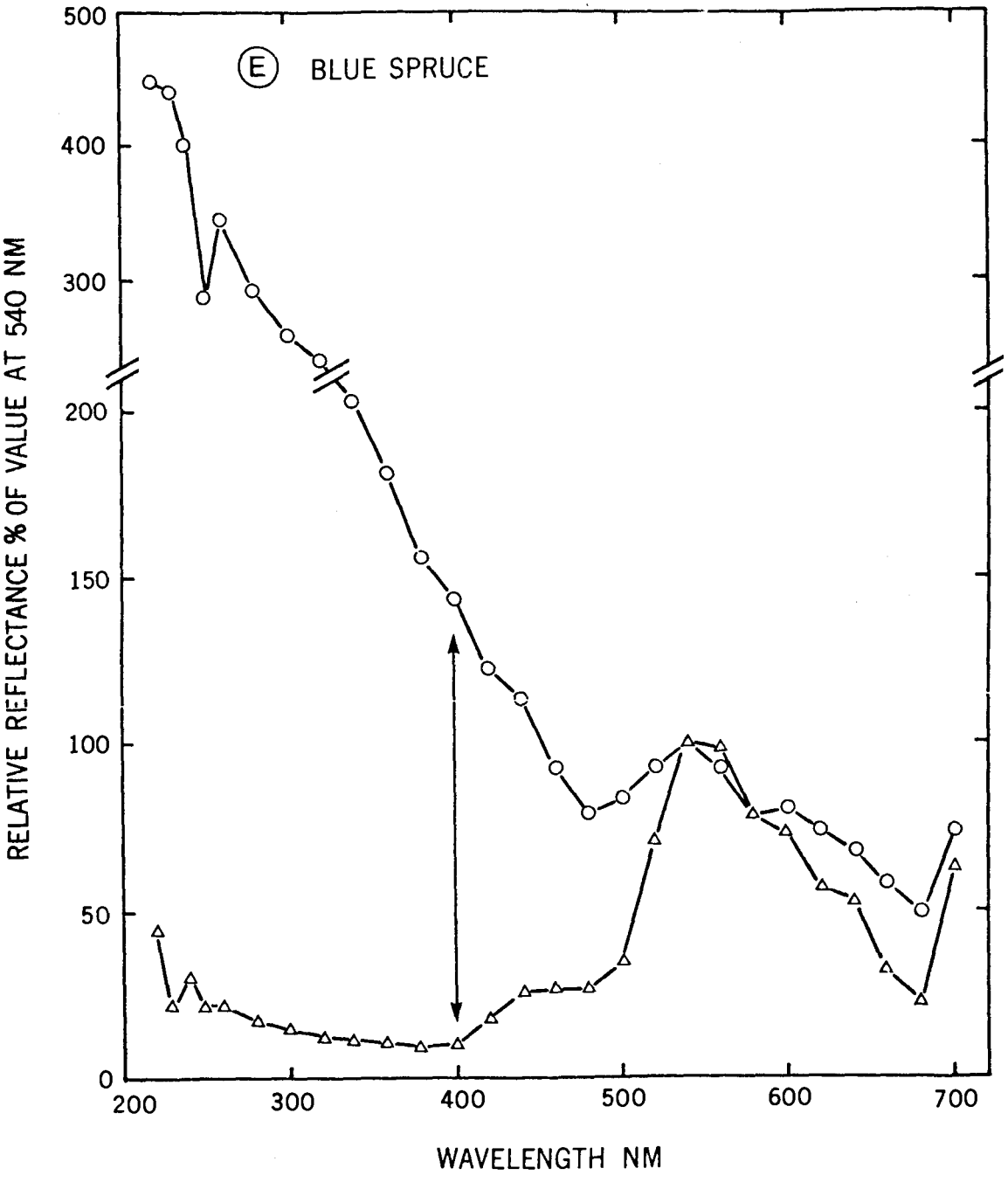
E. Blue spruce (page 58d).

Each point is the mean of four measurements (two needles from each of two trees of each species). The maximum range of variation between data from the same species was ±10% (220 - 460 nm) and ±5% (480 - 700 nm) of the mean value.

The arrows divide the visible and photosynthetically active region (400 - 700 nm) from the ultraviolet region ($\lambda < 400$ nm).


