The effect of increasing elevation on leaf cuticle thickness and cuticular transpiration in balsam fir

EVAN H. DELUCIA[1] AND GRAEME P. BERLYN
School of Forestry and Environmental Studies, Yale University, New Haven, CT, U.S.A. 06511

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First-year needles and stems of *Abies balsamea* were collected at the end of the growing season along an elevational gradient on Mt. Moosilauke, NH. Tissue was sampled from the base (732 m), midslope (1143 m), forest limit (1402 m), and tree line (1455 m). Mean cuticle thickness on the adaxial needle surface decreased with increasing elevation from 3.01 to 2.21 μm. A similar decline was observed for the cutinized cell wall at the lower three elevations. Associated with the decline in cuticular thickness was a 59.3% increase in the rate of cuticular water loss per gram dry weight from 732 to 1402 m. The amount of epicuticular wax and other features of leaf anatomy were also examined along the elevational gradient. The high rates of cuticular water loss in these subalpine trees increase the risk of desiccation damage at high elevations and support the concept that they can be a contributing factor in the formation of alpine tree line.

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Des aiguilles et des rameaux de première année d'*Abies balsamea* ont été récoltés, à la fin de la saison de croissance, le long d'un gradient altitudinal sur le mont Moosilauke au New Hampshire. Les tissus échantillonnés provenaient du pied de la montagne (732 m), du milieu de la pente (1143 m), de la limite des forêts (1402 m) et de la limite des arbres (1455 m). L'épaisseur moyenne de la cuticule sur la surface adaxiale des aiguilles diminue de 3,01 à 2,21 μm avec l'augmentation de l'altitude. La paroi cellulaire cutinisée présente une diminution semblable aux trois altitudes inférieures. De concert avec la diminution de l'épaisseur de la cuticule, il y a, de 732 à 1402 m, une augmentation de 59,3% dans le taux de perte cuticulaire d'eau par gramme de poids sec. La quantité de cires épicuticulaires ainsi que d'autres caractéristiques de l'anatomie foliaire ont aussi été examinées le long de ce gradient altitudinal. Le taux élevé de perte d'eau à travers la cuticule chez les arbres subalpins augmente le risque de dommages dûs à la dessication aux altitudes élevées, et appuie l'hypothèse que ce facteur puisse contribuer à l'établissement de la limite des arbres en milieu alpin.

[Traduit par le journal]

Introduction

A striking feature of montane vegetation is the occurrence of an upper elevational limit to tree growth referred to as the tree line. Several attempts have been made to explain alpine tree line in terms of a single limiting factor such as low CO_2 concentration, low temperatures, or high wind speeds, to name a few. Daubenmire (1954) reviewed several of these unidimensional arguments and indicated their shortcomings. A more comprehensive theory of tree line was proposed by Wardle (1971). Wardle suggested that the altitudinal limit of tree growth is determined by the inability of tissue to fully mature in terms of the production of lignin, cuticle, and epicuticular wax. Death occurs as a result of desiccation during periods of unfavorable weather, usually during the winter. His studies with *Nothofagus solandri* showed that the length of the growing season and the degree of tissue maturation decreased with increasing elevation in the Craigieburn Range of New Zealand. This hypothesis is also supported by Tranquillini (1979).

Several environmental parameters such as wind, frost, and low air and soil temperatures combine to shorten the growing season at high altitudes. As a consequence, the total energy available for plant growth as measured in degree-days above 0°C is greatly reduced in alpine habitats (Billings 1973, 1974). In New England, energy input and plant productivity is further reduced by frequent cloud cover and fog (Bliss 1966).

The effect of limited energy availability on subalpine trees is evident as a decrease in leaf dimensions, declining radial growth, and incomplete lignification and cell wall development (Tranquillini 1979). Baig and Tranquillini (1976) found that the thickness of needle cuticle and cutinized cell wall of *Pinus cembra* and *Picea abies* decreased with increasing elevation in the Austrian Alps. They also reported an inverse relationship between cuticle thickness and the rate of cuticular transpiration. It was hypothesized that extensive needle damage in Krummholz vegetation and ultimately the formation of alpine tree line is a result of high rates of cuticular water loss during the winter when uptake of water from the soil is greatly reduced by frozen stems and substrate. More recently, Baig and Tranquillini (1980) found that the relative water content of *Picea abies* twigs from the tree line decreases to a greater degree during the winter than does that of twigs from the forest line. Decreases in both leaf water potential and the relative water content of resting buds during the winter have also been reported for other high-elevation species, suggesting that faster rates of cuticular transpiration occur at high elevations (Lindsay 1971; Hansen and Klikoff 1972; Barclay and Crawford 1982).

Balsam fir, *Abies balsamea* (L.) Mill., rapidly increases in importance above 732 m (Bormann et al. 1970) and is the dominant tree-line species in New England (Hart 1959; Fowells 1965). In this investigation we examined the effect of increasing elevation on the amount of epicuticular wax, cuticle thickness, leaf structure, and the rate of cuticular water loss in balsam fir on Mt. Moosilauke, New Hampshire.

