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Photosynthetic Action Spectra of Trees

II. THE RELATIONSHIP OF CUTICLE STRUCTURE TO THE VISIBLE AND ULTRAVIOLET SPECTRAL PROPERTIES OF NEEDLES FROM FOUR CONIFEROUS SPECIES[1]

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ABSTRACT

The relative reflectance spectra for control and treated (surface wiped) current-year foliage of Douglas fir, and Sitka, Colorado, and Blue spruce (*Pseudotsuga menziesii* [Mirb.] Franco, *Picea sitchensis* [Bong.] Carr., *Picea pungens* Engelm., and *Picea pungens* Engelm. var. hoopsii, respectively) were obtained from 220 to 700 nm. The green color of the control foliage of both Douglas fir and Sitka spruce was unaffected by the treatment whereas the blue-green and blue-white foliage of control Colorado and Blue spruce, respectively, became "green" as a result of the wiping. The relative reflectance curves for all green foliage, including the treated Colorado and Blue spruce, were all very similar with a peak in the green (540–560 nm), minima in the red (660–680 nm) and blue (450–500 nm), and very low reflectivities in the ultraviolet ($\lambda < 400$ nm). In contrast, the control foliage for Colorado and Blue spruce both showed a generally higher relative reflectance over most of the visible spectrum (400–700 nm) with a marked increase in the blue region (400–500 nm). At wavelengths below 420 nm, their relative reflectances increased sharply with decreasing wavelength, the reflectance at 220 nm for Blue spruce being over four times that at 540 nm.

Scanning electron microscope examination of the needles' surfaces revealed a system of wax filaments whose complexity correlated with the degree of ultraviolet and blue reflectance.

It is concluded that both the bluish appearance (glaucous bloom) and the low relative efficiencies of blue light in photosynthesis of Colorado and Blue spruce result from the selectively enhanced reflection of blue light caused by the presence of the epicuticular wax deposits. The enhanced blue light reflection was shown to be the shoulder of a scattering effect which appeared to peak in the short ultraviolet region below 200 nm. The ecological implications of the results are discussed.

In a comparative study of one broadleaf and four coniferous tree species, Clark and Lister (5) showed that leaf form (broad *versus* needle) did not influence the ratio of net photosynthetic activity in blue light to that in red light. The effect of leaf coloration on spectral photosynthetic activity, however, remained to be clarified. Within the conifer group, in which foliage color ranged from green through blue-green to blue-white, the relative photosynthetic activity under blue light decreased with increasing blueness (or decreasing greenness) of the foliage. Since the transmittance of light is essentially zero (3, 7), the observed leaf color in material such as spruce or pine needles is attributable almost wholly to reflection. The bluish appearance of Colorado spruce, other factors being equal, might therefore be caused by a selectively enhanced reflection of blue light resulting in a reduction of the energy available for photosynthesis.

The blue color of Colorado spruce foliage, known as a bloom, is easily dispelled by gently wiping the needles to reveal a typical green foliage appearance. The bloom is caused by the extensive development of waxy epicuticular projections which scatter and reflect incident light. For a bloom to be observed, it seems that one or more dimensions of the waxy projections must be of the same order of size or slightly larger than the wavelength of light (11).

The purpose of the present comparative study of conifers with green and "blue" foliage was: (*a*) to determine the effects of the presence or absence as well as the architecture of the epicuticular wax deposits on the respective spectral reflectance characteristics of the needles, and (*b*) to examine the ecological implications of these results.

MATERIALS AND METHODS

Plants. The experimental material was detached, fully expanded, current year needles from lateral branches of 4-year-old plot grown potted trees of the following types: Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), coastal, low altitude type (courtesy of B. C. Forest Service Green Timbers Nursery); Sitka spruce (*Picea sitchensis* (Bong.) Carr.); Colorado spruce (*Picea pungens* Engelm.); and Blue spruce (*Picea pungens* Engelm. var. *hoopsii*). Sitka, Colorado, and Blue spruce were from Glassbergens's Ornamental Tree Nursery.

