DEVELOPMENTAL AND PHYSIOLOGICAL ASPECTS OF SURFACE WAXES OF BLUE SPRUCE

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This is to certify that the

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ABSTRACT

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By

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The development of surface waxes and the effects of these waxes on the physiological functions of blue spruce foliage were studied. The scanning electron microscope was used to observe the morphological and chronological development of epicuticular waxes. Structural waxes develop first in the epistomatal chambers of needles in an expanding bud. However as the needles are exposed to the atmosphere the structural waxes develop over the entire needle surface on glaucous foliage and only in stomatal areas on non-glaucous foliage. The epistomatal chambers are occluded with surface waxes as the needles emerge from the fascicle sheath and remain occluded. Structural waxes occlude the epistomatal chambers but as these waxes degrade the occlusion becomes amorphous. Structural waxes are degraded to an amorphous layer over the entire needle surface by weathering.

Foliage reflectance, energy budget, photosynthesis, transpiration, and moisture retention were studied on glaucous and non-glaucous foliage of blue spruce. Glaucous current-year-needles had significantly higher foliage reflectance than non-glaucous needles from 350-800 nm. The largest difference between the reflectance of glaucous and non-glaucous foliage was in the ultraviolet and blue region of the light spectrum. One-year-old and seedling needles had lower foliage reflectance than current-year needles, but the general pattern of reflectance was similar. However, differences in reflectance of glaucous and non-glaucous seedling foliage were only significant at three of the eight wavelengths of light measured.

The theoretical energy budget analyses demonstrated that the glaucous character of leaves could be both a selective advantage and a disadvantage depending on the environmental conditions. Glaucous leaves should have a lower leaf temperature regardless of the environmental conditions. The greatest differences in leaf temperature between glaucous and non-glaucous leaves should be under still air and high light conditions. Calculation of theoretical photosynthetic rates indicate that non-glaucous leaves would have higher photosynthetic rates than glaucous leaves at low temperatures and low light intensities. However glaucous leaves should have the advantage in photosynthesis at high temperatures and high light intensities. From theoretical calculations transpiration rates should be lower for glaucous leaves than non-glaucous leaves under all environmental conditions.

In the photosynthetic experiments glaucous foliage tended to have a lower net photosynthetic rate than non-glaucous foliage at low light intensities, but higher net photosynthetic rates at high light intensities. Expression of the photosynthetic rates as a ratio of rate at low over rate at high light showed that glaucous foliage utilized light more efficiently at high light intensities and that non-glaucous foliage was more efficient in light utilization at low light intensities. Differences in light saturation points were not clearly demonstrated, although glaucous foliage should have a higher light saturation point than non-glaucous foliage. Dark respiration rates of glaucous and non-glaucous foliage were not significantly different.

Glaucous foliage tended to have higher transpiration rates than non-glaucous foliage. Material collected in the morning had higher transpiration rates than material collected in the afternoon. Use of branches detached in the morning may be a useful method of obtaining transpiration measurements of material which is not easily measured in any other way.

Glaucous foliage lost more water early in the moisture retention experiments, but as desiccation increased it lost less water. No significant differences were found between glaucous and non-glaucous foliage in total water loss, fresh weight, needle dry weight, number of days to 50% of needle drop, and number of days to 100% of needle drop.

From the data presented in this study a hypothesis is suggested that a major role of the surface waxes may be to reduce the amount of energy entering the leaves which should reduce solarization damage under high light intensities.

DEVELOPMENTAL AND PHYSIOLOGICAL ASPECTS

OF SURFACE WAXES OF BLUE SPRUCE

By

David Arthur Reicosky

A DISSERTATION

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INTRODUCTION

The development of a cuticle represents a major step in the evolution of land plants by providing a protective coating which enabled plants to live out of water. Since this major evolutionary change the process of natural selection brought about the large array of anatomical and chemical variations of plant surfaces that exist in our present flora. Waxy bloom or glaucousness on the surface of plant stems, leaves, and fruits is undoubtedly a manifestation of this selective process.

The early work of DeBary (1871) showed that the cuticle was a surface deposit which covered the outermost layer of cells but was distinct from these cells and was impregnated with wax. He also believed that wax was actively secreted from canals in the cuticle and showed that granular wax films and wax rods developed without any apparent alteration of the underlying cuticle. DeBary (1884) described the waxes of many plant species and classified the types of wax coatings as (1) single layers of granules; (2) small rodlets perpendicular to the cuticle; (3) several layers of very small needles or granules, or (4) membrane-like layers or incrustations.

Glaucousness of plant surfaces has been described for many species and in certain cases genetic studies have been carried out. Harland (1918) showed that the waxy bloom on the stem of castor bean (Ricinus communis) was a simple allelomorphic character symbolized by

BB or Bb, presence of bloom and bb, absence of bloom. The mode of inheritance of double bloom which was the extension of wax bloom to the underside of the leaf is according to Peat (1928), due to the dominant allele C, which conditions the presence of bloom only when B is also present. The further extension of wax to the upper surface of the leaf, termed triple bloom, was also noted but genetic analyses were not carried out for this character. Mutations of waxes were induced in barley by ionization radiation and chemical mutagens resulting in the determination that <u>eceriferum</u> (wax mutations) loci control the synthesis and or excretion of organ specific wax components (Lundquist <u>et al</u>. 1968). VonWettstein-Knowles (1972) demonstrated the genetic control of B-diketone and hydroxy-B-diketone synthesis in epicuticular waxes of barley.

Clinal variation of leaf glaucousness in Tasmanian eucalyptus has been reported by Barber and Jackson (1957) with the more glaucous types being associated with higher altitudes and a higher incidence of frost conditions. They feel that more than one pair of alleles control the expression of glaucousness along the cline. Considerable variation exists in needle glaucousness of blue spruce (<u>Picea pungens</u>). In its natural range the foliage of trees tends to be more glaucous in Colorado, New Mexico and Arizona than in Wyoming and Northern Utah (personal observation). Cram (1968) has made experimental observations of the inheritance of foliage color in blue spruce and has suggested that some trees carry dominant factors for needle glaucousness. These observations indicate that the latitudinal variation of foliage glaucousness of blue spruce may be the result of the process of natural selection and that glaucousness may confer an adaptive advantage to the individuals

possessing these characters.

Many functions have been proposed for the surface waxes including regulation of the rate of moisture and gas exchange with the atmosphere, protection from mechanical and pest damage and protection from ultraviolet radiation and air pollutants. Despite the potential significance of these and other postulated functions, only recently has scattered information become available on the physiological action of the surface waxes. Development of chromatographic and electronmicroscopy techniques, particularly the scanning electron microscope, have greatly aided the attainment of information on surface waxes by workers interested in adaptive physiology and foliar application of nutrients and growth regulators.

Surface waxes are complex mixtures of long chain alkanes, alcohols, ketones, aldehydes, acetals, esters and acids and they may be useful as taxonomic criteria due to the chemical variation that exists between species (Eglinton and Hamilton, 1967). For example Herbin and Robins (1968a and b) have utilized surface waxes in chemotaxonomic studies of Liliaceae, Cupressaceae, and Pinaceae.

Deposition of the cuticle in oat (<u>Avena sativa</u>) and barley (<u>Hordeum vulgare</u>) was found to be directly proportional to light intensity and inversely proportional to relative humidity, although lipid constituents of the cuticle remained constant (Tribe <u>et al.</u>, 1968). Not only did surface wax deposition increase with an increase in light intensity in <u>Brassica napus</u> but the dimensions and complexity of the wax crystals also increased (Whitecross and Armstrong, 1971). A general decline in the density of surface coverage with increasing temperature

was noted in Eucalyptus viminalis (Banks and Whitecross, 1971). Baker (1973) found that an increase in radiant energy, a decrease in temperature, or a decrease of relative humidity increased the quantity of cutin, cuticular wax, and epicuticular wax on foliage of several species. The effect of radiant energy on epicuticular wax deposition was not greatly affected by temperature but was influenced by relative humidity. Morphology of the epicuticular waxes was changed by varying the environmental conditions. Low temperatures favored rod, tube, filament or ribbon wax structure oriented vertically from the cuticle surface, while high temperatures favored plate waxes and dendrites lying parallel to the cuticle. An increase of radiant energy increased the size and density of the above structures. Although the amount and the morphology of wax deposits varied with changes in the environment, only under widely divergent conditions were difference in the chemical composition of epicuticular and cuticular wax relatively substantial (Baker, 1973).

Glaucousness is due to the light reflecting characteristics of foliage surfaces where the structure, arrangement, and density of wax bodies largely determine the degree of glaucousness (Hall, <u>et al.</u>, 1965 and Hanover and Reicosky, 1971). Hanover and Reicosky (1971) also found species, plant and individual needle variation in the quantity, quality and distribution patterns of structural and amorphous wax of six conifer species. They also confirmed Rhine's (1924) report of wax in the stomatal pits of Austrian pine (<u>Pinus nigra</u>) which was originally thought to be soot and found considerable variation in the degree of stomatal occlusion by surface waxes.

In 1948, Schmucker tried unsuccessfully to establish a relationship between habitat conditions and foliage glaucousness. Stomatal and cuticular transpiration rates of non-glaucous and glaucous varieties of Douglas-fir (<u>Pseudotsuga menzeisii</u>) and blue spruce were similar, but for white spruce (<u>Picea glauca</u>) an intense blue variety had twice the rate of transpiration as a green variety. Priestly (1943), Kurtz (1958), Schieferstein and Loomis (1959), and others also were unable to find a relationship of cuticle thickness and or surface waxes with xeromorphic conditions. However Hall and Jones (1961) demonstrated that removal of the wax bloom from clover leaves increased cuticular water loss and Denna (1970) found in lines of <u>Brassica oleracea</u> that glaucous plants had less cuticular transpiration than non-glaucous plants. These contrasting results indicate that uncertainties exist in the relationship between surface waxes and the regulation of the water balance of plants.

The spectral properties of plants were investigated by Gates <u>et</u> <u>al.</u>, (1965) who found that desert species had higher light reflection values than non-desert species and that this represented an adaptation to their respective habitats. However Sinclair and Thomas (1970) reported that although surface reflection of light was higher for desert species so was light absorption. They concluded that changes in optical properties of leaves were not a significant adaptation to arid conditions. A reduction in reflectance of glaucous juvenile leaves of <u>Eucalyptus</u> <u>bicostata</u> caused by wiping the leaves with cotton wool increased the rate of photosynthesis at low light intensities but decreased the rate of photosynthesis at high light intensities (Cameron, 1970). This

indicates that alteration of foliage reflectance by surface waxes may represent an adaptation to certain climatic conditions.

The general objective of this study was to investigate the development of surface waxes and the effects of these waxes on the physiological functions of blue spruce foliage. Blue spruce was chosen as the experimental material because it has several unique and desirable characteristics for such work. Some of these include geographic and genetic variation in foliage glaucousness which can be vegetatively reproduced by grafting parent material, long needle retention, ease of transplanting, and a high resistance to cold, drought and most pests. Blue spruce is also valued as an ornamental species, a shelterbelt species, and a Christmas tree species in certain parts of North America and it is hoped that the information gained in this study will be useful in the genetic improvement of the species and in understanding its evolutionary development.

The specific objectives of this study were:

- To describe the morphological development of epicuticular waxes in blue spruce.
- 2. To determine the variation in occulusion of epistomatal chambers by epicuticular waxes in blue spruce.
- 3. To determine the theoretical effects of foliage glaucousness on:
 - (a) leaf temperature.
 - (b) photosynthesis.
 - (c) transpiration.
- 4. To measure the effect of foliage glaucousness on the following physiological processes of blue spruce:
 - (a) the reflection of light by foliage.