Materials and methods

Mt. Moosilauke is located at the southwestern edge of the higher White Mountain peaks, in Grafton County, NH. Its summit rises 1466 m above sea level and the total relief is approximately 1158 m (Brown 1941). Vegetation on the mountain includes three physiognomic regions: deciduous hardwood forest, evergreen coniferous forest, and alpine tundra (Reiners and Lang 1979). Fir needles were

[1]Present address: Department of Botany, Duke University, Durham, NC, U.S.A. 27706.

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sampled at four elevations along the Gorge Brook Trail on the southwestern side of the mountain. The lowest elevation samples (732 m) were collected at the transition between the deciduous and coniferous forest. Samples were also collected from a subalpine spruce–fir forest (1143 m), the middle of the "Kampfzone"[2] (1402 m), and from exposed fir Krummholz at the tree line (1455 m). At the upper two elevations fir is overwhelmingly dominant.

First-year needles produced during the 1981 growing season were sampled at three times during the following winter. Duplicate samples of the last 2 years growth were cut from between 5 and 15 trees at each elevation depending on the experiment. Unfortunately, severe weather prevented us from collecting tissue from Krummholz (1455 m) for the transpiration experiment and some of the anatomical measurements. Branches were chosen from vigorous dominant trees, and were cut with a pole cutter from unshaded canopy having a southwest exposure. The samples were then immediately sealed in plastic bags and put into a cooler for transport back to the laboratory.

Tissue for anatomical study was prepared in the following manner. One 1st-year branch from each of four trees at each elevation was chosen. Ten needles were selected from the center of each branch providing a sample of 40 needles for each elevation. The tissue was fixed in cold Formalin – acetic acid – alcohol, dehydrated in a *tert*-butyl alcohol series (Berlyn and Miksche 1976), and embedded in paraffin. Using a cryotome at −25°C, 15-μm sections were cut one-third back from the needle tip. Sections were mounted on slides and the cuticle was stained for 1 h with Sudan III in ethylene glycol (Jensen 1962). Prior to staining, the tissue was immersed in 10% chromic acid for 10 min to facilitate differentiation between the cuticle and the cutinized cell wall (Baig and Tranquillini 1976). Cuticle and cutinized cell wall thickness were measured with an ocular micrometer at 400× under oil immersion. Measurements were made at the center of an epidermal cell halfway between the needle margin and the midneedle groove on the adaxial surface. The degree of hypodermal development was also assessed.

Epicuticular wax on several randomly selected needles from 732 and 1455 m was examined with an ETEC Autoscan scanning electron microscope. Care was taken in handling the tissue to avoid damaging surface waxes. The needles were dried for 24 h in a desiccator under mild vacuum and were coated with gold–palladium (60:40) in a Polaroid sputter coater. The sputter coater was modified by the addition of a concentric magnet behind the cathode to confine the discharge and reduce heating of the specimen. The needle surface was examined at an accelerating voltage of 10 kV and lens current of 1.8 A.

Stomatal slit length and density were measured from negatives taken of the stomatal strip on the abaxial surface of between 20 and 43 randomly selected needles from each elevation. Epicuticular wax was removed by dipping the needles in chloroform and photographs were taken one-quarter back from the needle tip at 110× using Leitz Ultropak incident light optics. The 35-mm negatives were projected onto an enlarging easel and measurements were made directly from the easel. The easel was also fitted with a 10 × 10 grid for sampling the stomatal density within the stomatal strip.

The relative quantity of epicuticular wax per gram dry weight of needles collected from each elevation was determined with a colorimetric assay developed by Ebercon et al. (1977). Each sample consisted of 25 needles from the center of a 1st-year twig. Ten twigs from each elevation were used. Each sample was immersed in 10 mL of redistilled chloroform for 45 s to remove the surface waxes. The wax extract was then filtered through Whatman No. 1 filter paper into a 25 mL test tube, and the chloroform was removed by evaporation in a hot water bath. A quantitative color change was produced by the addition of 5 mL of an acidified $K_2Cr_2O_7$ reagent to each test tube. After cooling, 12 mL of deionized water was added and 10 min were allowed for color development. Optical density was then read at 590 nm. A standard curve was generated by using polyethylene glycol 400.

The rate of cuticular water loss was measured gravimetrically. Samples of between 11 and 15 first-year twigs from each of the lower three elevations were recut under degassed distilled water immediately after returning from the field. They were then placed upright into test tubes of distilled water and left in a humidified chamber under diffuse low light for 72 h prior to the experiment. At the beginning of the experiment the twigs were removed from the water, blotted dry, weighed, and placed horizontally on screen racks in a growth chamber. The twigs were reweighed periodically over the next 14.5 h. The intensity of photosynthetically active radiation and temperature in the chamber were 0.59 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 26.5°C, respectively. Relative humidity in the chamber was not controlled and rose from 15 to 22% by the end of the experiment.

The data collected for each elevation were grouped and the means were compared using a single factor analysis of variance (ANOVA) and the Student–Newman–Keuls multiple-range test (Zar 1974).

Results

The cuticle on balsam fir needles forms a waxy layer of relatively uniform thickness over the abaxial and adaxial surfaces and circumscribes the entire needle (Figs. 1,2). The thickness of the cutinized cell wall is more variable and becomes thicker and thinner over the anticlinal and periclinal cell walls of the underlying epidermis (Fig. 4). Differentiation of hypodermal cells is observed in needles from all elevations; however, in no case was a continuous hypodermal layer found. The epidermis and distribution of hypodermal cells are readily visualized under partially polarized light. Hypodermal cells are typically observed in transverse section in the midrib and at both edges of the needle (Fig. 2).