Determination of Relative Spectral Reflectance. The needle reflectance at a given wavelength was determined by measuring the intensity of the radiation scattered perpendicularly to the incident beam (15) using a Farrand Optical Company Mk II Spectrofluorimeter. Single needles were rotated in the sample chamber until a maximum output signal was obtained (light incident at 45°) with both the excitation and analyzer monochromators set at 540 ± 2 nm. The maximum entrance and exit slit widths on the excitation monochromator were 10 nm. The reflectance spectrum was determined by resetting the excitation

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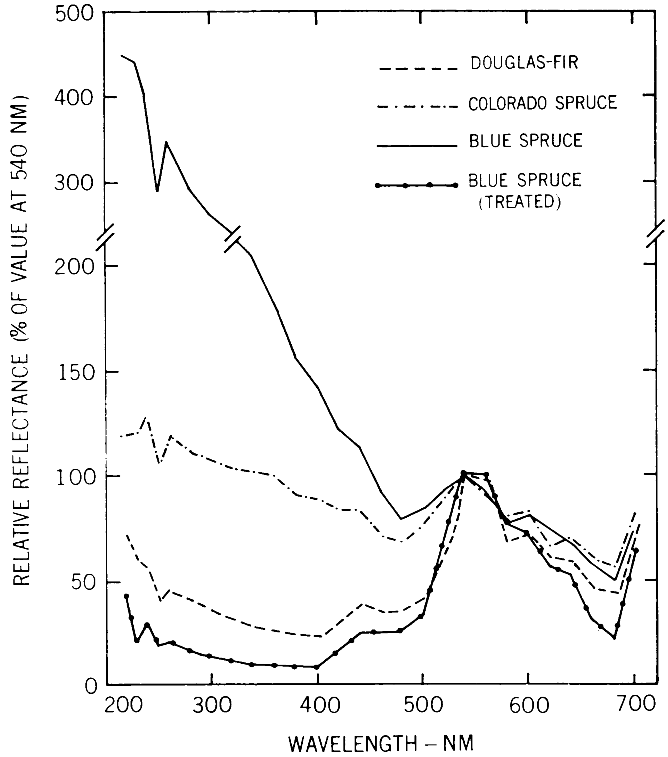

FIG. 1. Relative reflectance spectra, normalized at 540 nm, for four types of conifer needles. Each point is the mean of four to six measurements on replicate samples from replicate trees. Maximum variation is ±10% (220–460 nm) and ±5% (480–700 nm) of the mean value. Comparable data were obtained over two seasons.

monochromator at 10 or 20 nm intervals from 220 to 700 nm and scanning from $\lambda - 5$ to $\lambda + 5$ nm with the analyzer monochromator. The maximum signal obtained at each wavelength setting of the excitation monochromator (λ) was found to lie within the calibration limits of the two monochromators. Because the needle surfaces are irregular and their widths variable, some incident light is lost by diffuse scattering and noninterception. Although this limitation precluded the determination of absolute reflectance data, the method nonetheless was useful for a comparative study of relative spectral reflectance. The spectral reflectance data for each specimen was standardized against that of the same needle coated with magnesium oxide and then normalized at 540 nm, the wavelength of maximum reflectance in the region of photosynthetically active light for the majority of samples. This procedure yielded relative spectral reflectance results that were not only consistent for the same tissue types but were also readily comparable to those for other tissue types.

Treated needles had any surface bloom dispelled by wiping with a dry cotton-tipped swab.

Scanning Electron Microscope Examination of Needle Epicuticular Wax Deposits. Short needle sections, 5 mm long, were mounted on pegs, gold shadowed, and viewed in a Cambridge stereoscan Model S4 microscope operated at 20 kv.

RESULTS AND DISCUSSION

Relationship of Leaf Color to Spectral Reflectance. The range of relative reflectance spectra obtained for control and treated foliage of green and "blue" conifers are represented by the curves in Figure 1. There are two important features to be noted. First, for all naturally green foliage as well as for all green treated material, *i.e.*, including Colorado and Blue spruce, the relative spectral reflectance curves are essentially the same. There is a peak of reflectance (100%) at 540 to 560 nm which decreases sharply to minima (20–40%) at 450 to 500 nm and 660 to 680 nm. At 440 to 450 nm there is a minor but consistent peak of reflectance. Below 440 nm the relative reflectance remains low (10–35%) even into the UV region ($\lambda < 400$ nm). Above 680 nm the reflectance rises sharply (55–80%) in the near infrared region ($\lambda > 700$ nm). Second, for control foliage of Colorado and Blue spruce, the increased relative reflectances for blue light (400–500 nm) for the blue-green foliage (65–80%) and the blue-white foliage (75–130%) are accompanied by even greater parallel increases in the UV waveband (80–150% and 130–450%, respectively). This is in direct contrast to the low UV reflectance observed in the corresponding treated foliage as well as in all other green foliage.