- (b) net photosynthesis and respiration.
- (c) transpiration.
- (d) the ability of detached branches to retain moisture.

CHAPTER I. DEVELOPMENTAL ASPECTS OF SURFACE WAXES OF BLUE SPRUCE

The surface waxes of plant leaves have been the object of study in recent years by workers interested in adaptive physiology, plant responses to air pollutants, and factors affecting the penetration of chemicals applied to leaves. An understanding of the patterns of natural variation in the development and chemical composition of surface waxes is needed in all such studies. <u>The Cuticles of Plants</u> by Martin and Juniper (1970) is an excellent review of plant surfaces.

In 1924 Rhine reported that black deposits found in the stomatal pits of certain conifers and thought to be soot from coal smoke were actually fine granular waxes which were permeable to gases. Only recently has it been shown that variation exists in the degree of occlusion of the epistomatal chambers of conifers by surface waxes from the partially occluded condition in Austrian pine (<u>Pinus nigra</u>) to the completely occluded condition shown by blue spruce (<u>Picea pungens</u>) (Hanover and Reicosky, 1971).

Eglinton and Hamilton (1967) reviewed the chemical properties of plant surface waxes which are complex mixtures of long chain alkanes, alcohols, ketones, aldehydes, acetals, esters and acids. They suggest that these waxes can be used as taxonomic criteria because of the substantial chemical variation that exists between species. For example variation in the visible glaucousness of Tasmanian Eucalypts is genetically controlled and correlated with the environment in the native

habitat, especially frost conditions (Barber, 1955). Leyton and Juniper (1963) observed that the portion of Scotch pine (<u>Pinus sylvestris</u>) needles which is enclosed by the basal sheath lacked the waxy projections which occur on the exposed surface of the needle. Hanover and Reicosky (1971) examined needles of six conifer species, and showed that the quantity, quality, and distribution patterns of structural and amorphous surface waxes varied between species, within species, within plants, and within individual leaves. Structural wax is closely associated with needle glaucousness in that the structure, arrangement, and density of the wax bodies largely determines the degree of glaucousness (Hall <u>et</u> al., 1965 and Hanover and Reicosky, 1971).

This study focuses on the seasonal and morphological development of the epicuticular waxes with emphasis on the degree of stomatal occlusion by these waxes.

MATERIALS AND METHODS

Current and one-year-old needles were removed with forceps from glaucous and non-glaucous varieties of blue spruce at four different times during the growing season. Samples were taken from the same branch of each tree in mid-May, the end of June, the end of July, and mid-November. Fresh foliage was affixed to aluminum stubs with Tube-koat cement, coated with a thin layer of carbon, and then coated with a gold-palladium alloy (60:40) about 200A thick. The samples were then examined directly with an AMR 900 scanning electron microscope at 21 KV. Three needles from each of eight trees were examined at levels of magnification ranging from 200x to 20,000x at the first sampling time.

However within tree variation of the surface waxes on needles of the same age was minimal and henceforth one needle per tree was observed. Variation patterns in the surface waxes were recorded photographically for analytical and illustrative purposes.

The influence of the structural wax on the microenvironment of the leaf was investigated by comparing height measurements of the structural waxes with calculated boundary layer thicknesses at wind speeds of 10, 100, 500, and 1,000 cm sec⁻¹. Height measurements of structural waxes were obtained by mechanically measuring photographs of areas where the clusters of wax rods were orientated perpendicular to the plane of view (Fig. 8). Measurements for glaucous foliage were unobtainable because the cuticle was obscured by the dense covering of structural wax.

Boundary layer thicknesses for laminar and turbulent air flow which is perpendicular to the long axis of the needle was calculated after Eckert (1959) as follows:

Laminar flow:

d = 4.64 (
$$x^{0.5}$$
) ($\mu^{-0.5}$) ($\gamma^{0.5}$)

Turbulent flow:

d = 0.384 (x^{0.8}) ($\mu^{-0.2}$) ($\gamma^{0.2}$)

where:

d = boundary layer thickness (cm)

x = characteristic length which is the width of the needle

_1

(0.1 cm) in this case (cm)

$$\mu$$
 = wind velocity (cm sec⁻¹)

 γ = kinematic viscosity of air.

RESULTS

Repeatable and consistent results were obtained despite some shriveling by young succulent tissue which was dissected from the bud or which had just emerged from the fascicle sheath due to dessication in the microscope column. Epicuticular waxes varied within years, between years, within species, within trees and within individual needles. Fig. 1-24 illustrate the developmental pattern of the epicuticular waxes of blue spruce.

Structural wax (wax rods) first develops in the epistomatal chambers of needles which are just about to emerge from the bud (Fig. 1-4). Needles which are emerging from the bud have structural wax in the epistomatal chambers of those portions of the needles which are still in the bud (Fig. 1-4) and have structural wax in both stomatal and nonstomatal areas of needles which have been exposed to the atmosphere (Fig. 5-6). Although structural wax is beginning to develop in nonstomatal areas while in the bud, it is not readily found until the needles emerge from the fascicle sheath.

Wax structures protrude from the surface of the guard cells and subsidiary cells of the epistomatal chambers (Fig. 1-4) and from the surface of epidermal cells in nonstomatal areas (Fig. 7-9). The base of the wax rods merges with the cuticle and the tip projects randomly outward forming blunt-tipped rods (Fig. 8-9). Several wax rods appear to originate from the same point forming wax clusters (Fig. 7-8). The uniform distribution of wax clusters in non-glaucous varieties of blue spruce (Fig. 10-11) suggest that micropores may exist from which structural wax could be exuded in a liquid form and then crystalized

Fig. 1-8. Electronmicrographs of leaf surfaces of current-year needles of Picea pungens. -- Fig. 1. Needle of cv. 'Koster' dissected from the fascicle sheath. Structural wax occurs on the surface of guard cells and subsidiary cells of the epistomatal chamber. x500. -- Fig. 2. Same as Fig. 1 showing one stomate with its stomatal slit (arrow) partially occluded with structural wax. x1,500. -- Fig. 3. Needle of cv. 'pendula' dissected from the fascicle sheath. Note structural wax in the epistomatal chamber. x750. -- Fig. 4. Same as Fig. 3. x2,000 -- Fig. 5. Newly expanded glaucous needle has structural wax over the entire needle surface. Arrows indicate location of stomates. Round white spherical particles in the lower half of Figure 5 and in other figures are contaminants. x500. -- Fig. 6. Expanded needle in mid-June with structural wax occluding epistomatal chamber (arrow). x1,500. -- Fig. 7. Wax rods are in clusters and protrude from epidermal cells. x5,000. -- Fig. 8. Profile view of wax cluster looking down the edge of the needle surface. Note randomness of wax rod upward projection. x5,000. -- Scale for Fig. 1-32: x200, 1 cm = 68.7μ ; x500, 1 cm = 27.5 μ ; x750, 1 cm = 18.3 μ ; x1,000, 1 cm = 13.7 μ ; $x1,500, 1 \text{ cm} = 9.2\mu; x2,000, 1 \text{ cm} = 6.87\mu; x5,000, 1 \text{ cm} = 2.75\mu; x20,000,$ $1 \text{ cm} = .687 \mu$.



Fig. 9-16. Electronmicrographs of fully expanded current-year needles of Picea pungens. - Fig. 9. Wax structures are rod shaped and protrude from the cuticle. x20,000. -- Fig. 10. Expanded non-glaucous needle has more structural wax in the stomatal rows (arrows) than in nonstomatal areas. x200. -- Fig. 11. Non-glaucous needle. Note clusters of structural wax in nonstomatal areas and heavy deposits of structural wax in epistomatal chambers (arrows). x1,000. -- Fig. 12. Cv. 'Hoopsii' needle with dense deposit of structural wax and occluded epistomatal chamber (arrow). x1,000. -- Fig. 13. Glaucous needle of Picea pungens. Structural wax occludes the epistomatal chamber (arrow) and also occurs in nonstomatal areas. x1,500. -- Fig. 14. The epistomatal chambers (arrows) of a glaucous variety are almost obscured by the structural wax. x500. -- Fig. 15. Structural wax of non-glaucous needles in nonstomatal areas has started to degrade while structural wax in stomatal areas is not degraded to the same extent by mid-August. x500. -- Fig. 16. By mid-November, even the structural wax in the epistomatal chamber of a non-glaucous needle is degraded but the epistomatal chamber is still occluded. x1,000.



into rods as suggested by Hall (1967), although other methods of wax deposition may exist.

Non-glaucous varieties of blue spruce have structural wax deposits in stomatal areas while in nonstomatal areas the clusters of wax rods are uniformly but thinly scattered over the needle surface (Fig. 10-11). In glaucous varieties, the structural wax deposits are very dense and uniformly cover the entire needle surface (Fig. 12-14).

There was additional wax deposition on the needle surface from May to June with the majority of the surface waxes having been exuded by the end of June (Fig. 13). Glaucous varieties of blue spruce have a uniform increase of structural wax over the needle surface (Fig. 12-14), while the majority of the increase of structural wax in non-glaucous varieties is in stomatal areas (Fig. 15-16).

Varieties of blue spruce which readily retain their glaucous appearance showed little signs of structural wax degradation on the needles by the end of July (Fig. 14 and 17). However in varieties which do not readily retain their glaucous appearance such as cv. 'pendula', structural wax has begun to degrade from weathering and physical damage (Fig. 18-19). By mid-November the structural wax has started to degrade in all varieties (Fig. 20). The wax structures in the epistomatal chambers are not degraded to the same extent that they are in nonstomatal areas (Fig. 16 and 19). This may be due to the sheltering of the wax structures in the depression of the epistomatal chambers or the wax structures in the stomatal areas may be more resistant to degradation.

Further degradation of the surface waxes were studied during the same year on one-year-old foliage from the same branches as the

Fig. 17-24. Electronmicrographs of needles of Picea pungens. Fig. 17. Structural wax has started to degrade by mid-August on a glaucous needle but the epistomatal chambers remain occluded (arrow). x500. -- Fig. 18. Glaucous cv. 'pendula' sampled in mid-August. Note degradation of structural wax and occlusion of the epistomatal chamber (arrow). x1,000. -- Fig. 19. Glaucous needles with poor color retention have structural wax degradation by mid-August. Arrow indicates occluded epistomatal chamber. x1,000. -- Fig. 20. Glaucous needle illustrating general structural wax degradation by mid-November. Arrow indicates occluded epistomatal chamber. x1,000. -- Fig. 21. One-year-old glaucous needle with good color retention has little structural wax degradation. Occluded epistomatal chamber (arrow). x1,000. -- Fig. 22. Structural wax of a one-year-old non-glaucous needle or a needle with poor color retention has structural wax degraded to an amorphous layer. x500. -- Fig. 23. Degradation of structural wax in one-year-old non-glaucous needles or needles with poor color retention is almost complete. Arrow indicates occluded epistomatal chamber. x1,000. -- Fig. 24. Complete degradation of structural wax in epistomatal chamber (arrow) in a one-year-old needle. x1,500.



current-year foliage. Little evidence of surface wax degradation was found in mid-May on one-year-old needles of varieties with good color retention or needles which were sheltered from weathering and physical damage (Fig. 21). However needles of poor color retention varieties or needles exposed to weathering and physical damage had surface waxes which were degraded to an amorphous layer (Fig. 22-24). The surface waxes are continually degraded throughout the year in nonstomatal areas resulting in an amorphous wax layer. Although the structural wax in the epistomatal chambers appears to be more durable than the waxes in the nonstomatal areas it is also degraded to an amorphous form (Fig. 23-24).