Three different wax morphologies are found on the surface of an individual fir needle. On needles collected from the base of the mountain, a dense covering of epicuticular wax is associated with the five to eight parallel rows of stomata on either side of midrib (Fig. 7). The wax in the stomatal strip is composed of a dense lawn of entangled rodlets (Fig. 12) extending from the midrib approximately two-thirds of the way to the needle margin (Fig. 6). Small platelike regions can be seen in several areas over the wax rodlets (Figs. 7,9). It is apparent at high magnification that these plates are formed by coalescence of the subjacent rodlets (Fig. 10). On the needle margins and midrib the surface wax forms a laminate covering through which the outline of the underlying epidermal cells can be seen (Fig. 6). Isolated tufts of rodlets are occasionally seen on the laminate covering. Epicuticular wax on the adaxial surface is a combination of the laminate covering and tufts of rodlets. The density of the tufts is quite variable; however, they never become as dense as on the abaxial surface (Fig. 5). The stomatal antechamber of balsam fir needles is occluded with a dense plug of wax rodlets (Fig. 9). The guard cells are only visible after the wax has been removed by dipping the needles in chloroform (Figs. 3,11).

A dramatic difference in the wax morphology on the abaxial surface of several needles collected from the tree line was evident. The dense mesh of rodlets found between the stomata in the low-elevation needles is replaced by predominantly laminar wax at tree line (Fig. 8). However, typically low elevation wax morphology was also observed on some of the high-elevation needles.

The mean cuticle thickness of balsam fir needles collected along the elevational gradient from 732 to 1455 m decreased by over 25% (Fig. 13*a*). With the exception of needles from exposed Krummholz, the cutinized cell wall also decreased

[2]The Kampfzone is the transition zone between the closed stand (forest line) and the zone of severely stunted trees (Krummholz).