These results agree closely with the spectral reflectance of needles in the visible waveband for Douglas fir (17), pine (2), and other green leaves (7, 13). The low UV reflectance of green leaves in general (4, 7) is also supported by these results. Figures for absolute spectral reflectance, unavailable because of the limitations mentioned, require that all scattered light be collected, *e.g.*, with an Ulbricht sphere. However, for Douglas fir and Sitka spruce, an absolute reflectance of 10 to 20% at 540 nm would be indicated by the work of Billings and Morris (2). Pending experimental confirmation, the corresponding figures for Colorado and Blue spruce, because of the waxy deposits, are expected to be somewhat higher.

The glaucous bloom or bluish appearance of Colorado and Blue spruce foliage is therefore the result of a selectively enhanced reflection of blue light caused by the waxy deposits on the cuticular surface. Beyond the visible wavelengths this selective reflectance may reach its peak in the short UV just below 200 nm. The sequence of conifer species with respect to increasing relative blue and UV reflectance (Douglas fir, Sitka spruce, Colorado spruce, Blue spruce) is identical to that for decreasing relative photosynthetic efficiency of blue light (5).

Attempts were also made with intact Blue spruce foliage to determine the effect of wax removal on the utilization of blue light in photosynthesis. The results, although indicating a slight increase, were not conclusive. The problem however, is mainly one of technique. The mechanical removal of wax from 30 to 40 intact attached needles is not accomplished as easily nor effectively as was removal from single detached needles. Even when the wax appeared to have been completely removed, closer examination revealed that the stomatal antechambers had become plugged with displaced wax, which is likely to have increased their diffusive resistances as compared to those before treatment.

Effect of Needle Treatment on Architecture of Wax Deposits. The needle-surface architecture before and after treatment is shown in the series of scanning electron micrographs. Under low magnification a control Colorado spruce needle (Fig. 2) shows recognizable surface features such as stomatal pits. The medium and high magnification micrographs illustrate the differences in the surface architecture between the various tree types (Figs. 3, 4, and 5).

The waxy sculpturing on the control needle surfaces appears to have the same basic structure in each species, *viz.*, anastomosing or reticulate filaments of various lengths, but all approximately 125 nm in diameter. However, the degree of surface cover, depth of the filamentous mat, and density of packing of the individual elements appear to be specific differ-