The epistomatal chambers are partially occluded with structural wax while the needles are in the bud (Fig. 1-4) and are completely occluded by the time the needles emerge from the bud (Fig. 5-6). This occlusion of the epistomatal chambers is permanent and occurs in glaucous or non-glaucous varieties, although the occlusion appears greater in glaucous varieties due to the heavier structural wax deposit (Fig. 12-16). Throughout the first growing season occlusion of the epistomatal chambers consists of undegraded structural wax (Fig. 12 and 16), however as degradation of the structural waxes proceeds the occlusion becomes more amorphous (Fig. 23-24). The effect of the degradation of the structural waxes in the epistomatal chambers on the exchange of gases between the stomates and the atmosphere remains to be determined.

The height of the structural waxes ranged from .00116 mm to .0057 mm with an overall mean of .00263 mm. Calculated boundary layer thicknesses for turbulent and laminar air flow at 20°C are as follows:

Wind Speed	Laminar Flow	Turbulent Flow	
10 cm sec^{-1}	1.80 mm	.26 mm	
100 cm sec^{-1}	.57 mm	.17 mm	
500 cm sec ^{-1}	.25 mm	.12 mm	
$1,000 \text{ cm sec}^{-1}$.18 mm	.10 mm	

From these calculations it appears that the wax structures are too small to affect the boundary layer of the needle.

DISCUSSION

The results of the development of structural wax as reported here are consistent with other observations which show that a large portion of wax synthesis and deposition occurs with leaf expansion (Kolattukudy, 1965). Although the density of structural wax is greater in glaucous varieties of blue spruce, the non-glaucous varieties also have structural wax deposits which are located mainly in the stomatal rows as soon as the needles are exposed to the atmosphere.

The epistomatal chambers of both glaucous and non-glaucous varieties are occluded by the time the needles emerge from the fascicle sheath with highly structured epicuticular waxes (Fig. 5-10). With time, the structured waxes are degraded to an amorphous form, however the epistomatal chambers remain occluded by the epicuticular waxes (Fig. 23-24). Jeffree <u>et al.</u>, (1971) demonstrated by theoretical calculations that occlusion of the epistomatal chambers by structural waxes can be expected to reduce transpiration by two-thirds and photosynthesis by one-third and therefore act as an antitranspirant. Any effect of stomatal occlusion on the gaseous exchange of the stomates is present throughout the year but may be altered as the structural waxes degrade to an amorphous form (Fig. 13 and 23). The degradation of the structural waxes in the epistomatal chambers resulting in a reduction in the porosity of the diffusion path of gases may be one of the reasons for the increase of stomatal diffusion resistances in red pine (<u>Pinus resinosa</u>) with age as found by Waggoner and Turner (1971).

Structural wax found on guard cells and subsidiary cells of the epistomatal chambers of blue spruce is evidence that these chambers are filled with structural wax and not just covered with wax as suggested by Lehela, <u>et al</u>. (1972) for white spruce (Fig. 1-6). Particularly interesting is the fact that structural wax first occurs in the epistomatal chambers prior to exposure of the leaf to the atmosphere. This may be indicative of an adaptive role for the structural waxes in modifying the rate of gas exchange through the stomate.

The calculations of the boundary layer thickness indicates that the wax structures are not long enough to affect the boundary layer. However the structural waxes, particularly the dense coating of the glaucous foliage, can influence the microenvironment around the needle by raising the lowest level (i.e. zero level) of the boundary layer from the leaf surface. The resultant effect would be to increase the relative humidity in this micro-layer and decrease the vapor pressure gradient from inside to immediately outside the leaf. The effect of roughness on the boundary layer due to clusters of wax rods is not known.

The structural integrity of the epicuticular wax is maintained throughout the first growing season although progressive degradation occurs with time. This fact coupled with the early occurrence of the structural wax suggests that a physiological role of this wax may be

protective of the young seedling from desiccation during the first year and perhaps the first weeks of growth. This fact should be considered when experiments are designed to determine the physiological significance of the epicuticular waxes. Studies on the effect of structural waxes on the physiology and energy balance of the leaf should be helpful to fully explain the functional significance of these waxes.

CHAPTER II. THE EFFECT OF SURFACE WAXES ON THE PHYSIOLOGY

OF BLUE SPRUCE NEEDLES

The presence of foliage glaucousness, which is due to the light scattering properties of the surface waxes, has been described for many species and many functions have been proposed for these waxes. The most notable of the possible functions are the regulation of the rate of moisture and gas exchange with the atmosphere, protection from mechanical and pest damage, and protection from ultraviolet radiation and air pollutants. Despite the potential significance of these and other postulated functions experimental evidence for specific functions is lacking.

In 1931 McNair reported that more wax producing species are found in the tropics than in temperate zones, although the majority of xerophytes and succulents of Southern Arizona were found to contain small amounts of wax (Kurtz, 1958). Priestly (1943) concluded that there was no relation between cuticle thickness and xeromorphism whereas results of Schieferstein and Loomis (1959) indicate that sub-surface wax may be more important in withstanding desiccating conditions than surface wax. In Douglas-fir (<u>Pseudotsuga menziesii</u>), cuticular transpiration differences of non-glaucous and glaucous varieties were small although under certain conditions the rate of cuticular water loss was greatest in the glaucous variety (Schmucker, 1948). An increase in cuticular transpiration was noted for clover leaves when the wax bloom

was removed (Hall and Jones, 1961). Cuticular transpiration was significantly greater in non-glaucous lines of <u>Brassica oleracea</u> but stomatal transpiration rates were not significantly different from glaucous lines (Denna, 1970). Stomatal transpiration rates for non-glaucous and glaucous varieties of Douglas-fir and blue spruce (<u>Picea pungens</u>) were similar but an intense blue variety of white spruce (<u>Picea glauca</u>) had almost twice as much water loss as a pale green one. The results of this earlier work indicate that the role of surface waxes in regulating the water balance of plant foliage is still unclear.

An increase of foliage glaucousness in eucalyptus was found to be associated with an increase of elevation and an increase of frost conditions (Barber, 1955). Glaucous foliage of eucalyptus was also found to be more heat resistant and therefore thought to represent an adaptation to hot environments (Karschon and Pinchas, 1971).

Reflection, absorption and transmission of light by leaves of <u>Atriplex vesicaria</u> and <u>A</u>. <u>stipitata</u> were measured by Sinclair and Thomas (1970). They concluded that modifications of the optical properties of leaves are not a significant adaptation to the arid conditions of the Koonamore district. However Cameron (1970) demonstrated that removal of foliage glaucousness from juvenile leaves of <u>Eucalyptus bicostata</u> by wiping with cotton wool increased the rate of photosynthesis at low light intensities but decreased the rate of photosynthesis at the high light intensity. The juvenile glaucous leaf of <u>E</u>. <u>bicostata</u> reflected 11% and the wiped juvenile leaf reflected 6% of the incident light which indicates that differences in foliage reflectance may have an adaptive significance.

Reflection, photosynthesis, transpiration, moisture retention and energy budget analyses were carried out in the present study of glaucous and non-glaucous foliage of blue spruce to better understand the effects of foliage glaucousness on the physiology of conifer needles.

GENERAL MATERIALS AND METHODS

Material for the physiological studies was selected on the basis of the contrasting expression of foliage glaucousness from blue spruce trees planted as ornamentals which consistently displayed their respective foliage coloration. Five glaucous, 2 semi-glaucous, and 5 non-glaucous trees which ranged in age from 7-20 years, were compared as to foliage reflectance, photosynthesis, transpiration, and moisture retention to determine the effect of foliage glaucousness on the physiology of conifer needles.

The planting site was located 15 miles from the laboratory which necessitated the use of detached branches in all the physiological studies. In a study of balsam fir (<u>Abies balsamea</u>) and white spruce, Clark (1961) compared photosynthesis on attached and detached branches for 6.5 hours and found no significant difference due to severance of the branches. Neilson <u>et al.</u>, (1972) measured photosynthesis of Sitka spruce (<u>Picea sitchensis</u>) for 72 hours after detachment and obtained less than 5% deviation from the initial measurement in the photosynthetic rates. The effect of detachment on photosynthesis was established in this study for blue spruce and found to be nonsignificant for up to 8 hours which is the longest time required for the current photosynthetic measurements. The effect of severance on transpiration rates was also
determined for blue spruce and found to be nonsignificant for up to 4 hours.

Branches were detached from the upper 1/3 of the tree crown with the aid of tree trimmers and then immediately recut underwater to eliminate gas bubbles from the transpirational stream. The cut ends of the branches were kept in water while undergoing the 30 minute transport to the laboratory where the branches were again recut underwater. For the photosynthetic and transpiration studies the cut ends of the branches were sealed in vials containing water to insure a constant supply of water during the experiments.

Analysis of variance was performed where indicated and if warranted a Tukey's test was also utilized to determine statistically significant differences.

Details about the methods used in each physiological study will be presented at the beginning of each section.

REFLECTION

MATERIALS AND METHODS

In the older trees reflective characteristics of current-year needles, one-year-old needles and current-year and one-year-old needles washed in chloroform were examined. Current-year needles of seedlings were also examined. Needles collected in mid-July were mounted as close together as possible on a cardboard square to minimize the effect of light reflected from the cardboard surface. One square cm of the mounted foliage was placed at one port of the integrating sphere for reflectance measurements. Percent reflectance readings were taken at 3 different orientations for each sample with the mean of the 3 measurements used in all subsequent analyses.

A Zeiss PMQ II spectrophotometer fitted with an integrating sphere was used in all reflectance measurements. Monochromatic light with an incident angle of 90° was shown on the foliage and the diffused reflected light monitored by a photomultiplier tube inside the integrating sphere. The results obtained in this study represent minimum reflectance values since the degree of reflectance increases as the angle of incidence varies from 90° (Howard, 1967).

To establish the general pattern of light reflection of blue spruce needles, reflectance measurements of current-year needles of 2 glaucous and 2 non-glaucous trees were obtained every 10 nm from 350-800 nm. Additional replication and analysis of variance were carried out at 12 wavelengths selected along the established curve (Fig. 25).

Epicuticular waxes were removed from current-year and one-year-old needles of 2 glaucous and 2 non-glaucous trees by washing the branches in a beaker of chloroform for 1 minute. This treatment removed the surface waxes from the needle (Hanover and Reicosky, 1971).

Since no differences in reflectance were found between current-year and one-year-old dewaxed needles from the same tree these reflectance measurements were combined together for subsequent analyses.

RESULTS AND DISCUSSION

Glaucous and non-glaucous foliage had the greatest reflectance in the 750-800 nm region with values of 70.2% and 65.5%, respectively (Fig.

Figure 25. Reflectance curve of glaucous (G) and non-glaucous (NG) and the difference between these types (----) for current-year needles of blue spruce. Values for semi-glaucous determinations are also plotted (Δ). Glaucous and non-glaucous foliage reflectance is significantly different (1% level) at all wavelengths of light. Points not joined by a vertical line indicate significant differences (5% level) of reflectance between the semi-glaucous foliage and the other degrees of foliage glaucousness.



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25). The lowest reflectance values (15.9% and 7.9%, respectively) were found in the 670 nm region. Both glaucous and non-glaucous foliage had an increase of foliar reflectance in the 520-570 nm region where little absorption of light by leaf pigments occurs. This general pattern of reflectance is similar for current-year, one-year-old, and seedling foliage (Table 1).