A DOUGLAS-FIR
B SITKA SPRUCE
RELATIVE REFLECTANCE % OF VALUE AT 540 NM
WAVELENGTH NM
0
50
100
200
300
400
500
600
700


C COLORADO SPRUCE – INTERMEDIATE – GREEN
D COLORADO SPRUCE – BLUE – GREEN
RELATIVE REFLECTANCE % OF VALUE AT 540 NM
100
50
0
100
50
0
200
300
400
500
600
700
WAVELENGTH NM


E BLUE SPRUCE
RELATIVE REFLECTANCE % OF VALUE AT 540 NM
WAVELENGTH NM
500
400
300
200
150
100
50
0
200
300
400
500
600
700

For the green needles of Douglas-fir and Sitka spruce, the relative reflectance spectra are essentially identical regardless of the needle treatment (Figure 4.1 A,B). The treated foliage spectra for the Colorado and Blue spruces (Figure 4.1 C,D,E) are very similar to the results for the Douglas-fir and Sitka spruce. In each case, there is a peak of reflectance (100%) in the green portion of the visible spectrum at 540 to 560 nm which falls off rapidly to minima (20 - 40%) in the blue (450 - 500 nm) and the red (660 - 680 nm). At 440 to 450 nm there is a minor but consistent blue peak of reflectance. At wavelengths shorter than 440 nm, the relative reflectance remains low (10 - 35%) down to 300 nm except for the greenish Colorado spruce, below which it slowly begins to increase. On the longwave side of the red minimum, the reflectance rises dramatically (55 - 80%) as the near infrared region ($\lambda > 700$ nm) is approached. These results closely agree with the relative reflectance of needles in the visible waveband reported by Woolley (1971) for Douglas-fir and Billings and Morris (1951) for pine, and for other green leaf reflectances reported by Rabideau, French and Holt (1946) and Gates et al. (1965). The low UV reflectance of green leaves in general (Caldwell 1968, Gates et al. 1965) is supported by these results.

The effect of needle treatment on the spectral reflectance in Colorado and Blue spruce can be seen by comparing the control foliage curves with the treated foliage curves in Figure 4.1 (C, D and E). In each case, the relative reflectance of the control

foliage is greater in the ultraviolet, blue and red wavebands compared to the treated foliage. The results are summarized in the following table:

Foliage	Relative Reflectance (%) UV	Blue	Green	Red
Control				
Colorado spr.				
Greenish	75-125	60- 75	100	45
Blue-green	80-150	65- 80	100	55
Blue spruce	130-450	75-130	100	50
Treated and all other green species	10- 35	20- 40	100	20-40

The increased relative reflectance of the blue waveband (400 - 500 nm) for the greenish Colorado spruce, blue-green Colorado spruce and Blue spruce is accompanied by an even greater parallel increase in the UV waveband, in contrast to the low UV reflectance observed in the treated and all other green foliage. The slightly greater reflectance in the region of the red minimum (660 - 680 nm) does not follow this pattern; these variations are not significantly different.

These results show that the presence of the bloom does cause selective reflection of blue light in Colorado and Blue spruce, appearing to be the result of "carry over" scattering which has its peak effect in the 100 - 200 nm waveband. The sequence of conifer species with respect to decreasing photosynthetic efficiency of blue light is identical to that for increasing blue light reflectance (compare Figures 1.4 and 4.1).

The effect of the needle treatment at the physiological

level was checked by measuring the action spectrum of APS for control and treated foliage of Blue spruce since it would be expected that the treatment would show the greatest effect in this species (Figure 4.2). The action spectrum for the control foliage shows the same features as described previously; a broad red peak of activity from 620 - 665 nm and a much reduced shoulder of activity in the blue (400 - 500 nm). The effect of the treatment has raised the relative APS activity in the blue, although not as much as was expected. Considerable handling is required to treat each needle of an intact branch as compared to the relative ease in treating single detached needles for reflectance measurements. To minimize physiological damage, the intact attached needles did not receive as thorough a treatment as the detached needles, hence a reduced effect of the treatment could be expected. Furthermore, the stomatal antechambers are plugged with displaced wax (Figure 4.5 C) which alters the CO_2 diffusion resistances of the treated needles. With these restrictions, the treatment had at least a limited effect in the expected direction.