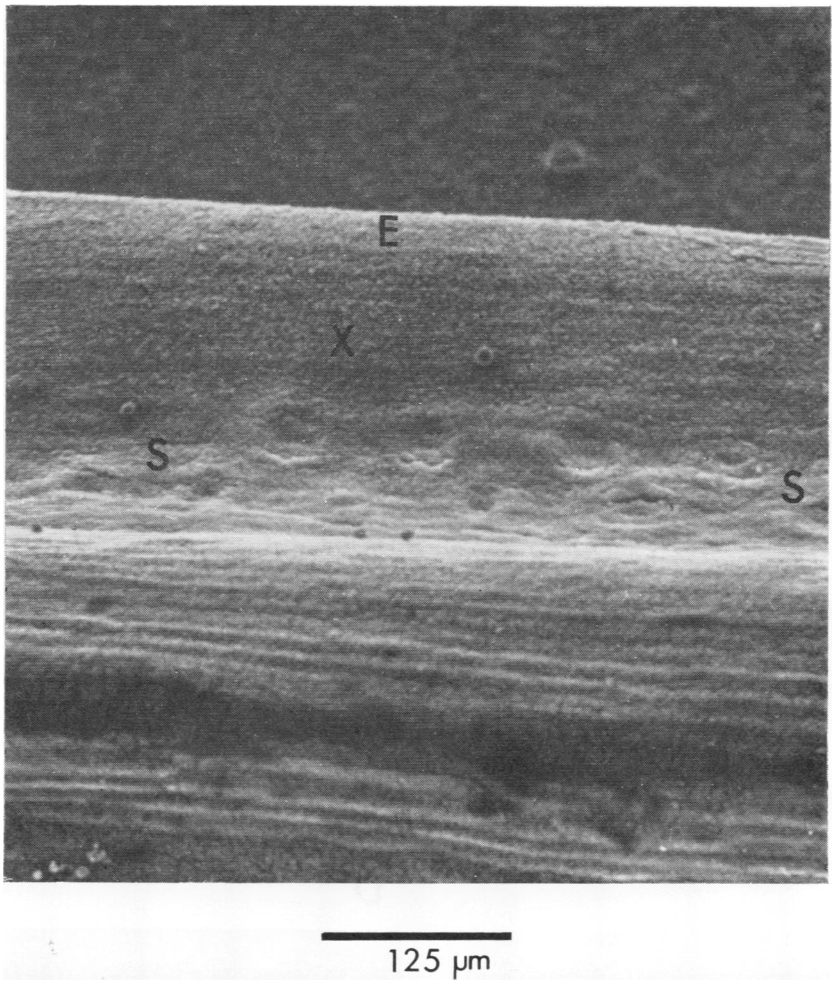

FIG. 2. Low magnification scanning electron micrograph of a control Colorado spruce needle. Note the row of stomatal pits (S-S) and the general flocculent appearance of the whole surface due to the epicuticular wax deposits. The region marked X indicates the portion of the needle surface subsequently examined at intermediate and high magnifications for each species. The horizontal line (E) is the needle edge.

ences. In Douglas fir (Fig. 3, A and B) the wax projections occur in clumps, leaving the needle cuticle surface clearly visible between clumps. In contrast, the "blue" spruces possess a deeper filamentous mat, a more extensive coverage of the general needle surface, and greater degree of packing of the filaments. The needle cuticle surface is visible only in a few places for Colorado spruce (Fig. 4, A and B) and is completely obscured in Blue spruce (Fig. 5, A and B).

Removal of wax had little or no apparent effect on the surface sculpturing in Douglas fir (Fig. 3, C and D) in contrast to the effect in the "blue" spruces in which the highly developed architecture of the wax deposits of the control needles was totally destroyed (Fig. 4, C and D; Fig. 5, C and D).

There can be no doubt that the differences in needle spectral reflectance, especially for the blue and UV wavebands, are attributable to the specific differences in the degree of complexity of the needle surface architecture. Similar epicuticular structures in the form of interlacing wax tubes (1 μm long × 150 nm o.d.) have been reported for Sitka spruce needles (9) where they are found mainly within the stomatal antechambers and in epidermal regions between the stomatal bands rather than on the other parts of the epidermis (8). Insofar as both the structure and the degree of development of the epicuticular needle sculpturing are concerned, the earlier observations (8, 9) for Sitka spruce show good agreement with the range of species considered in the present study.

Jeffrey *et al.* (8) have shown that such wax-filled stomatal antechambers are effective antitranspirants. The presence of the conspicuous bloom over the entire surface of Colorado and Blue spruce foliage suggests, however, that in these species this wax is more likely to be an adaptation to the relatively intense solar UV irradiation in their native habitat. MacMillan (10) reporting on "sunscald" of bean plants in Colorado, found that interposition of window glass prevented development of the typical sunscald leaf coloration changes, whereas a 30-min exposure to artificial sources of UV ($\lambda = 230$ nm) produced symptoms indistinguishable from those occurring naturally. Window glass effectively excludes radiation below 350 nm (4). Colorado spruce, native to the Rocky Mountains from Montana to New Mexico and Arizona, occurs at elevations ranging from 1,800 to 2,800 m in the north to 2,400 to 3,500 m in the south (12). Global UV-A (315–400 nm) irradiance increases linearly by 10% per 2,000 m elevation increase, and on a cloudless midsummer day, the global UV-B (280–315 nm) irradiance at 4,350 m is 26% greater than at 1,670 m elevation (4). Thus, it seems that the major function of the extensive epicuticular wax deposits in Colorado spruce spp. is to reduce, by reflective scattering, the intensity of the UV waveband to physiologically tolerable levels. It may also be expected that the "blue-shade" light characteristic of many conifer forests (6, 14, 16, 17) would be more pronounced under stands of Colorado spruce spp. The reduced photosynthetic activity resulting