Statistically significant differences at the 1% level were found when the reflectance of current-year needles of glaucous, and non-glaucous trees of blue spruce were compared. Semi-glaucous foliage was intermediate in reflectance between the glaucous and non-glaucous foliage but more closely resembled the glaucous type. Glaucous foliage has a higher percentage of light reflectance at all wavelengths of light from 350-800 nm. The largest difference of reflectance between glaucous and non-glaucous foliage was in the 350 nm region with a general decline in the difference to the smallest difference at the 800 nm region (Fig. 25). It is interesting that the greatest difference of reflectance of glaucous and non-glaucous foliage was in the ultraviolet and blue region of the light spectrum where the energy per photon was maximum for the wavelengths of light measured. The ability of glaucous foliage to reflect more energy than non-glaucous foliage may be a selective advantage in environments where solar radiation is intense or of long duration. An increase in reflectance should reduce the amount of energy that enters the leaves which means that there is less energy that must be dissipated by the leaves.

Removal of the epicuticular waxes and hence the glaucous characteristic of foliage by a chloroform wash greatly reduced the

Wavelength in nm	Current-year foliage		One-year- old foliage		S ee d fol	lling iage	Foliage washed in chloroform		
	G	NG	G	NG	G NG		G	NG	
		1							
350	25.6**	10.1	15.4 **	6.3	14.4	8.5	2.5	2.8	
400	_2	-	-	-	-	-	2.9	3.2	
450	24.0 *	10.3	15.6 **	7.4	14.5	** 10.2	6.0	5.6	
500	-	-	-	-	-	-	8.4	7.9	
540	29.6	* 19.8	21.7 **	14.8	21.6	21.3	-	-	
550	29.1	* 19.7	21.7 **	14.9	21.4	22.0	14.0	14.0	
600	22.4	13.5	17.1 **	10.8	15.3	15.2	13.9	12.0	
650	-	-	-	-	-	-	9.4	8.4	
670	15.9 *	* 7.9	11.9 **	6.5	9.7	* 7.7	-	-	
680	-	-	-	-	-	-	8.3	7.1	
700	27.8 *	* 21.0	23.2 **	17.5	21.9	24.0	-	-	
800	70.2 *	* 65.5	59.3 **	56.4	66.1	64.7	-	-	

Table 1. Reflectance values (%) of glaucous (G) and non-glaucous (NG) needles for current-year, one-year-old, seedling, and chloroform washed foliage at different wavelengths of light.

¹Tukey's test of significant differences: * = 5% level, ** = 1% level. ²- indicates no measurement made. reflectance of the leaf surface and eliminated significant differences in reflectance between glaucous and non-glaucous foliage (Table 1). When chloroform.washed and unwashed foliage was examined with the scanning electron microscope it was seen that the surface waxes were removed in the chloroform washed sample (Fig. 26-27). This indicates that a large portion of the observed foliage reflectance was due to the surface waxes. There was a change of foliage reflectance of chloroform washed foliage with light quality indicating that other components of foliage reflectance exist (Table 1). The change of reflectance with light quality is not associated with degrees of glaucousness and may be due to the differential absorption of various wavelengths of light by leaf pigments as shown by Gates, <u>et al</u>. (1965).

One-year-old foliage had significantly lower reflectance than current-year foliage but the general pattern of foliage reflectance was similar (Table 1 and 2). The glaucous appearance of the one-year-old foliage is also reduced suggesting that an alteration of the light reflecting properties of the leaves may have occurred. Current-year and one-year-old foliage rinsed in chloroform were not significantly different in foliage reflectance indicating that leaf pigmentation had no effect on foliage reflectance of material of different age. Cameron (1970) attributed the reduction of light reflectance of adult leaves of <u>Eucalyptus bicostata</u> to a change in density and clustering of the wax rods on the leaf surface. Changes in structural waxes due to weathering and degradation from a highly structured type in current-year foliage (Fig. 13) to an amorphous layer in one-year-old foliage (Fig. 24) were strongly correlated with the reduction of leaf glaucousness and

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Fig. 26. Scanning electron micrograph of a glaucous needle of blue spruce showing the dense deposit of epicuticular wax which almost obscures the epistomatal chambers (arrows). x 200.



Fig. 27. Scanning electron micrograph of a glaucous needle of blue spruce (from the same tree as in Fig. 26) washed in chloroform for 1 minute. The dense deposit of epicuticular waxes is removed exposing the sunken stomates. x 200.

Wavelength in nm	Curren foli	Current-year foliage		One-year- Seedling Foliag old foliage foliage washed chloro				e in form
350	18.4	A ¹	10.8	A,E ²	11.4	A ²	2.7	A ²
	a ³		Ъ		Ъ		_4	
400	-	-	-	-	-	-	3.0	A
							-	
450	17.6	A	11.5	A	12.4	A	5.8	В
	a		Ъ		Ъ		-	
500	-	-	-	-	-	-	8.1	B,C
							-	
540	24.8	В	18.2	В	21.4	В	-	
	a		Ъ		a,b			
550	24.6	В	18.3	В	21.7	В	14.0	D
	а		Ъ		a,b		-	
600	18.1	A	14.0	С	15.3	С	13.0	D
	a		Ъ		a,b		-	
650	-	-	-	-	-	-	8.9	С
							-	
670	12.0	С	9.2	Ε	8.7	E	-	
	а		a,b		Ъ			
680	-	-	-	-	-	-	7.7	B,C
							-	
700	24.4	В	20.4	В	23.0	В	-	
	a		Ъ		a,b			
800	68.3	D	57.8	D	65.4	D	-	
	а		Ъ		С			

Table 2. Reflectance values (%) for blue spruce needles summarizing wavelength and foliage type significant differences.

Wavelength differences at the 1% level.

²Wavelength differences at the 5% level.

³Foliage type differences at the 5% level.

4- indicates no measurement made.

reflectance in older foliage of blue spruce.

Observation of nursery and field plantings indicated that there was an intensification of foliage glaucousness with seedling age. This observation was substantiated by the fact that current-year needles of seedlings have lower reflectance than current-year needles of older trees (Table 2). Genotypic effects may cause the above differences although I suspect that there is a developmental effect of age. Cameron (1970) found differences of foliage reflectance between juvenile. intermediate, and mature leaves in eucalyptus species. Differences of reflectance of glaucous and non-glaucous foliage of blue spruce seedlings are significant only at 350 nm, 450 nm, and 670 nm wavelengths (Table 2). Lack of significant differences in reflectance at the other wavelengths indicates that glaucousness is probably not developed enough at the seedling stage to allow detection of differences between glaucous and non-glaucous foliage at the wavelengths of light where the differences are smaller. These results suggest that there is an apparent developmental change which acts to intensify the expression of the glaucous character as the seedling matures. The glaucous character can be expressed only if the genes for wax development are present in the genome.

ENERGY BUDGET ANALYSIS

THEORETICAL PROCEDURES

A theoretical energy budget analysis of a glaucous and non-glaucous needle was undertaken to determine the theoretical effects

of glaucousness on leaf temperature, latent heat transfer (transpiration) and photosynthesis. It was assumed that the only difference between the glaucous and non-glaucous leaf was a difference in their absorptivities of incident energy. The largest difference in reflectance of the glaucous and non-glaucous foliage was 15% (Fig. 25), which was the basis for establishing a difference in absorptivities. Leaves normally absorb 44-88% of the radiation received (Raschke, 1956). Because blue spruce needles are 0.1 cm thick, 90% was chosen as the absorptivity for the non-glaucous leaf and 75% absorptivity for the glaucous leaf due to its higher surface reflectance.

For the determination of the boundary layer resistance it was assumed that the shape of a blue spruce needle approximated a cylindrical body for which the formula for the boundary layer resistance is

$$R_{a} = \frac{2.88 \times 10^{-4}}{0.615 (Re)^{0.5} (2.57 \times 10^{-3})}$$
3.6

for Re (Reynolds number) between 40 ... 4000. Re = $\frac{ux}{\gamma}$ where u = wind velocity, x = width of leaf, and γ = kinematic viscosity of air. Theoretical consideration of the above formula can be found in Grigull (1963).

Energy budget calculations were carried out for all combinations of the following conditions for a glaucous and non-glaucous leaf; Incident radiation: 1.2, 0.8, and 0.2 cal cm⁻² min⁻¹ Temperature: 10, 20, and 30°C Stomatal resistance: 2.3, 10, and 17 cm⁻¹ sec. Leaf temperature was calculated by:

$$T_{1} - T_{a} = \frac{\frac{R_{a}J_{n}}{\pi c_{p}}\rho - [b \frac{R_{a}}{R_{a} + R_{s}} (E_{a} (1 - r))]}{1 + b \frac{R_{a}}{R_{a} + R_{s}} \frac{de}{dt}} (C^{\circ})$$

Latent heat transfer was calculated by:

$$-V = \frac{bC_{p}\rho[E_{a}(1-r) + (T_{1} - T_{a}) \frac{de}{dt}]}{R_{a} + R_{s}} (cal \ cm^{-2} \ sec^{-1})$$

Symbols for the above formulas are:

$$T_{1} = \text{leaf temperature (C^{\circ})}$$

$$T_{a} = \text{air temperature (C^{\circ})}$$

$$R_{a} = \text{boundary layer resistance (sec cm-1)}$$

$$J_{n} = \text{incident radiation (cal cm-2 sec-1)}$$

$$\rho = \text{density of the air}$$

$$\pi = \text{pi}$$

$$C_{p} = \text{specific heat of the air}$$

$$b = \text{a factor, converting gradient of water vapor pressure into gradient of latent energy (2 degree Torr-1)}$$

$$R_{g} = \text{stomatal resistance (sec cm-1)}$$

$$E_{a} = \text{vapor pressure of saturated air}$$

$$r = \text{relative humidity of the air expressed as a decimal fraction}$$

$$\frac{de}{dt} = \text{water vapor pressure change with temperature}$$

$$-V = \text{latent heat transfer (cal cm-2 sec-1)}$$

$$C_{p}\rho = 2.88 \times 10^{-4} \text{ cal cc}^{-1}$$

$$E_{a} \text{ and } \frac{de}{dt} \text{ change with temperature}$$

$$R_{a}, R_{g}, r, \text{ and } J_{n} \text{ are selected by the investigator.}$$

The above formulae were derived from those of Raschke, 1956 and 1960 (personal communication). Further discussions of energy budget analysis and heat transfer can be obtained from Eckert (1959) and Gates (1968).

No data were available for blue spruce on the response of photosynthesis to temperature, so a temperature response curve for Douglas-fir at 5,000 footcandles was used (Krueger and Ferrell, 1965). The rate of change of photosynthesis per degree centigrade for each temperature range was determined and then multiplied by the deviation of the appropriate leaf temperature. These values were then added to the photosynthetic rate at the given air temperature to arrive at the final photosynthetic rate. An estimate of the reduction of the photosynthetic rate due to the reduction of light intensity from 3,000 to 1,000 footcandles from each side of the chamber was made (Fig. 29) for glaucous (46%) and non-glaucous (59%) foliage and applied to the calculation of the photosynthetic rates at the low light intensity. No correction was made for the other light intensities since these light intensities would be near or above the light saturation point for photosynthesis.