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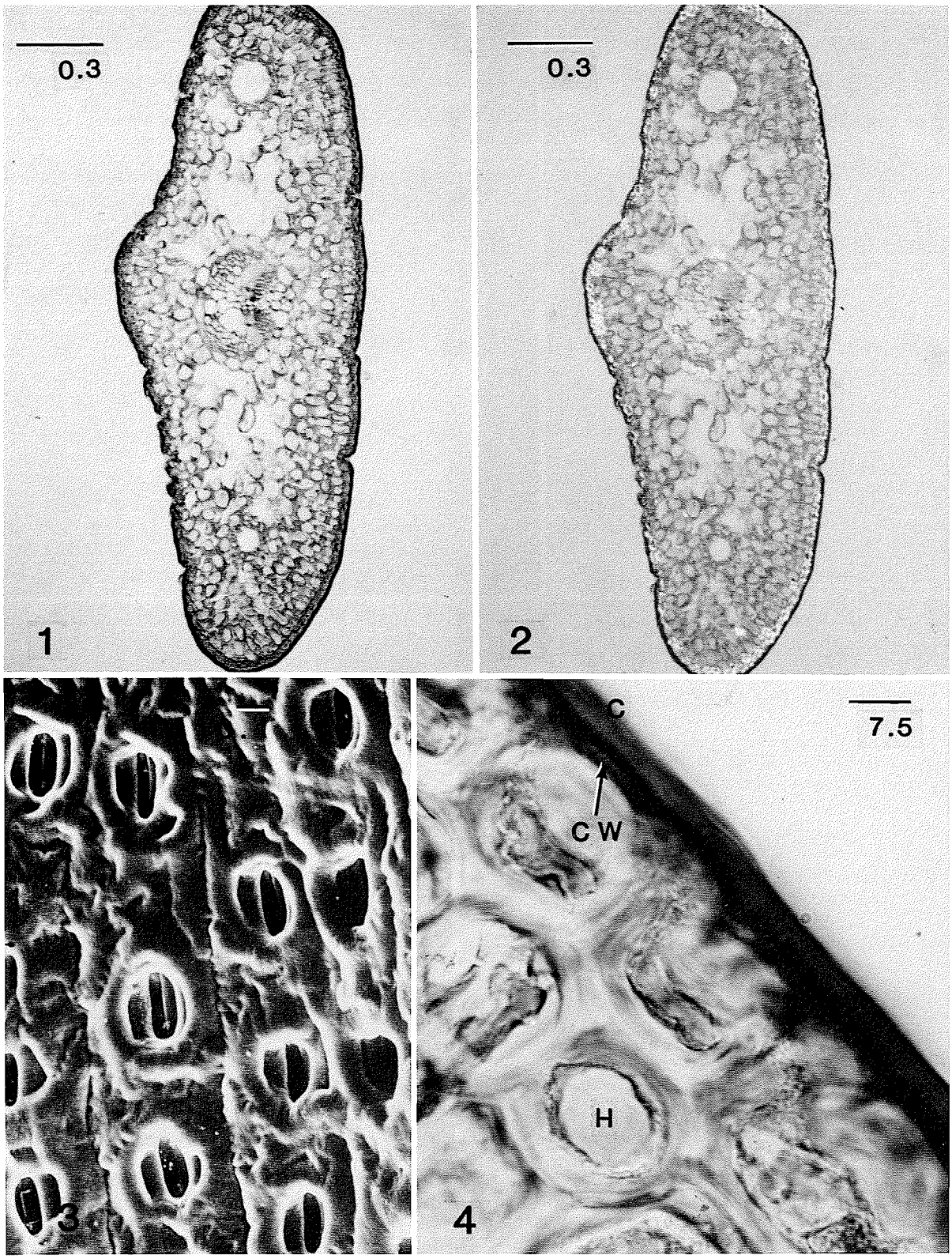
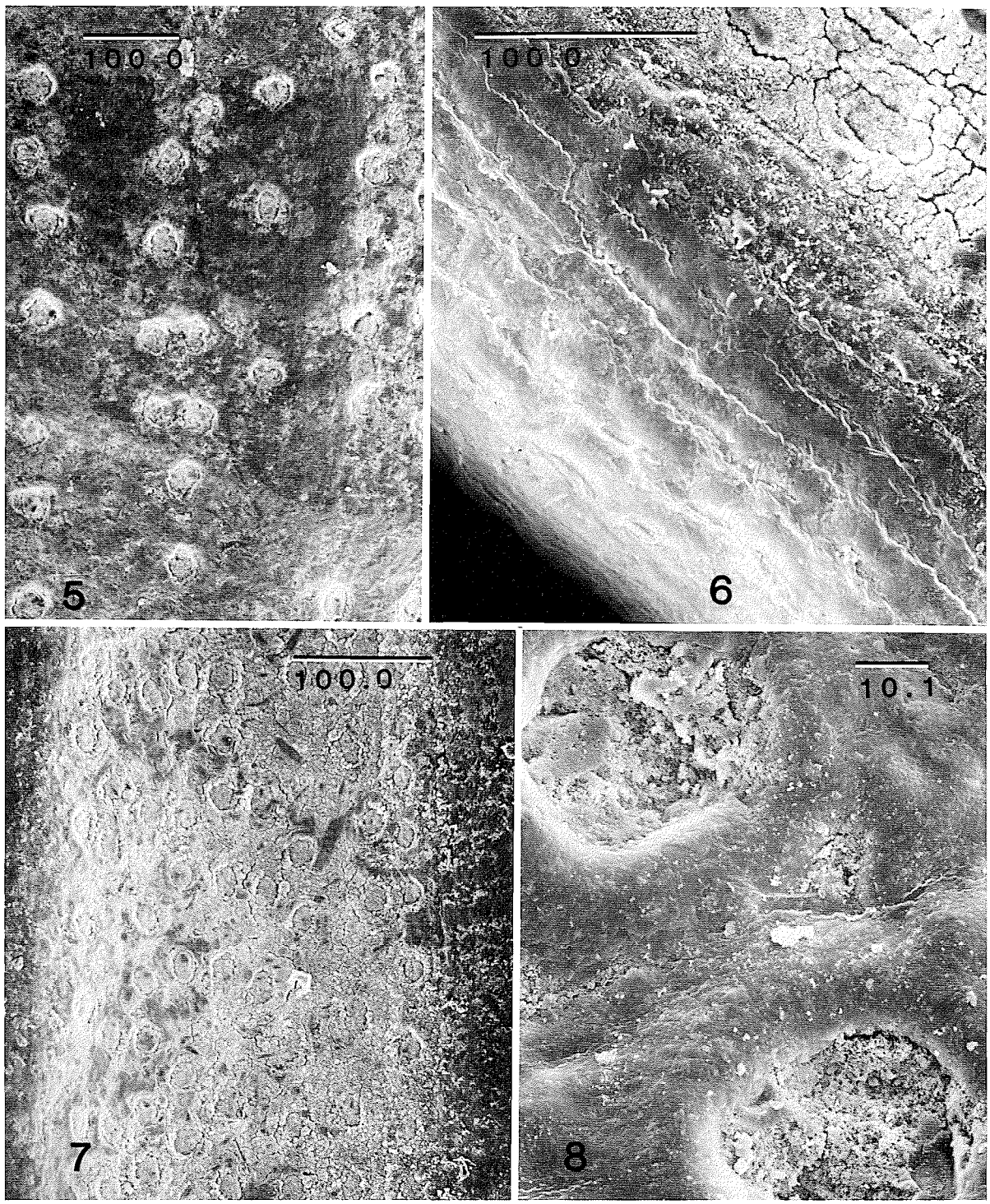


FIGS. 1–4. Morphology of the cuticle and epicuticular wax on 1st-year needles of *Abies balsamea*. The scale bars in Figs. 1 and 2 are in millimetres, those in Figs. 3 and 4 are in micrometres. Fig. 1. Section of fir needle one-third back from the needle tip. Fig. 2. Same as in Fig. 1, but under partially polarized light. Fig. 3. Dewaxed stomatal strip to the left of the midrib on the abaxial surface. Fig. 4. The cuticle (C), cutinized cell wall (CW), and hypodermal cell (H) on the abaxial surface.