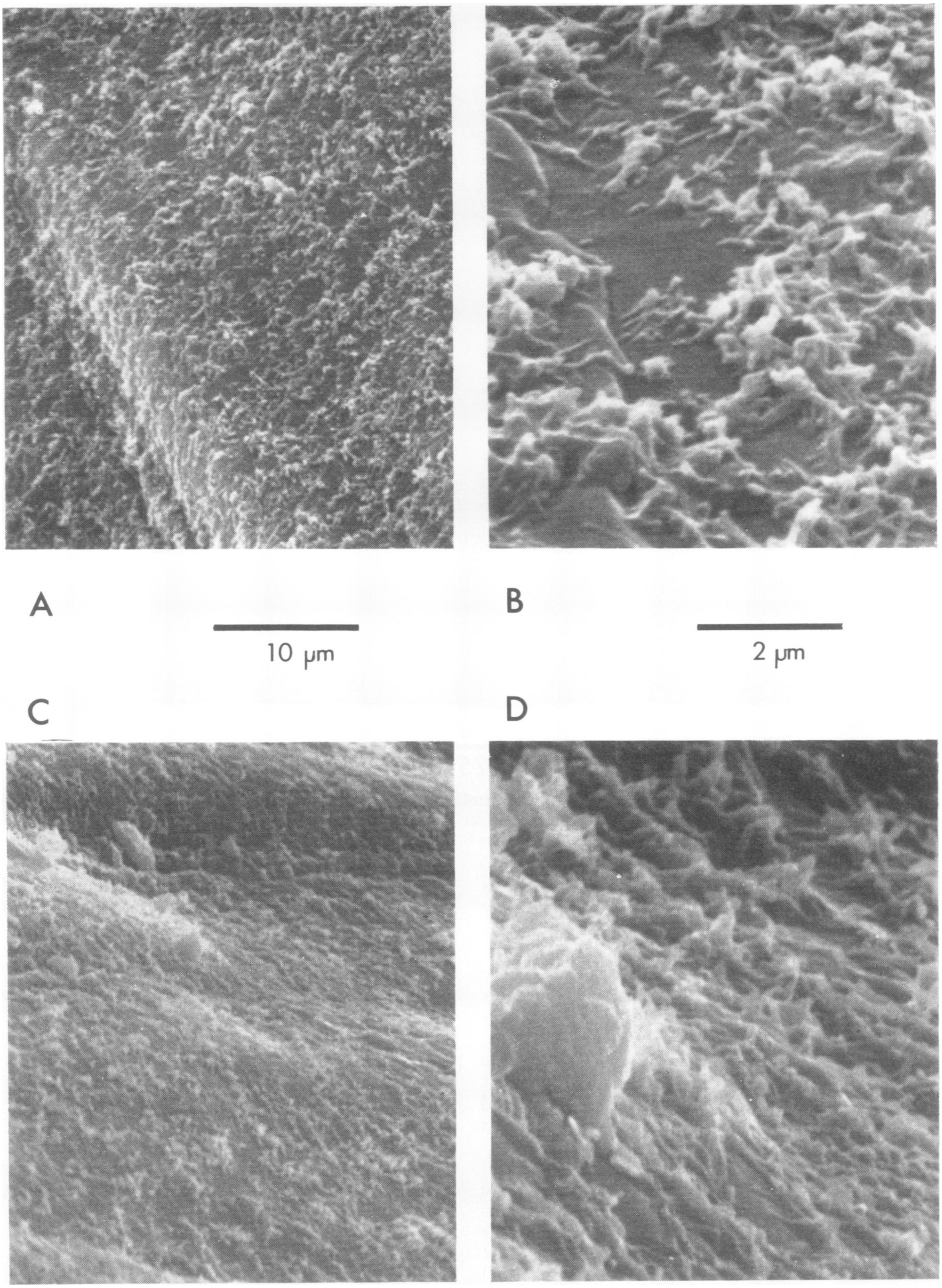

FIG. 3. Scanning electron micrographs of the wax projections on the surface of control and treated Douglas fir needles. A and B: control; C and D: treated. Note that the treatment has no apparent effect on the structure of the surface scuplturing.

from the concomitantly enhanced blue light reflectance in these species does not seem to be disadvantageous. The benefits from the self-protection (1) afforded by such a crudely selective UV screening system apparently outweigh the loss of potentially usable blue light in photosynthesis.