The surface architecture before and after needle treatment is shown in a series of SEM micrographs. The first (Figure 4.3) is a low power (X200) micrograph of a control Colorado spruce needle for orientation to easily recognizable surface features such as stomatal pits. The following medium (x2,400) and high (x12,000) power micrographs, taken from the area indicated in the low power micrograph, illustrates the differences in the

Figure 4.2 A. The action spectra of apparent photosynthesis for attached branches with control (O - O) and treated (Δ - Δ) current-year foliage of Blue spruce at 22C, 300 ppm CO_2, 21% O_2 and a constant incident light energy of 0.8×10^5 ergs cm^{-2} sec^{-1}.

B. The rate of apparent photosynthesis under an equal intensity of white light under the same experimental conditions as in A.

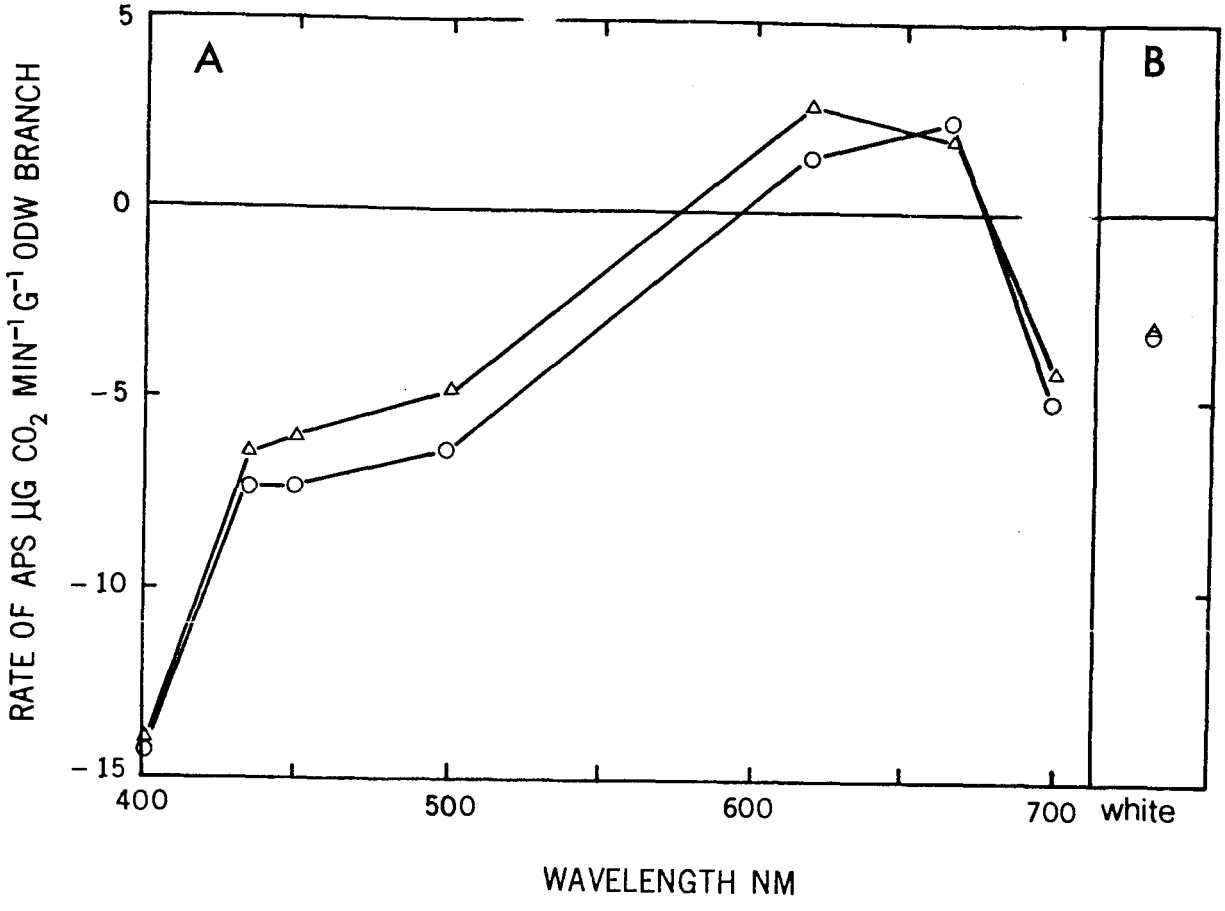
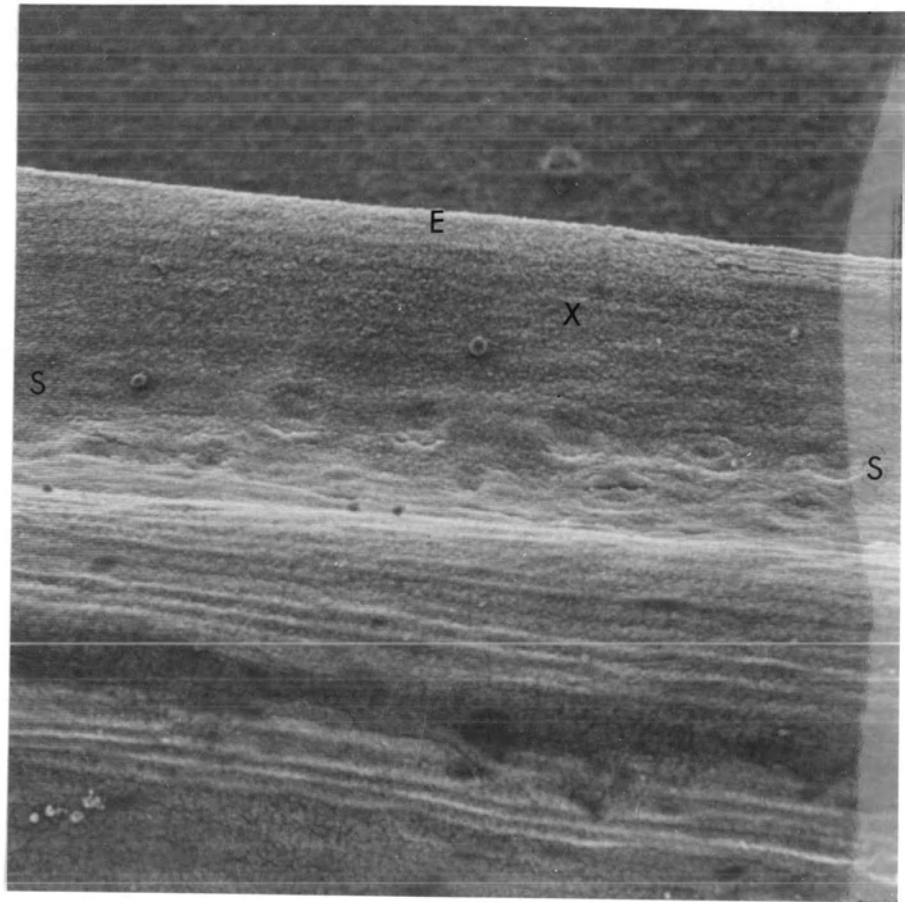