with rising elevation (Fig. 13*b*). However, the thickness of the cutinized cell wall in needles collected from Krummholz vegetation was considerably greater than in needles collected from trees growing in the kampfzone only 53 m downslope and was also thicker than in needles from the base of the mountain. Because of the greater thickness of the cutinized cell wall in Krummholz needles, the total cuticular layer was thicker in Krummholz than in kampfzone trees (Fig. 13*c*). In



FIGS. 5–8. Morphology of the cuticle and epicuticular wax on 1st-year needles of *Abies balsamea*. All scale bars are in micrometres. Fig. 5. Stomata one-quarter back from the needle tip on the adaxial surface. Fig. 6. The needle edge on the abaxial surface. Fig. 7. Epicuticular wax in the stomatal strip. Fig. 8. Abaxial surface of a needle within the stomatal strip, collected from exposed Krummholz.

contrast, the relative amount of epicuticular wax per gram needle dry weight increased along the elevational gradient, as did the needle and stomatal density (Table 1). However, differences in the quantity of epicuticular wax were not statistically significant.

Transpiration was measured as percent decrease in fresh weight of excised 1st-year stems collected from the lower three elevations and is shown in Fig. 14. It was assumed that the stomata were fully open at time 0, and that the initial rapid rate of water loss from 0 to 120 min represents stomatal transpiration. The transition phase, during which the stomata were closing, and the cuticular phase, when the stomates were closed, were from 120 to 317 min and 317 to 857 min, respectively (definitions as in Hygen 1951). A regression equation of percent of initial fresh weight versus time during the cuticular phase was determined for twigs from each elevation. The slopes of the regressions for 732, 1143, and 1402 m were -0.81×10^{-2}, -1.07×10^{-2}, and -1.40×10^{-2}, respec-

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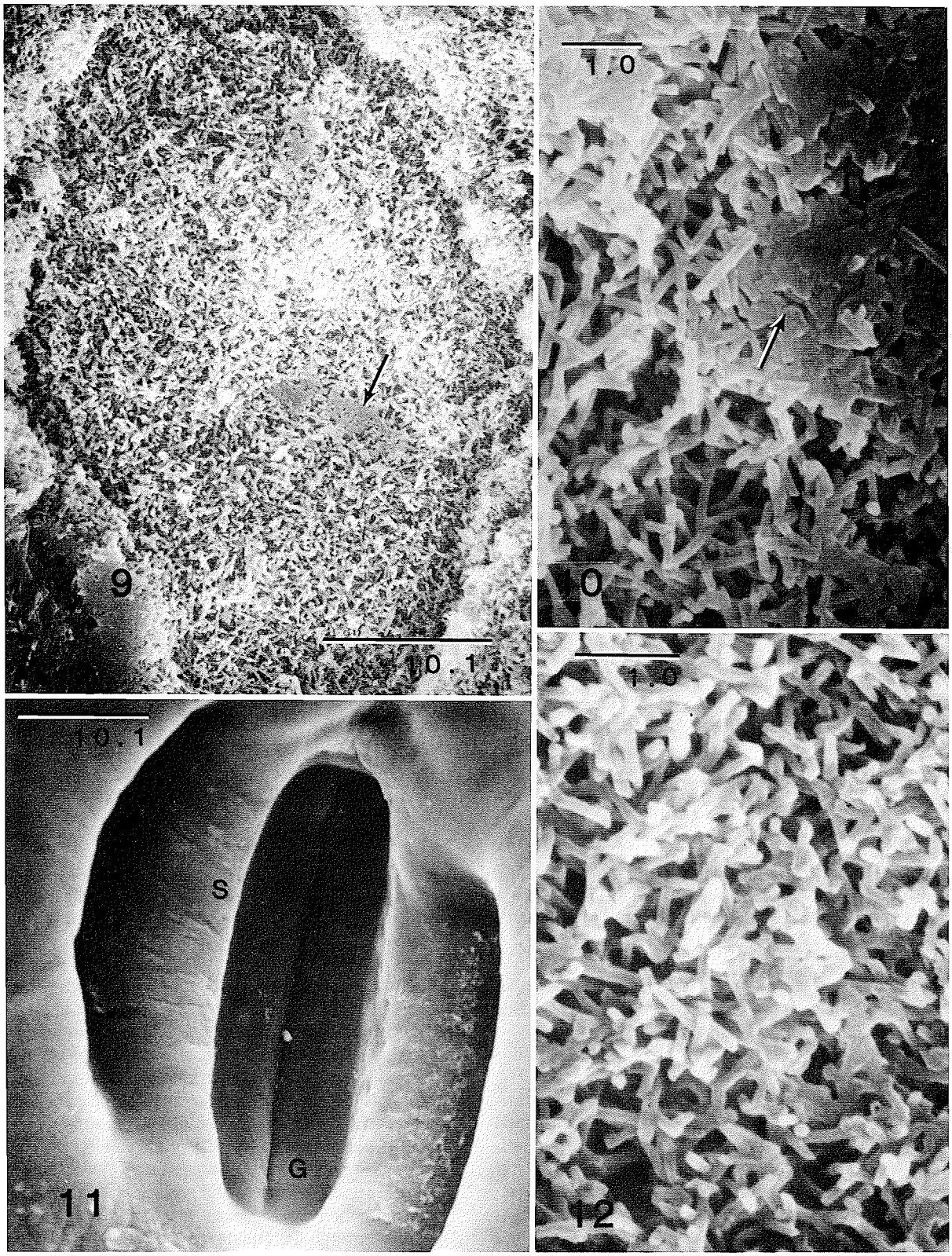