RESULTS AND DISCUSSION

The effect of incident radiation, air temperature, relative humidity and stomatal resistance, and wind velocity on leaf temperature is shown in Table 3 and 4. The effect of these variables on leaf temperature have been discussed elsewhere (Gates, 1968; Raschke, 1956 and 1960). Therefore only differences between glaucous and non-glaucous leaves will be emphasized here. The glaucous leaf (absorptivity .75) always had a lower leaf temperature than the non-glaucous leaf

Table 3. (A) Leaf temperature – air temperature differences $(x10^{-2})$ for a glaucous needle (absorptivity .75) and a non-glaucous needle (absorptivity .90) and (B) differences in leaf temperature between a non-glaucous and glaucous needle¹.

A

				10°C			20°C			30°C				
Incident radiation	Absorp- tivity	R.H. %	% Stomatal resistance											
			2.3	10	17	2.3	10	17	2.3	10	17			
0.2 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	-12 - 8 5 3	3 7 6 10	5 9 7 11	-29 -25 - 8 - 4	9 3 4 8	3 7 6 10	-57 -53 -20 -16	- 8 - 4 1 5	- 1 3 4 8			
0.8 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	20 27 31 38	35 43 38 45	37 45 38 46	2 10 22 30	31 38 36 43	34 42 37 45	-27 -20 10 17	23 31 32 40	30 38 35 43			
1.2 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	39 51 50 62	55 66 57 69	57 69 58 70	21 33 42 53	50 62 55 67	54 66 57 69	- 8 3 29 40	43 55 52 64	50 62 55 67			
B														
0.2 cal	.9075	30	4	4	4	4	4	4	4	4	4			
cm ⁻² min ⁻¹	.9075	70	4	4	4	4	4	4	4	4	4			
0.8 cal	.9075	30	7	8	8	8	7	8	7	8	8			
cm ⁻² min ⁻¹	.9075	70	7	7	8	8	7	8	7	8	8			
1.2 cal	.9075	30	12	11	12	12	12	12	11	12	12			
cm ⁻² min ⁻¹	.9075	70	12	12	12	11	12	12	11	12	12			

¹Wind velocity is 500 cm sec⁻¹ ($R_a = .0359$ sec cm⁻¹).

Table 4. (A) Leaf temperature - air temperature differences (x10⁻²) for a glaucous needle (absorptivity .75) and a non-glaucous needle (absorptivity .90) and (B) differences in leaf temperature between a non-glaucous and glaucous needle at different wind speeds.¹

A

Incident	Absorp-	10 ((R _a	cm se =.25	ec^{-1} 540) ²	100 (R _a	cm s = .(sec ⁻¹)803)	5000 (R _a	cm 80 = .(ec ⁻¹ 0359)	1,00 (R _a	0cm s = .0	ec ⁻¹ 254)
radiation	tivity					Degi	rees	centi	Igrad	ie			
		10	20	30	10	20	30	10	20	30	10	20	30
0.2 cal	.75	24	- 4	-50	7	- 2	-17	3	9	- 8	2	- 1	- 6
cm ⁻ min ⁻¹	.90	51	22	-24	16	7	- 9	7	3	- 4	5	2	- 3
0.8 cal	.75	241	208	156	75	68	52	35	31	23	25	22	17
cm ⁻ min ⁻¹	.90	296	262	207	92	85	69	43	38	31	30	27	22
1.2 cal	.75	377	34 2	284	122	112	95	55	50	43	39	36	30
cm ⁻ min ⁻	.90	459	421	362	148	138	121	66	62	55	47	44	39
В													
0.2 cal cm ⁻² min ⁻¹	.9075	27	26	26	9	9	8	4	4	4	3	3	3
0.8 cal cm ⁻² min ⁻¹	.9075	55	54	51	17	17	17	8	7	8	5	5	5
1.2 cal cm ⁻² min ⁻¹	.9075	82	79	78	26	26	26	11	12	12	8	8	9
$\frac{1}{R.H.} = 302$	and R _g =	10	sec	cm ⁻¹ .									
$R_a = \sec \alpha$													

regardless of the air temperature, relative humidity, stomatal resistance, wind velocity, or light intensity. The smallest difference in leaf temperature between the glaucous and non-glaucous leaves was at the low light intensity and high wind velocity while the greatest difference in leaf temperature was at the high light intensity and low wind velocity (Table 3 and 4). The reduction of energy entering the leaf by the light reflecting properties of the epicuticular waxes could result in a reduction of leaf temperature as shown here. Attempts were made to measure leaf temperature of glaucous and non-glaucous needles in this study. However the insensitivity of my technique prevented the detection of differences as small as those expected (Table 3 and 4). Thomas (1961) suggested that the reflection, by waxes of incident radiation and its effect on leaf temperature is of probable importance in the origin and maintenance of the clinal variation of glaucousness in eucalyptus. Additional measurements of leaf temperatures of glaucous and non-glaucous foliage types are needed to further test the above hypothesis.

The high leaf temperature of the non-glaucous needle is only advantageous in terms of photosynthesis at a low air temperature (Table 5). The increased rate of photosynthesis of the non-glaucous needle at the low air temperature was probably due to the enhanced reaction rates of the metabolic processes occurring in the plant (Salisbury and Ross, 1969). Depending on the magnitude of photosynthesis during the colder months it could be an important advantage for the non-glaucous foliage to have a higher leaf temperature than the glaucous foliage. At light intensities below light saturation the non-glaucous needle has a higher

Table 5. (A) Photosynthetic rates (mg CO₂ g⁻¹ dry wt hr⁻¹) calculated for a glaucous (absorptivity .75) and a non-glaucous (absorptivity .90) needle from leaf temperatures in Table 3 and (B) differences of photosynthetic rates of a glaucous and non-glaucous needle.¹

А

				10°C			20°C			30°C	
Incident radiation	Absorp- tivity	R.H. %				Istanco	nce				
			2.3	10	17	2.3	10	17	2.3	10	17
0.2 cal^2 $\text{cm}^{-2}\text{min}^{-1}$.75 .90 .75	30 30 70 70	4.67 6.00 4.69	4.70 6.04 4.71	4.70 6.04 4.71	6.84 8.78 6.88 8.84	6.90 8.86 6.91 8 87	6.91 8.87 6.91 8.88	4.50 5.78 4.42 5.65	4.39 5.62 4.37 5.59	4.37 5.60 4.36 5.58
0.8 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	10.30 10.33 10.34 10.38	10.37 10.41 10.38 10.42	10.38 10.42 10.38 10.42	14.99 14.96 14.89 14.86	14.85 14.82 14.83 14.79	14.84 14.80 14.82 14.78	9.63 9.60 9.45 9.42	9.39 9.35 9.35 9.31	9.36 9.32 9.33 9.29
1.2 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	10.39 10.44 10.44 10.50	10.46 10.52 10.47 10.53	10.48 10.53 10.48 10.54	14.89 14.84 14.80 14.75	14.76 14.70 14.74 14.68	14.73 14.68 14.73 14.67	9.54 9.49 9.36 9.31	9.29 9.24 9.25 9.19	9.26 9.20 9.24 9.18
В											
0.2 cal -2 -1	.9075	30	1.33	1.34	1.34	1.94	1.96	1.96	1.28	1.23	1.23
	.9075	70	1.34	1.34	1.34	1.96	1.97	1.97	1.23	1.22	1.22
0.8 cal -21	.9075	30	0.03	0.04	0.04	-0.03	-0.03	-0.04	-0.03	-0.04	-0.04
CM M1N	.9075	70	0.04	0.04	0.04	-0.03	-0.04	-0.04	-0.03	-0.04	-0.04
1.2 cal	.9075	30	0.05	0.06	0.05	-0.05	-0.06	-0.06	-0.05	-0.05	-0.06
cm min -	.9075	70	0.06	0.06	0.05	-0.05	-0.06	-0.06	-0.05	-0.06	-0.06

Rates of photosynthesis for the low light intensity were corrected for the reduction in photosynthetic rates due to a reduction of light intensity from light saturation as estimated for a glaucous needle and non-glaucous needle from Fig. 29.

²0.2 cal cm⁻²min⁻¹ = 3.6 x 10² microeinsteins m⁻²sec⁻¹ or 2,300 footcandles; 0.8 cal cm⁻²min⁻¹ = 7.2 x 10³ microeinsteins m⁻²sec⁻¹ or 10,200 footcandles; 1.2 cal cm⁻²min⁻¹ = >7.2 x 10³ microeinsteins m⁻²sec⁻¹ or >10,200 footcandles with 400 watt color improved mercury vapor lamp, (actual measurements). rate of photosynthesis than the glaucous foliage due to its more efficient utilization of incident light.

The increase of leaf temperature and more efficient utilization of light can additively act to increase the photosynthetic rate of the non-glaucous needle at the low air temperature. However at a high air temperature the increased leaf temperature of the non-glaucous needle could reduce the rate of photosynthesis but probably can not negate the positive effect of more efficient light utilization (Table 5).

At the highest temperatures and at light intensities above light saturation for photosynthesis the glaucous needle can have a clear advantage in net photosynthesis due to its reduced leaf temperature (Table 5). The high temperature optimum, of the respiration process coupled with the enyzme inactivation in the photosynthetic process at higher temperatures may account for the reduction of net photosynthesis at high air temperatures of the non-glaucous needle (Salisbury and Ross, 1969). Glaucous foliage, therefore could have a selective advantage in hot environments especially in areas with large amounts of solar radiation and low wind velocities where as shown by the energy budget analysis the difference of leaf temperature of glaucous and non-glaucous needles would be expected to be the greatest (Table 4). A temperature response curve for photosynthesis should be established for glaucous and non-glaucous foliage of blue spruce to test the above hypothesis.

Heat loss due to transpiration increases with an increase of temperature and light intensity but decreases with an increase of relative humidity and stomatal resistance (Table 6). The non-glaucous leaf (absorptivity .90) absorbs more energy than the glaucous leaf and

Table 6. (A) Latent heat loss (cal cm⁻² sec⁻¹ x 10^{-4}) for a glaucous (absorptivity .75) and a non-glaucous (absorptivity .90) needle and (B) differences of latent heat loss between a non-glaucous and glaucous needle.¹

Α

			10°C 20°C						30°C				
Incident radiation	Absorp- tivity	р- R.H. У %	Stomatal resistance										
			2.3	10	17	2.3	10	17	2.3	10	17		
0.2 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	15.69 15.75 6.80 6.85	3.71 3.72 1.61 1.62	2.19 2.20 0.94 0.96	29.46 29.57 12.75 12.86	7.04 7.06 3.04 3.07	4.16 4.17 1.80 1.82	52.31 52.50 22.63 22.81	12.70 12.74 5.49 5.53	7.53 7.55 3.25 3.28		
0.8 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	16.20 16.31 7.29 7.41	3.82 3.85 1.72 1.75	2.26 2.27 1.02 1.03	30.31 30.53 13.58 13.80	7.24 7.29 3.25 3.29	4.28 4.30 1.92 1.95	53.69 54.02 24.01 24.33	13.03 13.12 5.82 5.91	7.72 7.77 3.45 3.50		
1.2 cal cm ⁻² min ⁻¹	.75 .90 .75 .90	30 30 70 70	16.50 16.68 7.59 7.78	3.90 3.94 1.79 1.84	2.30 2.33 1.06 1.08	30.83 31.16 14.13 14.43	7.36 7.44 3.37 3.45	4.35 4.40 1.99 2.04	54.57 55.08 24.89 25.39	13.25 13.38 6.04 6.17	7.85 7.93 3.58 3.65		
В													
0.2 cal cm ⁻² min ⁻¹	.9075 .9075	30 70	0.06 0.05	0.01 0.01	0.01 0.02	0.11 0.11	0.02	0.01 0.02	0.19 0.18	0.04 0.04	0.02 0.03		
0.8 cal	.9075	30	0.11	0.03	0.01	0.22	0.05	0.02	0.33	0.09	0.05		
cm ⁻² min ⁻¹	.9075	70	0.12	0.03	0.01	0.22	0.04	0.03	0.32	0.09	0.05		
1.2 cal	.9075	30	0.18	0.04	0.03	0.33	0.09	0.05	0.51	0.13	0.08		
cm ⁻ min ⁻¹	.90 75	70	0.19	0.05	0.02	0.30	0.08	0.05	0.50	0.13	0.07		
¹ Wind velo	ocity is	500	cm sec	⁻¹ (R	 _ = .()359 ве	ec cm	⁻¹).	<u></u>				

because of its greater leaf temperature - air temperature differential, which steepens the vapor pressure gradient from leaf to air, it has a greater rate of transpiration under all conditions. The largest difference in transpiration rates between the glaucous and non-glaucous leaf is at the highest temperature, highest light intensity, and lowest stomatal resistance, while the smallest difference is at the lowest temperature, lowest light intensity, and highest stomatal resistance (Table 6). These results indicate that the glaucous character could be a selective advantage in environments where conservation of water is important to the survival of the individual especially environments with high temperatures and high light intensities.