A
B
5
0
-5
-10
-15
400
500
600
700
white
RATE OF APS µG CO_2 MIN^{-1} G^{-1} ODW BRANCH
WAVELENGTH NM

Figure 4.3 Low power (X 200) scanning electron micrograph of a control Colorado spruce needle. Note the row of stomatal pits (S---S) and the general flocculent appearance over the whole surface due to the presence of the bloom. The area marked X indicates the portion of the needle surface subsequently examined at intermediate (X 2,400) and high (X 12,000) power magnifications for each species. The horizontal line (E) is the needle edge. At low power (X 200) 1 mm on the micrograph is equivalent to 5,000 nm.


E
X
S
S

surface sculpturing between Douglas-fir, Colorado spruce, and Blue spruce, as examples of the extremes in the range.

The waxy sculpturing on the needle surfaces appears to have the same basic unit in each species; anastomosing or reticulate filaments of various lengths but all approximately 125 nm in width. However, the degree of surface cover, depth of the mat, and density of packing of the individual elements appears to be species dependent. In control foliage, where the wax has not been disarranged, these differences are apparent. In Douglas-fir (Figure 4.4 A, B) the wax projections occur in clumps leaving the needle surface between clumps completely devoid of projections; within the clumps the needle surface is still visible. In contrast, the blue spruce types show a much greater thickness of the filamentous mat, a more extensive coverage of the surface and a greater degree of packing of the filaments. The needle surface is only visible in a few places for the Colorado spruce (Figure 4.5 A, B) but is completely hidden from view in the Blue spruce (Figure 4.6 A, B).