The reflective scattering caused by the epicuticular filamentous mat, not only of blue and UV wavebands but also of other visible wavelengths, may have further ecological significance for alpine plants. Under the clear skies of the alpine environment, such plants are subjected to high irradia-

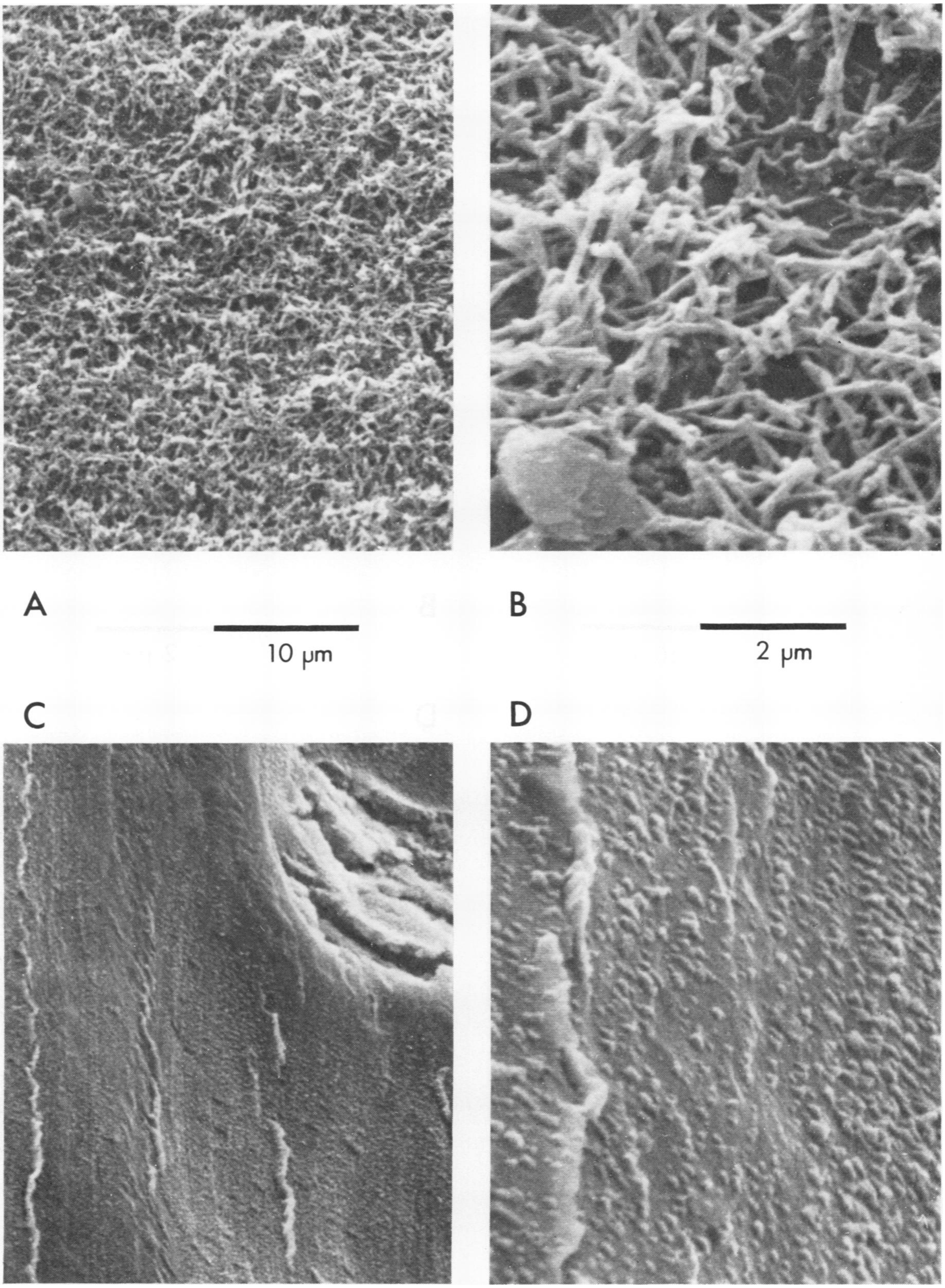

FIG. 4. Scanning electron micrographs of the wax projections on the surface of control and treated Colorado spruce needles. A and B: control; C and D: treated. Note the partially occluded stomatal pit and the striations of displaced wax as a result of the treatment in C.

tion levels, low relative humidities, and in many cases, low soil moisture levels. Although it may be possible for the alpine plant to limit the damaging effects of heat by transpirational dissipation of radiation, it seems that their higher potential for heat loading is additionally compensated for by the generally higher relative reflectance. In contrast, low altitude, green foliage conifers exhibit a development of epicuticular wax deposits which is relatively rudimentary in comparison.