The energy budget analysis has demonstrated that the glaucous character of leaves may be either a selective advantage or a disadvantage depending on the environmental conditions. Clark and Lister (1973) have shown that the waxy bloom of blue spruce needles selectively reflects blue light and therefore the needles are less efficient in photosynthesis. The reflective properties of the epicuticular waxes of the glaucous leaves may reduce the energy that is absorbed by the leaf and result in a reduction of leaf temperature (Table 3 and 4). Under conditions of low temperatures (i.e., winter conditions) a lower leaf temperature may be disadvantageous due to the reduction of the net photosynthetic rate of the leaf (Table 5). However a lower leaf temperature could be advantageous under these conditions if conservation of water is important because the glaucous leaf could have smaller transpirational losses as shown by the energy budget analysis (Table 6). In the summer when temperatures are high and photosynthetic rates are high a lower

leaf temperature could be advantageous due to the reduction of the rate of respiration and the more active photosynthetic process which result in an increase in net photosynthesis. Transpirational losses may also be reduced by a lower leaf temperature of the glaucous needle especially in hot sunny environments (Table 6). Although the expected differences between glaucous and non-glaucous leaves are small, as indicated by the energy budget analyses with time (i.e. a growing season or a generation) these factors could have a large and significant additive effect on the survival of the individual. Experimental evidence is needed to test the above hypotheses.

PHOTOSYNTHESIS

MATERIALS AND METHODS

The photosynthetic response of current-year glaucous and non-glaucous foliage of blue spruce to increasing light intensities was measured in a closed environmental control system. Measurements of net photosynthesis on material collected at 7:30 A.M. started at the end of July and were continued into mid-August. A single-stemmed branch for each sample was sealed in a water jacketed plexiglass chamber. Illumination was provided by two 400 watt color improved mercury vapor lamps positioned opposite each other on either side of the photosynthetic chamber. Light intensity was varied from 1,000 footcandles per side to 8,000 footcandles per side by adjusting the distance of the mercury lamps from the photosynthetic chamber. Light quanta measurements were made between 400 and 700 nm at the various light intensities with a Lambda LI-185 Quantum sensor. Photosynthetic measurements started at a light intensity of 1,000 footcandles from each side and were increased by 1,000 footcandles for each successive measurement until 8,000 footcandles was reached from each side. Each branch was acclimated for at least 20 minutes before the rate of photosynthesis was recorded for each light setting. Net photosynthetic rates were measured with a Beckman infrared CO_2 gas analyzer by following the rate of depletion of CO_2 between 300 and 270 ppm in the system. CO_2 was replenished by opening the environmental control system to the laboratory air. Temperature was 24 ± 1°C, relative humidity was 55 ± 2%, and the wind velocity was 112 cm sec⁻¹ for all photosynthetic measurements.

Dark respiration was measured at the end of the photosynthetic determinations by placing a black cloth over the photosynthetic chamber and following the rate of increase of CO_2 in the system. Dark respiration and photosynthetic rates are both expressed as mg CO_2 dm⁻² hr⁻¹ and as mg CO_2 g⁻¹ dry wt hr⁻¹.

Surface area measurements were made using a non-destructive technique. The number of needles on a branch was determined by multiplying the needles in a 1 cm band around the median portion of the branch by the total length of the branch. Mean needle length was determined by measuring 3 needles each at the base, middle, and tip of the branch. Mean needle width was similarly determined by measuring needle widths of the middle portion of the needle on two of its 4 sides. The product of needle number, needle length, needle width, times 4 was taken as the foliage surface area of a blue spruce branch. A factor of 4 is used in the formula since blue spruce needles are 4 sided. Foliage

surface areas estimated by the above procedure were highly correlated (r = .95) with photoelectrically determined areas of the same material.

Foliage was oven dried at 105° C until a constant dry weight was obtained. Oven dry weights were also highly correlated with photoelectrically determined surface areas (r = .98).

RESULTS AND DISCUSSION

No statistical significance could be found for the differences in net photosynthetic rates due to foliage glaucousness at any light intensity. However certain trends are indicated in the data (Fig. 28 and 29). The photosynthetic rate for the glaucous branches was lowest at the low light intensity and highest at the high light intensity. The non-glaucous net photosynthetic rate was highest at the low light intensity and intermediate at the high light intensity while the semi-glaucous photosynthetic rate was intermediate at the low light intensity and low at the high light intensity. Cameron (1970) found a similar pattern in his investigation of waxed and dewaxed leaves of Eucalyptus bicostata. Since the photosynthetic rates were determined first on the normal glaucous leaf and then the same leaf was dewaxed and remeasured, the change in the photosynthetic rates were directly attributable to wax removal. The observed change in photosynthetic efficiency of glaucous and non-glaucous foliage with the change in light intensity is expected when the reflection data are considered. The glaucous foliage reflects more light and thus presumably absorbs less light than the non-glaucous foliage in blue spruce (Fig. 25). Therefore glaucous foliage would be expected to have lower rates of net

Fig. 28. Net photosynthesis (mg CO₂ dm⁻² hr⁻¹) for glaucous, semi-glaucous, and non-glaucous foliage and differences in light saturation between glaucous and non-glaucous foliage of blue spruce at increasing light intensities. Differences in net photosynthetic rates at any light intensity were not significant (5% level). ¹Differences in light saturation. Points not connected by the same line are significantly different at the 5% level.





Fig. 29. Net photosynthesis (mg CO₂ g⁻¹ dry wt hr⁻¹) of glaucous, semi-glaucous, and non-glaucous foliage and differences in light saturation between glaucous and non-glaucous foliage of blue spruce at increasing light intensities. Differences in net photosynthetic rates at any light intensity were not significant (5% level). ¹Differences in light saturation. Points not connected by the same line are significantly different at the 5% level.



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photosynthesis at light intensities below light saturation and higher rates of net photosynthesis at light intensities above light saturation. The reason for the change in positions of the semi-glaucous and non-glaucous categories at high light intensities is not understood, but may be due to sampling error because only 2 trees were sampled in the semi-glaucous group.

The percent difference in net photosynthetic rates of glaucous and non-glaucous foliage at the low and high light intensities was determined to see if this difference could be explained in terms of reflectance differences. Glaucous needles reflect 16-30% while non-glaucous needles reflect 8-20% of incident light in the photosynthetically active region (Fig. 25). Therefore non-glaucous foliage would be expected to absorb more energy than glaucous foliage and be more efficient in photosynthesis at light intensities below light saturation. At a light intensity of 1,000 footcandles per side non-glaucous foliage had a 29% greater net photosynthetic rate per unit area than the glaucous foliage (19% greater if expressed on a dry weight basis). However above the light saturation level (8,000 footcandles per side) glaucous foliage had a 15% greater net photosynthetic rate per unit area than non-glaucous foliage (18% if expressed on a dry weight basis). The percent differences of net photosynthetic rates of glaucous and non-glaucous foliage at the low and high light intensities are within the range and in the direction of values that would be expected on the basis of the reflectance data. Using Cameron's (1970) data I made similar calculations of the photosynthetic rates of glaucous and dewaxed juvenile leaves of

eucalyptus which reflect 11 and 6% of incident light respectfully. The dewaxed leaves were 7% more efficient than normal glaucous leaves in net photosynthesis at an incident radiation of 2×10^{-3} ergs cm⁻² sec⁻¹. But at an incident radiation of 12×10^{-3} ergs cm⁻² sec⁻¹ the normal leaves were 8% more efficient than the dewaxed leaves. These data on eucalyptus also closely agree with that expected from the reflectance results. Although other factors may be involved in blue spruce, the difference in photosynthetic rates of glaucous and non-glaucous foliage at high and low light intensities could be the result of the differential reflection of light by the epicuticular waxes.

To further study light utilization by glaucous and non-glaucous foliage net photosynthetic rates were expressed as a ratio of the rate of photosynthesis at the lowest light intensity over the rate of photosynthesis at the highest light intensity (Table 7). Expressing the data in this manner not only removes the large variation due to individual trees but it also measures the relative efficiency of the photosynthetic mechanism of the trees at the respective light intensities. The small ratio of the glaucous individuals was significantly different (1% level) from the ratio of the non-glaucous and semi-glaucous individuals. These results indicated that, (1) either the rate of photosynthesis of the glaucous individuals at the low light intensity was low compared to the photosynthetic rate at the high light intensity, or (2) the rate of photosynthesis at the high light intensity was high compared to the rate of photosynthesis at the low light intensity, or (3) a combination of the above cases was occurring in relation to the non-glaucous individuals. Inspection of the data shown in Fig. 28 and

Table 7. Dark respiration and ratios of the rate of net photosynthesis at the low light intensity to the rate at the high light intensity for glaucous, semi-glaucous, and non-glaucous foliage expressed on both an area and dry weight basis.

	<u>Glaucous</u>	<u>Semi-glaucous</u>	Non-glaucous
Ratios			
$mg CO_2 dm^{-2} hr^{-1}$.40	.54	.54
	Α	В	В
mg CO ₂ g ⁻¹ dry wt hr^{-1}	.40	.54	.54
	A	В	В
Dark Respiration			
$mg CO_2 dm^{-2} hr^{-1}$	1.6	1.4	1.4
	а	а	а
mg CO ₂ g ⁻¹ dry wt hr^{-1}	1.6	1.5	1.3
	a	a	a

¹Values without common letters in each row are significantly different. A = 1% level. a = 5% level. 29 indicated that glaucous branches had a lower rate of photosynthesis at the low light intensity and a higher rate of photosynthesis at the high light intensity than the non-glaucous branches resulting in the smaller ratio. Under environmental conditions of low light the non-glaucous individual would be more efficient in fixing CO₂, while in high light conditions the glaucous individuals would be more efficient.

Harland (1947) demonstrated that natural selection in a specialized climate acted differentially on the allelomorphic pair of genes, B (presence of waxy bloom on the stem) and b (absence of bloom) of castor bean (<u>Ricinus communis</u>) in Peru, where it is adventive. At low elevations along the coast where a thick fog bank and a drizzling rain is present from June to September, BB or Bb plants do not fruit and set seed while the bb plants grow normally. With increasing elevation, the fog bank disappears, sun light increases, and the proportion of BB or Bb plants in the wild population increases from 22.2% near the coast to 100% at 84 kilometers from the coast. Although a reciprocal transplant study was not carried out at the high elevations it is clear that BB or Bb plants have an advantage at the high elevation since no bb plants are present.