The needle treatment had no apparent effect on the surface sculpturing in Douglas-fir (Figure 4.4 C, D) in contrast to the effect in the blue spruces where the bloom was removed or smeared (Figure 4.5 C, D and 4.6 C, D). The effect of needle treatment in these latter species was to destroy the highly developed architecture of the epicuticular wax of the control needles. There can be no doubt that the differences in leaf reflectivity (especially in the UV and blue portions of the

Figure 4.4. Intermediate and high power scanning electron micrographs of the wax projections on the surface of control and treated Douglas-fir needles.

A. Control, X2,400 (1 mm = 416 nm)

B. Control, X12,000 (1 mm = 83.5 nm)

C. Treated, X2,400

D. Treated, X12,000

Note: Treatment has had no apparent effect on the structure of the surface sculpturing.

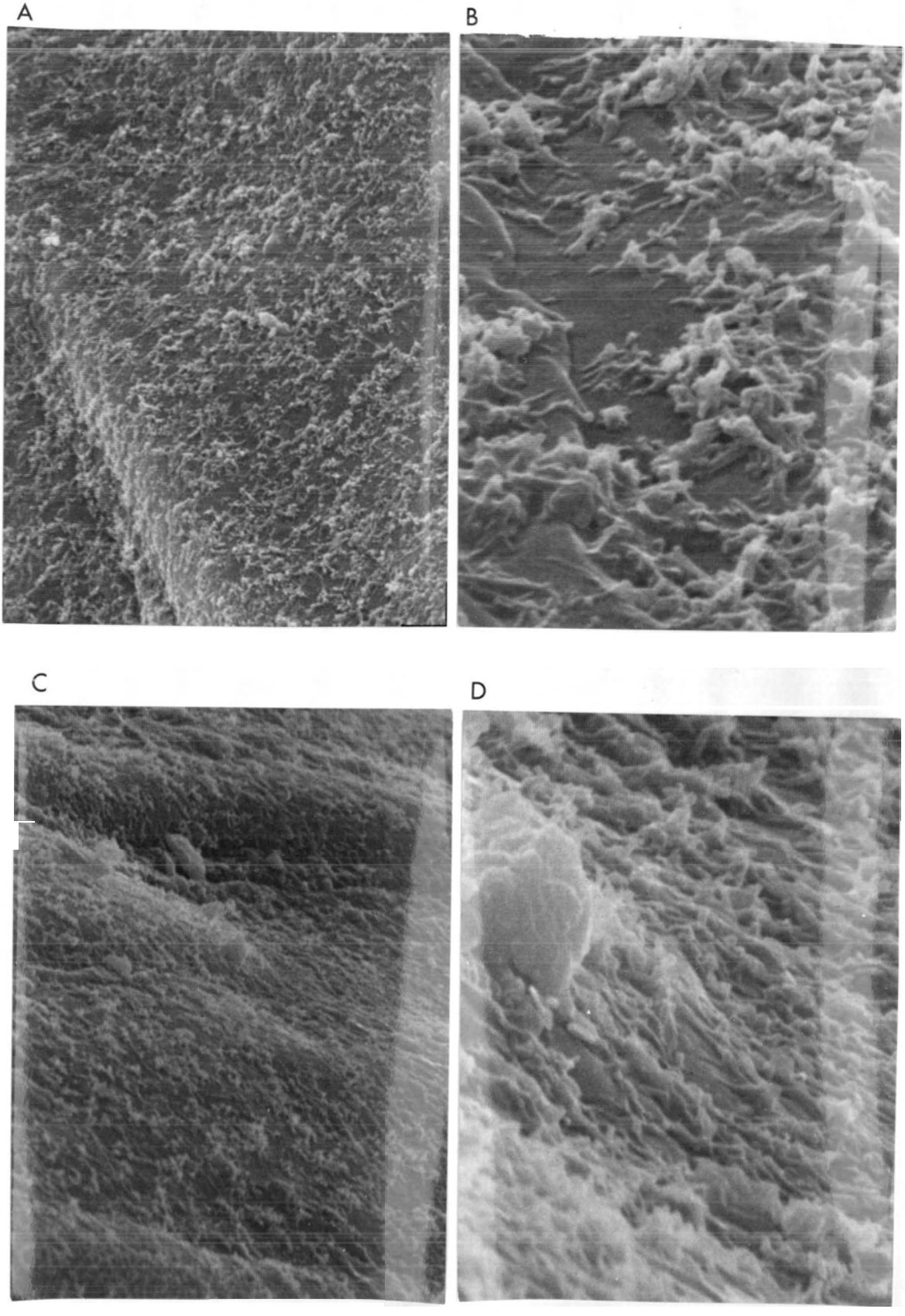

A
B
C
D

Figure 4.5. Intermediate and high power scanning electron micrographs of the wax projections on the surface of control and treated Colorado spruce needles.