FIGS. 9–12. Morphology of the cuticle and epicuticular wax on 1st-year needles of *Abies balsamea*. All scale bars are in micrometres. Fig. 9. Stomate on the abaxial surface occluded with epicuticular wax. The arrow points to a coalesced wax plate. Fig. 10. Higher magnification of the wax plate in Fig. 9. Fig. 11. Abaxial stomate with wax removed from the stomatal antechamber. G, guard cells; S, subsidiary cells. Fig. 12. Interstomatal wax morphology on the abaxial surface.

tively. The slopes for tissue collected at 732 and 1402 m were significantly different ($p = 0.01$) indicating that the rate of cuticular transpiration increased with rising elevation up to 1402 m. The rate of water loss on a per gram dry weight basis was also calculated (Fig. 15). The mean rate of water loss during the stomatal and cuticular phase in tissue from each elevation was compared with a single-factor ANOVA. Needles from 1402 m showed a higher rate of water loss per

TABLE 1. Needle morphology of *Abies balsamea* along the elevational gradient

Morphologic traits	732 m	1143 m	1402 m	1455 m	Level of significance (p)
Needle length, mm	19.6±0.49*a* (270)	16.9±0.38*a* (270)	13.8±0.35*a* (296)	13.1±0.30 (270)[a]	0.001
Needle density, no. of needles/mm stem	2.06±0.19*a* (8)	2.26±0.30 (8)	2.51±0.23 (8)	2.87±0.51 (9)[a]	0.025
Stomatal slit length, μm	2.43±0.07*a* (94)	2.27±0.07*b* (104)	2.17±0.06*ab* (95)		0.001
Stomatal density, no. of stomata/mm^2	163.26±5.54*a* (43)	169.80±11.70 (20)	174.79±8.47*a* (41)		0.1
Epicuticular wax, g wax/g dry weight	0.0251±0.0023 (10)	0.0263±0.0029 (10)	0.0278±0.0081 (10)		ns

NOTE: Values are means ± 2 SE; sample size is in parentheses. Values followed by the same letter are significantly different at the probability level shown in the last column. ns, not significant.

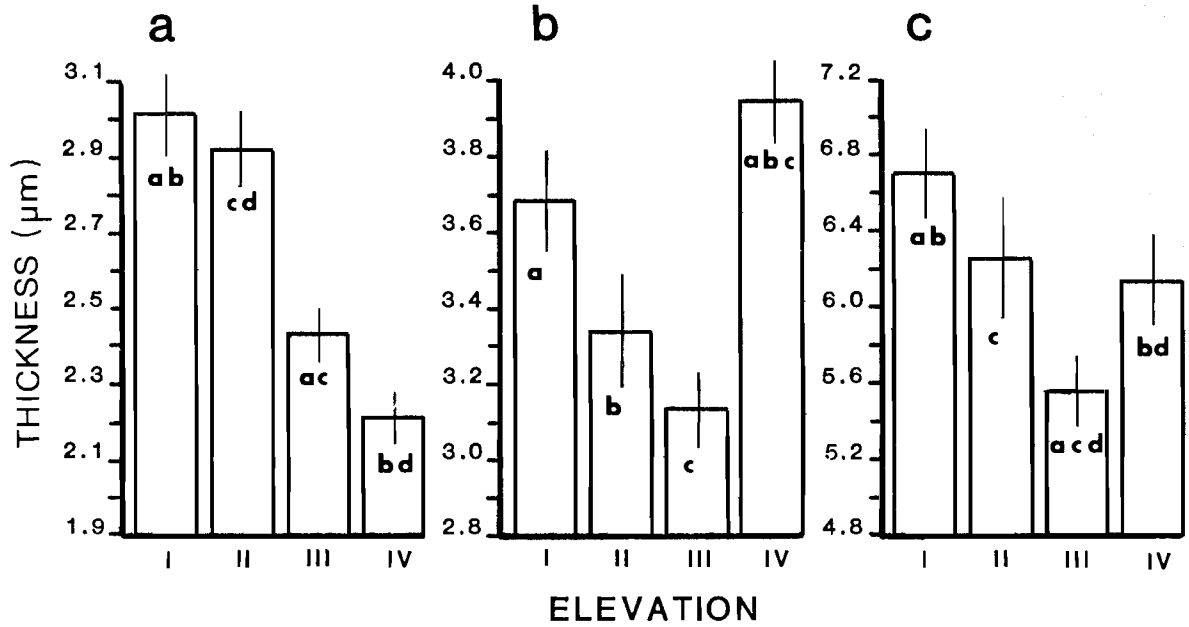

FIG. 13. The thickness of (*a*) the needle cuticle, (*b*) the cutinized cell wall, and (*c*) the total cuticular layer in *Abies balsamea* along the elevational gradient. Error bars represent ± 2 SE and $n = 40$ for each elevation. Bars designated by the same letter are signifcantly different at the $P = 0.001$ level, except for I and IV in Fig. 13*b* which are different at the $P = 0.1$ level. The elevations are as follows: I, 732 m; II, 1143 m; III, 1402 m; IV, 1455 m.

gram dry weight during the stomatal ($p = 0.5$) and cuticular phase ($p = 0.005$) than tissue collected from the lower two elevations.