Light saturation for single-stemmed branches was statistically determined by separate comparison of the rates of net photosynthesis at each light intensity for glaucous and non-glaucous foliage (Fig. 28 and 29). When photosynthetic rates were expressed on an area basis (Fig. 28), the glaucous foliage appeared to have a light saturation point between 2,000 and 4,000 footcandles of light incident on each side of the chamber, while the non-glaucous foliage appears to have light saturation between 1,000 and 3,000 footcandles. However on a dry

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weight basis (Fig. 29) there is no statistical difference in light saturation of photosynthetic rates between glaucous and non-glaucous foliage with general light saturation occurring between 2,000 and 4,000 footcandles per side. Glaucous foliage would be expected to have a higher light saturation point than non-glaucous foliage based on the reflection data, but this result was not clearly seen in the two ways I expressed the data. A clear identification of the light saturation point is probably being masked by the large individual tree component of variance and self-shading factors of the branch. Individual needles would be expected to have lower light saturation points due to the elimination of self-shading that occurs on the branch and whole trees would have a higher light saturation point due to the increase of self-shading. Although it was unclear if there was a difference in the light saturation point for glaucous and non-glaucous branches, on the basis of the reflection data presented here it would be expected that glaucous foliage has a higher light saturation point as indicated in Fig. 28.

Dark respiration rates of glaucous, semi-glaucous, and non-glaucous foliage were not significantly different at the 5% level (Table 7).

TRANSPIRATION

MATERIALS AND METHODS

Transpiration rates were measured by a weight-loss method on detached single-stemmed branches sealed in water-filled vials. Four
branches were collected at the end of August from each of the 12 trees, weighed at the beginning of the experiment, and then periodically during the experiment to obtain weight loss per unit time. At the end of the experiment foliage dry weights were determined and transpiration rates expressed as g of $H_{2}O$ g⁻¹ dry weight hr⁻¹ x 10.

Sample branches were detached in the morning and afternoon, sealed in the water supply vials, and subjected to 2 different temperatures in growth cabinets giving 4 treatments. Temperatures in the cabinets were 24°C and 30°C and relative humidity was 40%. The light intensity was held constant a 4,300 footcandles (6.6 x 10^2 microeinsteins m⁻² sec⁻¹) by ten 4-foot fluorescent tubes and eight 25 watt incandescent bulbs.

An attempt was made to measure cuticular transpiration by placing the branches in the growth cabinet in darkness at 30°C. However transpiration rates measured in this manner did not differ from those obtained with the lights on indicating that the stomates did not close. Hence these data are not presented. It was difficult to determine if the stomates were closed in blue spruce because the stomates were sunken beneath the leaf surface and heavily occluded with waxes.

RESULTS AND DISCUSSION

No significant differences in transpiration rate were found between degrees of glaucousness in any of the treatments or at any of the measurement times (Table 8). However only in treatment 2 at the 7.5 hour measurement time was the mean transpiration rate for the non-glaucous branches greater than that of the glaucous branches.

Elapsed time		Treatments															
		A.M., 24°C			A.M., 30°C				P.M., 24°C			P.M., 30°C					
		G	SG	NG		G	SG	NG		G	SG	NG		G	SG	NG	
								mg	н ₂ 0	g ⁻¹	hr ⁻¹	^L x10					
1.5 hr	8	3.9	4.7	3.2	al	6.2	5.6	5.2	A	3.0	3.2	2.2	A	4.8	4.1	4.4	A
				A				B				С				D	
3.5 hr	8	4.0	4.6	3.3	a	6.5	6.0	5.4	A	3.3	3.5	2.6	A	5.6	5.1	5.1	B
				A				B				С				D	
7.5 hr	8	4.2	4.6	3.6	a	5.9	5.7	5.1	A	4.0	3.9	3.3	B	5.8	5.7	5.8	B
				a				Ъ				с				d	
13.0 hr	8	4.1	4.4	3.5	a	4.8	4.8	4.2	В	-	-	-	-	-	-	-	-
				a				Ъ				-				-	
18.0 hr	:8	-	-	-	-	-	-	-	-	4.1	3.8	3.7	B	4.6	4.1	4.3	A
				-				-				a				Ъ	

Table 8. Transpiration rates of detached branches of glaucous (G), semi-glaucous (SG), and non-glaucous (NG) foliage at 4,300 footcandles and 40% relative humidity.

Letters not common in a row indicate significant differences due to collection time and letters not common in a column indicate differences due to measurement time. A = 1% level and a = 5% level.

Schmucker (1948) found that transpiration rates did not differ much between glaucous and non-glaucous foliage of blue spruce or Douglas-fir but a glaucous variety of white spruce had twice the transpiration rate of a non-glaucous one. These results are contrary to the trends of stomatal transpiration rates reported for seven glaucous and non-glaucous sibling lines of <u>Brassica oleracea</u> by Denna (1970). This suggests that the epicuticular waxes, which cause foliage glaucousness, may either vary among species in regulating water loss through stomates or other factors such as stomate frequency or chemical differences of the waxes influence the rates of stomatal transpiration and obscure the effect of the epicuticular waxes.

Hall and Jones (1961) found an increase in cuticular transpiration in clover leaves when the bloom was removed by brushing detached leaves. Cuticular transpiration differed among seven glaucous and non-glaucous sibling lines of <u>B</u>. <u>oleracea</u> with glaucous foliage losing less water through the cuticle (Denna, 1970). These data indicate that foliage glaucousness (i.e. structural waxes) may function in reducing cuticular water loss. Cuticular water loss of glaucous and non-glaucous foliage should be measured on blue spruce to see if it follows the above general pattern.

An increase of temperature increased the rate of transpiration in both the afternoon and morning treatments (Table 8). Material collected in the afternoon exhibited a significant increase in transpiration rate from 1.5 hours to 7.5 hours. However from 7.5 to 18.0 hours the transpiration rate decreased. The material collected in the morning tended to have higher transpiration rates within a temperature but less

of a total increase in transpiration rate from 1.5 to 7.5 hours. Material collected in the morning did show a statistically significant decrease in transpiration rate at the 30°C treatment from 7.5 to 13.0 hours. Sucoff (1972) showed that leaf water potential fell from -6 bars at 4:00 A.M. to -14 bars at 12:00 P.M. in red pine (Pinus resinosa) needle fascicles because transpired water exceeded absorbed water. Leaf water potential started to rise at 4:00 P.M. and by 8:00 P.M. the leaf water potential had risen to -9 bars. Apparently, material collected in the afternoon in the present study had a lower water potential and a lower transpiration rate than material collected in the morning. In the afternoon material, when the tension on the transpirational stream is released by detaching and when the branches were placed in the growth cabinets they recovered from the low water potential resulting in an increased transpiration rate. The water potential of the morning material should be higher than the afternoon material and therefore would not show as great a recovery of water potential and the resultant increase of the transpiration rate as indicated in Table 8. The decline in transpiration rates from 7.5 to 13 or 18 hours is not fully understood but may be due to the clogging of the transpirational stream by oleoresin seeping from the cut end of the branch or from prolonged effects of detachment. Use of branches detached in the morning before the plant water potential drops may be a useful method of obtaining transpiration measurements over a 7.5 hour period of material which is not easily measured in any other way.

MOISTURE RETENTION

MATERIALS AND METHODS

The ability of the glaucous, semi-glaucous, and non-glaucous foliage to retain moisture was determined by using detached branches collected at the end of July. Fresh weights of 4 branches for each of the 12 trees were obtained at the start of the experiment and then the branches were placed in open seed envelopes under the following conditions:

> Treatment 1: 22°C, 37% R.H. Treatment 2: 22°C, 64% R.H. Treatment 3: 34°C, 24% R.H.

The loss of water was determined by periodically weighting the branches and the results were expressed as a percent of fresh weight.

RESULTS AND DISCUSSION

The general pattern of moisture retention for the 3 treatments is shown in Fig. 30, 31 and 32. Treatment 3 resulted in the largest and fastest loss of water which was expected as the high temperature and low relative humidity enhance the rate of water loss. Treatment 2 which had a relative humidity of 64%, had a much slower rate of water loss than treatment 1 which had a relative humidity of 37%. These results were expected since a decrease in relative humidity increases the rate of water loss. Fig. 30. Treatment 1 (22°C, 37% R.H.). Change in branch weight as a percent of fresh weight for glaucous, semi-glaucous, and non-glaucous foliage. Differences due to glaucousness are not significant at the 5% level.





Fig. 31. Treatment 2 (22°C, 37% R.H.). Change in branch weight as a percent of fresh weight for glaucous, semi-glaucous, and non-glaucous foliage. Differences due to glaucousness are not significant at the 5% level.







Fig. 32. Treatment 3 (34°C, 24% R.H.). Change in branch weight as a percent of fresh weight for glaucous, semi-glaucous, and non-glaucous foliage. Differences due to glaucousness are not significant at the 5% level.





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The relation between glaucousness and moisture retention is uncertain because no significant differences were found between glaucous and non-glaucous foliage in any treatment. In addition in all treatments the glaucous foliage at the beginning appeared to lose more water than the non-glaucous foliage but as time went on (the 9th, 29th and 6th days, respectively) the glaucous foliage lost less water than the non-glaucous foliage (Fig. 30, 31, and 32). A very similar pattern was reported by Schmucker (1948) for non-glaucous and glaucous twigs of Douglas-fir from which he concluded that under certain conditions, cuticular transpiration could be greater in the glaucous foliage than the non-glaucous foliage. In treatment 3 at the end of the experiment glaucous foliage lost more water than non-glaucous foliage (Fig. 32). Parker (1968) was able to show species differences in ability to retain moisture using similar methods, however the method may not be precise enough to detect differences within species.

Total water loss, fresh weight, needle dry weight, number of days to 50% of needle drop, and number of days to 100% of needle drop were also determined in conjunction with the moisture retention data (Table 9). Differences between glaucous, semi-glaucous and non-glaucous foliage for the above variables were not significantly different. The number of days to 100% of needle drop for glaucous twigs tends to be longer than for non-glaucous twigs. Further study of this character with more precise methods may be needed to illucidate a real relationship which could prove useful to the Christmas tree industry.

Table 9. Effects of temperature and relative humidity treatment on total water loss, fresh weight, percent moisture, needle dry weight, number of days to 50% of needle drop, and number of days to 100% of needle drop for glaucous (G), semi-glaucous (SG), and non-glaucous (NG) foliage.¹

Verdeblee	Treatment									
measured	1 (22°C, 37% R.H.)	2 (22°C, 64% R.H.)	3 (34°C, 24% R.H.)							
<u>Total water loss</u>	<u>(g)</u>									
G	2.55	2.60	2.40							
SG NG	2.26 2.93	2.55 2.84	2.34 2.82							
Fresh weight (g)										
G	4.07	4.16	3,84							
SG	3.60	4.05	3.74							
NG	4.63	4.47	4.44							
Percent moisture										
G	62.5	62.4	62.4							
SG	62.8	63.1	63.1							
NG	63.3	63.5	63.4							
Needle dry weight (g)										
G	1.04	1.08	0.98							
SG NS	0.94 1.17	0.99 1.11	0.93 1.12							
Number of days to 50% of needle drop										
G	17.0	30.0	10.8							
SG	17.8	31.1	11.7							
NG	12.2	29.9	9.8							
Number of days to 100% of needle drop										
G	19.6	37.7	12.0							
SG	19.4	35.5	13.0							
NG	13.7	33.7	11.0							

No significant differences were detected.