A. Control needles at X2,400 (1 mm = 416 nm)

B. Control needles at X12,000 (1 mm = 83.5 nm)

C. Treated needles at X2,400. Note the partially occluded stomatal pit and the striations of displaced wax as a result of stroking the surface with the Q-tip.

D. Treated needles at X12,000.

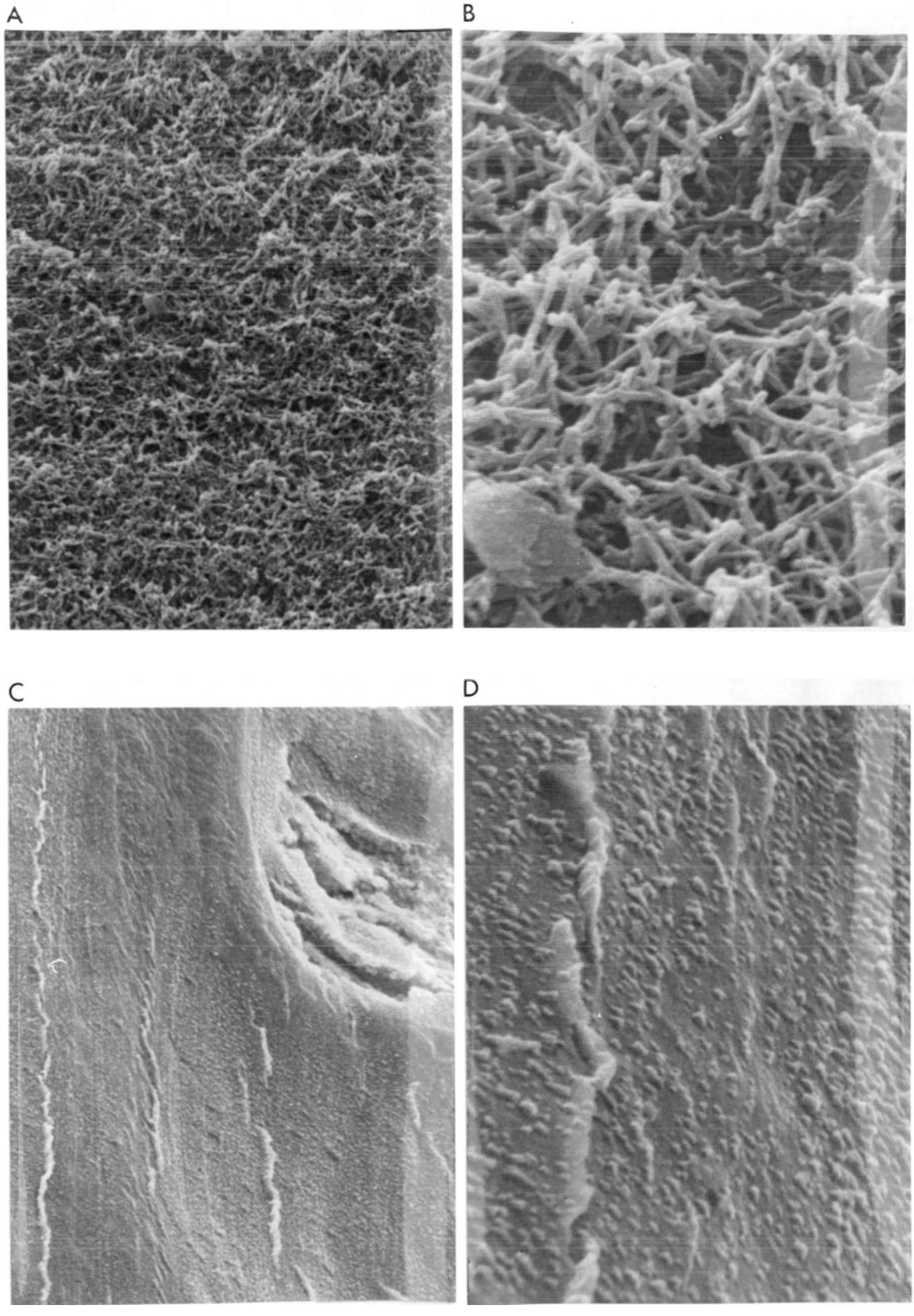

A
B
C
D

Figure 4.6. Intermediate and high power scanning electron micrographs of the wax projections on the surface of control and treated Blue spruce needles.