Discussion

A great many environmental factors contribute to a shortening of the growing season with rising elevation. A contracted growing season, in addition to frequently unfavorable conditions during the growing season, limits the time available for carbon assimilation and maturation in subalpine trees. Although no phenological data were collected, budbreak for balsam fir growing at tree line on Mt. Washington occurs in June, a full 2−3 months later than in trees growing at a lower elevation in a cool greenhouse (Chabot and Chabot 1975). In this study, the shortening of the growing season with increasing elevation is evident in the decrease in cuticle thickness and needle, guard cell, and twig length in balsam fir growing along the elevational gradient. There is no evidence that fir is able to partially compensate for the short duration of the growing season at high elevations by occasional winter photosynthesis. In fact, Chabot and Chabot (1977) showed that profound ultrastructural changes involving the chloroplasts accompany the onset of dormancy and there was no evidence of starch accumulation in the chloroplasts from November until March.

In this investigation a clear relationship between leaf cuticle thickness, elevation, and the rate of cuticular water loss was observed. Cuticle thickness decreased and cuticular transpiration increased with rising elevation. This strongly suggests that the thickness of the leaf cuticle is extremely important in determining the rate of winter transpiration. While the relationship between cuticle thickness and permeability has come into question (Schonherr 1976), recent results indicate that over 50% of the flux through the cuticle can be accounted for by thickness alone (Reed and Tukey 1982). Furthermore, our results are consistent with those found for *Picea abies* and *Pinus cembra* in the Austrian Alps (Baig and Tranquillini 1976).

Sowell et al. (1982) also found a decrease in cuticle maturation in *Pinus albicaulis* growing at tree line. They measured seasonal changes in cuticular resistance *in situ* and found that the resistance began to increase later in the growing season in Krummholz trees and never attained the same level as trees growing at the forest limit 210 m downslope. In contrast to the

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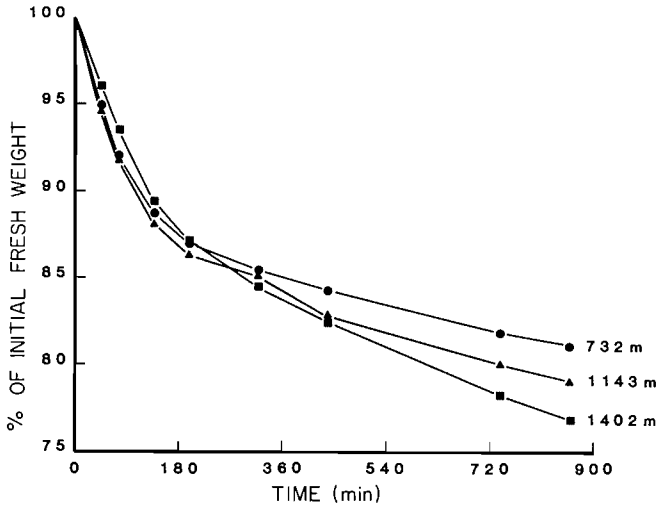

FIG. 14. Water loss from 1st-year twigs of *Abies balsamea* measured as the change in fresh weight as a percent of initial fresh weight over time. Each point represents the mean percent of initial fresh weight of 14, 11, and 15 samples from 732, 1143, and 1402 m, respectively.

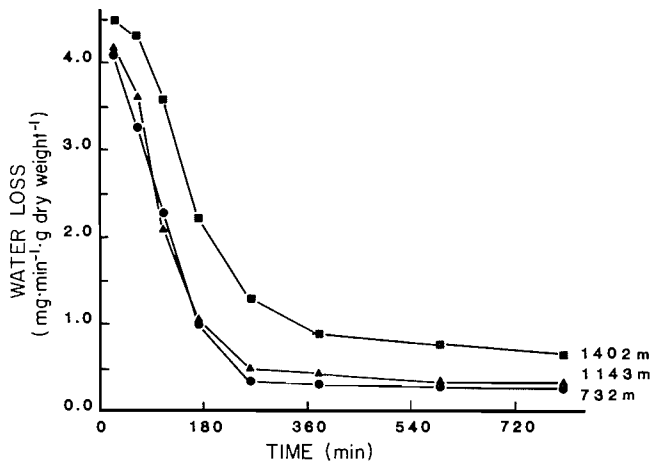

FIG. 15. The rate of water loss per gram dry weight of needles from excised 1st-year twigs of *Abies balsamea*. Each point represents a mean rate for that time interval and was plotted at the center of the interval. Means were calculated from 14, 11, and 15 samples from 732, 1143, and 1402 m, respectively.

trend observed for the cuticle, we found a marked increase in the thickness of the cutinized cell wall in balsam fir needles collected from Krummholz vegetation. The thickness of the cutinized cell wall decreased with rising elevation up to the forest limit and then increased dramatically in Krummholz trees. The increase in thickness occurred in only a 53 m rise in elevation and may represent an adaptation of Krummholz trees to the severe conditions prevalent above the forest limit.