SUMMARY AND CONCLUSIONS

Glaucous foliage reflected more light than non-glaucous foliage at the wavelengths of light measured in this study. The difference in reflectance was greatest in the ultraviolet and blue region of the light spectrum where glaucous foliage reflected 26% and non-glaucous foliage reflected 10% of incident light. Clark and Lister (1973) have shown that the waxy needle bloom of blue spruce selectively reflects blue light and results in low apparent photosynthesis. This data indicates that the glaucous character could be selectively disadvantageous by reducing photosynthetic efficiency. However, the ability to reduce the amount of energy entering a leaf by reflecting light, especially light of high energy, could have an adaptive significance in environments of high light intensities.

Results of the energy budget analysis suggest that glaucousness could be both an advantage and a disadvantage depending on the environmental conditions. At low light intensities and low temperatures a non-glaucous needle could have a higher leaf temperature and a greater rate of net photosynthesis than non-glaucous foliage. But at high temperatures and high light intensities the glaucous needle could have the advantage over the non-glaucous needle due to its reduced leaf temperature and more efficient photosynthetic process. The transpiration rate of the glaucous needle should be lower than the non-glaucous needle under all conditions because less energy must be dissipated by the glaucous needle. Results of the photosynthetic experiments agree with the above hypotheses. The non-glaucous foliage is more efficient in photosynthesis at low light intensities but the glaucous foliage is more efficient at high light intensities. The differences of the photosynthetic rates at the low and high light intensities agree with the differences that you would predict on the basis of the reflectance data. No clear difference in light saturation points was found between glaucous and non-glaucous foliage but glaucous foliage would be expected to have a higher light saturation point.

Stomatal transpiration of glaucous foliage tended to be greater than non-glaucous foliage, although it would be expected to be less based on the energy budget analysis. Perhaps other factors are interacting to obscure the effect of the surface waxes. Glaucous foliage lost more water early in the moisture retention experiments, but as desiccation increased it lost less water than non-glaucous foliage. These results indicate that the role of the glaucous character in regulating the water balance of foliage is unclear.

Ronco (1970 a and b) has attributed the high mortality of Engelmann spruce (Picea engelmannii) seedlings planted in open areas in the Rocky Mountains to solarization effects. Seedlings grown in the shade are normal green and have high rates of photosynthesis, while seedlings grown in full sunlight are chlorotic and have low rates of photosynthesis. Moisture stress and nutrient deficiencies have been eliminated as possible causes of the above phenomena. Glaucous and non-glaucous needles have surface wax deposits in the stomatal areas but the glaucous needles also have a relatively dense deposit of

structural wax in the nonstomatal areas. This indicates that the effect of stomatal occlusion on gaseous exchange by the surface waxes may be similar for both types. Therefore the main difference between the glaucous and non-glaucous foliage is an increase of reflectance by the additional surface wax deposits of glaucous foliage, although these deposits may also regulate cuticular water loss. The data presented in this present study suggests that a major role of the surface waxes may be to reduce the amount of energy entering the leaves and reduce solarization damage.

CONCLUSIONS AND RECOMMENDATIONS

The results of the developmental study of epicuticular waxes on blue spruce foliage show that the formation of surface waxes is closely associated with needle development. Structural waxes are present in epistomatal chambers of needles in the expanding bud. As the needles expand and are exposed to the atmosphere, the process of wax deposition increases. The wax structures observed are best developed during the first part of the growing season and it appears that wax formation decreases or ceases as needle growth is completed. This suggests that the epicuticular waxes may act as a protective mechanism for the young succulent growth as it is exposed to the rigors of the environment. This could be an effective mechanism for the prevention of desiccation, frost damage, abrasion by wind and wind blown particles, protection from solarization, disease or insects, or a combination of the above factors.

Both glaucous and non-glaucous foliage have structural waxes in the stomatal areas where the potential for transpiration is maximum but only glaucous foliage has a dense deposit of structural wax in the nonstomatal areas. The structural wax layer is too small to affect the boundary layer of the needle but may increase the diffusion resistance of water vapor from the cuticle by raising the zero level of boundary layer from the cuticle. These results indicate that the glaucous and non-glaucous foliage might differ in cuticular transpiration rates but not in stomatal transpiration. Cuticular transpiration measurements

were attempted for blue spruce foliage, however the stomates apparently did not close in darkness and the results were invalid. Schmucker, (1948) measured cuticular water loss for non-glaucous and glaucous varieties of Douglas-fir and under certain conditions the glaucous variety lost more water. This result is very similar to those reported in the present study for moisture retention of blue spruce branches with the glaucous foliage losing more water early in the experiment and then as desiccation increased the glaucous foliage lost less water than the non-glaucous foliage. The significance of this pattern is not understood. Denna (1970) and Hall and Jones (1961) have shown that glaucousness retards cuticular water loss in Brassica oleracea and red clover. This suggests that there may be species differences in the action of glaucousness in controlling the loss of water from foliage. Cuticular transpiration should be measured for glaucous and non-glaucous foliage of blue spruce and other conifer species with controls that would assure that the stomates would close. This would provide further information on the effect of glaucousness on cuticular water loss and verification of the trends reported here and by Schmucker (1948).

Glaucous foliage reflects more light than the non-glaucous foliage and since the glaucous foliage has less energy to dissipate it would be expected to have a lower transpiration rate. Results of the energy budget analysis support this hypothesis, however the measurements of stomatal transpiration in blue spruce are contrary to this expected result. In all but one case the glaucous foliage had greater transpiration rates than the non-glaucous foliage although differences could not be statistically attributed to glaucousness. Schmucker (1948)

found no transpiration differences for glaucous versus non-glaucous Douglas-fir and blue spruce varieties, but for white spruce the glaucous variety had twice the transpiration rate of the non-glaucous variety. Further stomatal transpiration studies are warranted on other conifer species to test the consistency of the trends reported here.

Cuticular and stomatal transpiration measurements obtained here represent only one point midway in the active growing season for blue spruce. As suggested earlier the primary significance of the glaucous character may be early in the growing season when the young succulent needles are expanding or in the winter time when the needles are exposed to the dry cold winds. A seasonal study of both cuticular and stomatal transpiration of glaucous and non-glaucous foliage would be quite helpful in determining the role of glaucousness in regulating the water balance of conifer foliage throughout a season. Stomatal frequency and chemical variation in the surface waxes of glaucous and non-glaucous foliage of blue spruce should also be studied to determine their effect on water loss.

The reflective properties of structural waxes of glaucous foliage could be of adaptive significance in a hot sunny environment such as found in the southern Rocky Mountains. The possible reduction of energy by the increased reflection of glaucous foliage could reduce leaf temperature, increase photosynthetic rates at high air temperatures, and reduce the transpirational load of the leaves as demonstrated by the energy budget analyses. Glaucous foliage as reported here and by Cameron (1970) has higher rates of photosynthesis at high light intensities than non-glaucous foliage. It is not known whether this

effect is due to solarization, increased leaf temperature, or complementing effects.

Engelmann spruce seedlings have high mortality when planted in clear cut areas or undergoing natural regeneration in open areas in the Rocky Mountains. Ronco (1970a) found that severe chlorosis developed and persisted for many years in open grown seedlings of Engelmann spruce while seedlings grown in the shade were a normal green. Photosynthesis was found to be greater at all light intensities for the shaded seedlings than for the unshaded seedlings which suggests that the photosynthetic mechanism in the open grown seedlings had been damaged, presumably by solarization. Clark (1961) showed similar photosynthetic results for white spruce and Douglas-fir. Ronco also showed that water stress was not the major cause of mortality in open grown Engelmann spruce but that a combination of water stress and exposure to direct sunlight could result in irreversible injury from solarization. He concluded that solarization was responsible for the high mortality of Engelmann spruce seedlings.

Kung and Wright (1972) found that foliage glaucousness was most intense in the southern Rocky Mountains for ponderosa pine (<u>Pinus</u> <u>ponderosa</u>), limber pine (<u>P. flexilis</u>), southwestern white pine (<u>P.</u> <u>strobiformis</u>), Douglas-fir and white fir (<u>Abies concolor</u>) than in any other part of their natural range. The southern Rocky Mountains have a greater annual mean daily solar radiation than the northern Rockies (i.e. Albequerque, New Mexico has 512 langleys and Lander, Wyoming has 443 langleys) and a lower precipitation-evaporation ratio (Smith, <u>et</u> <u>al</u>., 1968). From these results Kung and Wright suggested that

glaucousness could be of selective value by reducing desiccation and reducing the photo-destruction of auxin.

For algae the light efficiency of the photosynthetic process is only 22% and the rest of the light energy is wasted as heat. The quantum requirement of photosynthesis is the same for blue light as red light even though there is more energy per photon for blue light. Therefore blue light is more wasteful of energy in the photosynthetic process (Salisbury and Ross, 1969). It is not known if the quantum requirement is the same for blue spruce. The greatest difference in foliage reflectance of glaucous and non-glaucous foliage is the ultraviolet and blue region of the light spectrum. Clark and Lister (1973) have shown that the blue color and low apparent photosynthetic rates of blue spruce are the result of the enhanced selective reflection of blue light by the waxy needle bloom. The photosynthetic results reported here for blue spruce indicate that the glaucous foliage is more efficient in photosynthesis at high light intensities than non-glaucous foliage. These results indicate that the glaucous foliage could have a selective advantage in environments where solar radiation is intense by not only selectively reflecting high energy blue light but by reducing the total amount of energy received by the leaves throughout the light spectrum as shown here.

Surface waxes of plant foliage represent the perimeter defense of the plant to the various climatic factors, diseases, insects, and to man's own air pollution. By the nature of their position they must be multifunctional to assure the survival of the plants which produce them. The hydrophobic nature of the surface waxes indicates that they may play

a role in preventing desiccation. However, the evidence presented here and elsewhere does not support this view and perhaps as Schieferstein and Loomis (1959) have suggested the subsurface wax may be more important than surface wax in preventing water loss. The chemical and physical nature of the surface wax represents a formidable barrier to pathogens and insect pest. Grose (1960) suggested that foliage glaucousness (i.e. structural waxes) reduces the wetability of eucalyptus leaves and prevents their penetration by water and therefore provides resistance to frost or winter-kill. Good experimental evidence for the above functions which illustrate a consistent trend for the surface waxes in several species is still lacking.

I feel that the reflective characteristics of the surface waxes may be one of their more important functions by reducing the amount of energy entering the leaves and protecting the leaves from solarization. Harland's work in 1947 in castor bean provides the first evidence which associates the occurrence of glaucousness with increasing sunlight. Barber (1955) showed an increase of glaucousness with increasing elevation where solar radiation is intense. High light conditions also have been shown to enhance cuticle and surface wax development (Baker, 1973). Ronco (1970a) demonstrated that solarization was a major factor in the poor establishment Engelmann spruce seedlings in open areas in the Rocky Mountains. Kung and Wright (1972) have described the variation of foliage glaucousness for 5 tree species and determined that the more glaucous types are found in the southern Rocky Mountains where the incident solar radiation is greatest. The whitish-blue color of glaucous foliage is due to the enhanced selective reflectance of blue

color by the waxy needle bloom. The reflectance data and energy budget analyses presented here indicate that glaucous foliage could have a selective advantage in hot sunny environments. The photosynthetic data also indicate that glaucous foliage may have a selective advantage at high light intensities. All of the above evidence for the various species suggest that foliage glaucousness occurs with high solar radiation and that a major function of the structural waxes may be in preventing solarization.

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