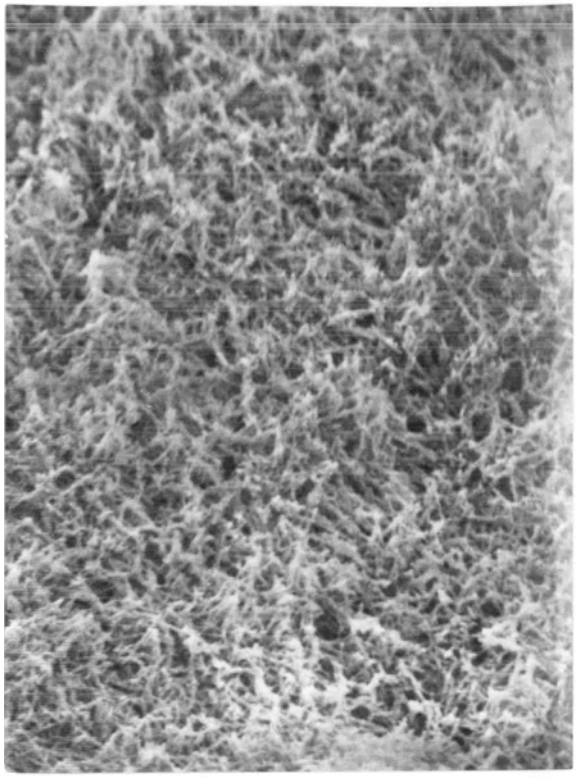
A. Control needles at X2,400 (1 mm = 416 nm)