Our data for excised stems suggest that the rate of cuticular transpiration is considerably greater for trees growing at high elevations up to the forest limit. Although water is generally not limiting during the summer in the White Mountains, frozen stems and soil can greatly reduce its availability during the winter. In a year with little snowfall, the soil on Mt. Moosilauke remained frozen from the middle of November until the beginning of May (Fahey and Lang 1975). High rates of cuticular transpiration during the winter when the availability of water is reduced can lead to dangerously low water potentials in subalpine trees (Lindsay 1971; Hansen and Klikoff 1972; Barclay and Crawford 1982). High wind speeds further contribute to the increased rate of cuticular water loss from exposed tissue growing near tree line (Hadley and Smith 1983) and can also lead to deterioration of the cuticle by ice blast and actual mechanical breakage.

Even when stems are frozen, limited water movement continues (Hygen 1965; Havis 1971). However, when climatic conditions produce unusually high evaporative demands, such as on a clear winter day having low relative humidity, high irradiance, and high wind speed, the rate of cuticular water loss can greatly exceed transport to the leaves. Sakai (1970) suggested that these conditions were responsible for the extensive desiccation damage to spruce and fir overwintering in areas of Hokkaido, Japan, where soils remained frozen for extensive periods of time.

It is important to recognize that periodic measurements of needle water potential at high elevations do not always indicate the development of water stress during the winter. Marchand and Chabot (1978) studied balsam fir on Mt. Washington and found no evidence of a seasonal decline in relative water content of tissue sampled near tree line. In view of the intermittent nature of days which produce a large vapor pressure deficit and assuming a slow but continuous replacement of water, it is reasonable to expect that exceedingly low water potentials can occasionally develop during discrete periods in the winter and early spring. Under these circumstances measuring water potentials at fixed intervals during the winter may not provide evidence of winter water stress.

The epidermis of balsam fir needles is composed of a series of small, slightly rectangular, thick-walled cells. Chabot and Chabot (1977) observed a well developed hypodermal layer of thick-walled cells lying directly below the epidermis. It is believed to provide the needle with additional structural support and protection against cuticular transpiration (Busgen and Munch 1929). In this investigation a continuous hypodermal layer was not observed in tissue from any of the four elevations sampled. The absence of a continuous hypodermis may be a result of the position on the needle from which sections were taken. Bazukis and Hansen (1965) reported that a hypodermis is rarely found in cross sections taken above midneedle.

The needles of many conifers including balsam fir have sunken stomates. The guard cells are suspended below the epidermis by specialized subsidiary cells. Chabot and Chabot (1977) found that the depth of the stomatal antechamber formed by the subsidiary cells increased with increasing elevation. In addition, the stomatal antechamber of balsam fir needles is occluded with a dense mesh of wax rodlets. Jeffree et al. (1971) calculated that the combination of a wax plug and sunken stomates functions as a very effective antitranspirant. Although the above-mentioned morphological features potentially reduce the rate of stomatal transpiration, in our study the needles collected from the forest limit had higher rates of stomatal and cuticular transpiration per gram dry weight than tissue collected at lower elevations. This higher rate of stomatal water loss is probably a result of the greater number of stomates per unit surface area in high-elevation needles. This higher rate of stomatal water loss is probably a result of greater number of stomates per unit surface area in high-elevation needles. Depending on the geometry and distribution of pores, a greater number of small stomates potentially lead to an increased efficiency of diffusion (Bidwell 1974). Au (1966) found that alpine

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populations of *Oxyria digyna* had a greater stomatal frequency than arctic populations, and suggested that this difference accounted for the higher rate of photosynthesis observed in the alpine populations. An increased capacity for gas exchange per unit leaf surface area would be an important adaptation of high-elevation plants confronted with a greatly shortened growing season. Fryer and Ledig (1972) found that balsam fir on Mt. Moosilauke adapted to increasing elevation by having a lower temperature optimum for net photosynthesis at high elevation.

Epicuticular wax also plays an important role in reducing water loss from plant tissue. Hall and Jones (1961) found mechanical removal of surface wax from *Trifolium* leaves dramatically increased the rate of cuticular transpiration. Though no statistically significant difference was found, our data suggest that the amount of epicuticular wax per gram dry weight of needle increases with increasing elevation between 732 and 1402 m. A significant increase in the quantity of surface wax in trees growing at tree line has been demonstrated for other species (Gunthardt and Wanner 1982). The quantity of epicuticular wax has been shown to increase in response to a decrease in soil moisture and large vapor pressure deficits (Hunt and Baker 1982). Artificially induced water stress and high light intensity also influence wax chemistry and morphology (Baker 1974; Bengston et al. 1978). Our data suggest that the quantity of epicuticular wax on balsam fir needles increases along the elevational gradient. Although a greater amount of wax on the needle surface would probably reduce transpiration, the physiological significance of these changes remains to be tested.

In this investigation we demonstrate that cuticle thickness of balsam fir needles decreases and the rate of cuticular water loss increases along the elevational gradient. The observed changes in leaf morphology are largely a manifestation of the shortened growing season at high elevations. Our findings support the hypothesis proposed by Wardle (1971) and Tranquillini (1979) for the formation of the alpine tree line.

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