In Colorado and Blue spruce then, the glaucous bloom seems to make it possible for the plants to tolerate high light inten-

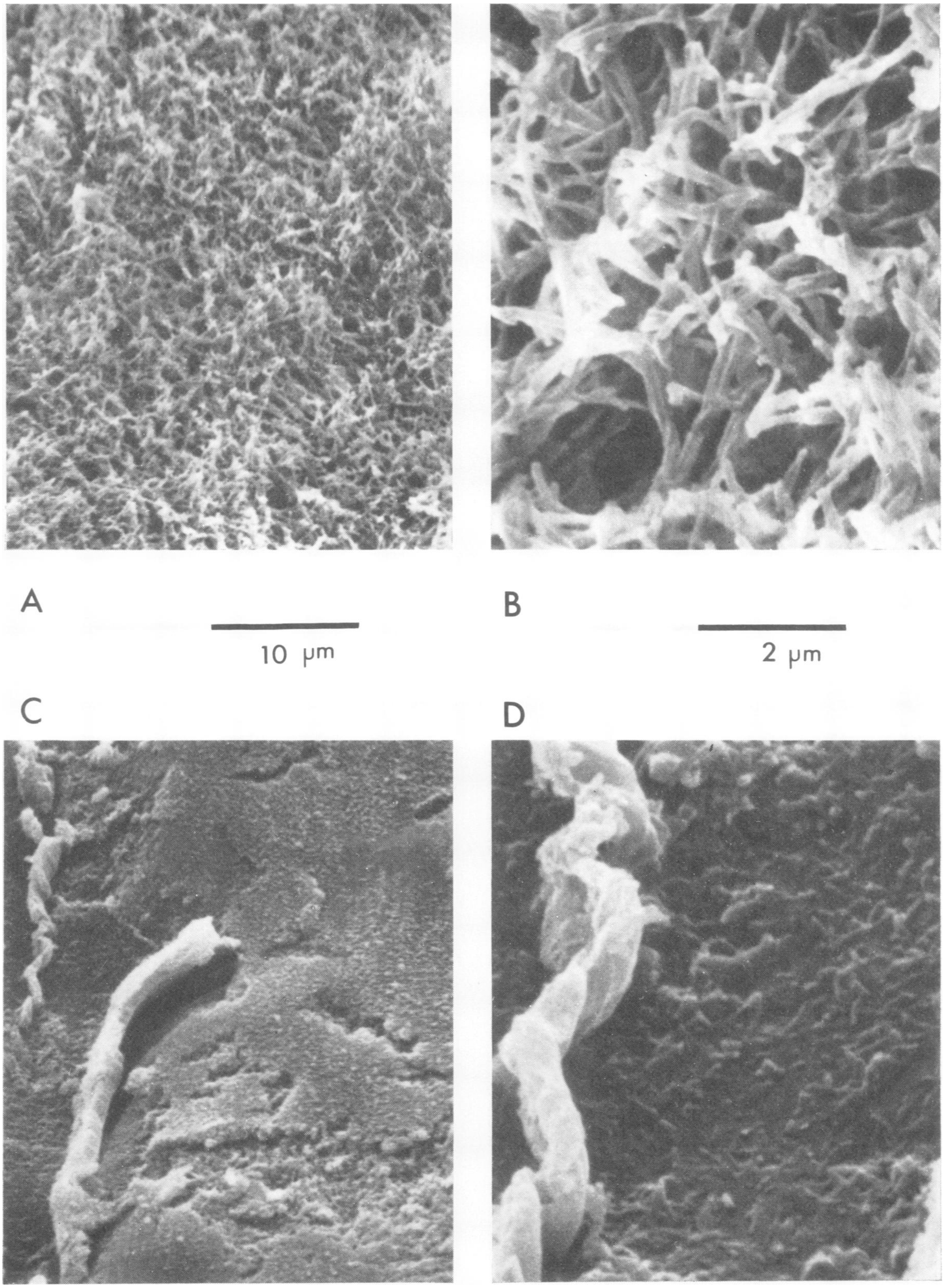

FIG. 5. Scanning electron micrographs of the wax projections on the surface of control and treated Blue spruce needles. A and B: control; C and D: treated. The treatment has completely destroyed the complex architecture of the wax deposits.

sities without damage, particularly with respect to the shorter wavelengths. The reduced photosynthetic utilization of blue light that this entails is not detrimental under the high irradiance conditions of their usual habitat.

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