B. Control needles at X12,000 (1 mm = 83.5 nm)

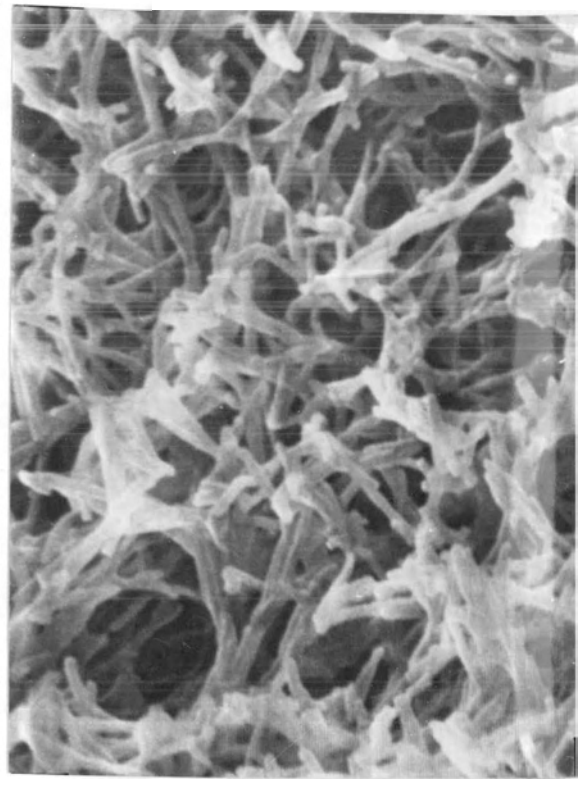
C. Treated needles at X2,400

D. Treated needles at X12,000

A

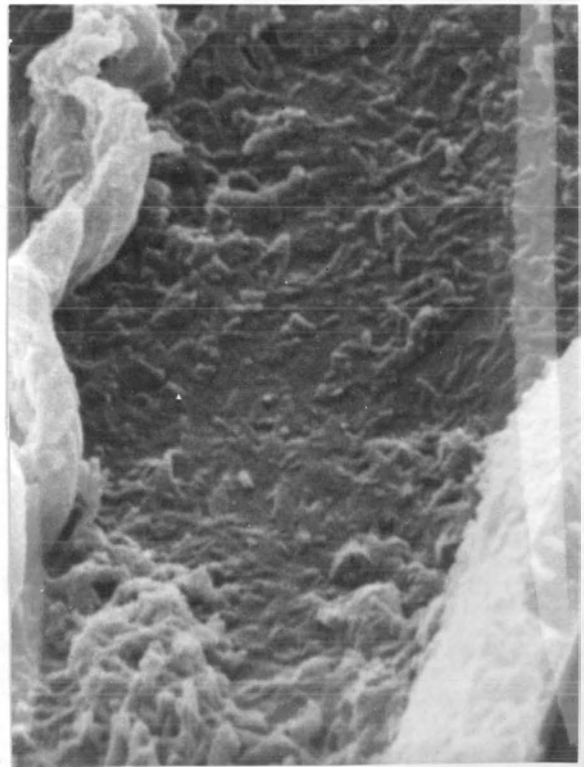


B



C

D



spectrum) of the blue spruces compared to the green foliage of Douglas-fir and Sitka spruce arose from the species differences in the degree of complexity of needle surface sculpturing.

Similar epicuticular structures in the form of intermeshing wax tubes (1 μm long X 150 nm outside diameter) have been reported on Sitka spruce needles by Johnson and Jeffree (1970) and Jeffree, Johnson and Jarvis (1971). The distribution of the layer of wax tubes, being present mainly within the stomatal antechambers and epidermal regions between the stomatal bands in contrast to the other parts of the epidermis (Jeffreee et al. 1971), corresponds well with the results of this study insofar as the structure and degree of development of the epicuticular sculpturing in the various conifer species is concerned.

Granting that the wax-filled antechambers are effective antitranspirants (Jeffree et al. 1971), the presence of the conspicuous bloom over the entire surface of Colorado and Blue spruce needles suggests that this development is an adaptation to the relatively intense solar UV irradiation in their native habitat in contrast to the low-altitude conifers, ie. Douglas-fir and Sitka spruce. Colorado spruce, a native of the Rocky Mountains from Montana to New Mexico and Arizona, occurs at elevations ranging from 6,000 - 9,000 ft in the north to 8,000 - 11,000 ft in the south (Preston 1968). The intensity of the UV-A waveband (280 - 315 nm) increases linearly by 10% per 2,000 m elevation increase (Caldwell 1968). On a cloudless midsummer day Caldwell (1968) measured a 26% increase in the

global UV-B (315 - 400 nm) irradiation at 4350 m as compared to 1670 m. The dramatically high UV reflectivity of control Colorado and Blue spruce foliage has been shown by this study.

From this discussion it is concluded that the primary function of the extensive needle bloom in Colorado and Blue spruce is to reduce the intensity of UV wavelengths by scattering, to physiologically tolerable levels as evidenced by the very low UV reflectivities for their own treated and other naturally green (non-bloomed) foliage. The secondary "carry-over" scattering into the blue portion of the visible spectrum accounts for the bluish colour, which in turn results in a reduced intensity of photosynthetically available blue light.

The differences between the photosynthetic action spectra for the "green" vs "blue" conifers in the blue waveband, can thus be attributed to the physical light screening mechanism just described which serves to filter light at the needle surface.

OVERALL SUMMARY AND CONCLUSIONS

The action spectra of apparent photosynthesis (APS) for a broadleaf and four coniferous tree species were determined on a consistent and comparable basis (isoenergetic beams) from 400 - 710 nm by CO_2 exchange analysis. The blue (400 - 500 nm) peak of APS activity in the green deciduous leaf of alder was reduced to a plateau in the green needles of Douglas-fir and Sitka spruce, a shoulder in the blue-green needles of Colorado spruce and a reduced shoulder in the blue-white needles of Blue spruce.

There was no evidence of a metabolic screening mechanism in the form of a differential stimulation of photorespiration by blue light or of differential absorption screening by non-chloroplast pigments. The carotenoid/chlorophyll ratios of the four conifers were very similar with 40 - 50% more carotenoid relative to chlorophyll than in alder. The higher APS activity of alder as compared to the conifer group can be attributed to the greater amounts of blue light being absorbed directly by the main photoactive pigments as a result of comparatively less absorption screening by the accessory pigments.

The presence of a highly developed epicuticular architecture on Colorado and Blue spruce needles was shown to selectively reflect blue light as compared to the non-bloomed foliage of Douglas-fir and Sitka spruce. The physical screen-

ing of light effected by this structure accounts for the relatively low APS activities in the blue waveband of the "blue" conifers as compared to the green conifers.

From these results it is also concluded that the basic photosynthetic apparatus of these broadleaf and conifer plants are the same.

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