FIRST RESULTS ON CHEMICAL COMPOSITION DIFFERENCES OF CUTICLES FROM *PINUS UNCINATA* MILL. NEEDLES BETWEEN FOREST AND TREELINE POPULATIONS

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RESUMEN.—La brevedad del verano en los niveles más altos del piso subalpino, es considerada en la actualidad, la causa última de la existencia de un límite superior para las especies arbóreas. Las hojas con cutículas desarrolladas de forma insuficiente se ven sometidas, durante el siguiente invierno, a un exceso de transpiración cuticular, lo que puede conducir a su muerte por desecación. En el presente trabajo, se compara la cantidad relativa de ciertos compuestos grasos que forman parte de las "ceras" cuticulares en ejemplares de pino negro (*Pinus uncinata*) situados en el interior del bosque y cerca de su límite altitudinal. Se observa un incremento de ácidos, alcoholes y ésteres grasos en las hojas de las poblaciones más altas, discutiéndose la relación que este hecho puede guardar con los elevados valores de transpiración cuticular medidos en brotes pertenecientes a árboles del *treeline*.

SUMMARY.—The short growing season in high subalpine levels is considered the reason for the existence of an alpine treeline. Needles with "unripe" cuticle are subjected to excesive cuticular water loss during the next winter and it can produce the death by desiccation. The present study tries to compare the relative proportions of certains fatty compounds of the cuticle in leaves belonging to exemplars of "mountain pine" (*Pinus uncinata*) growing within the forest and near their upper limit. An increase of fatty acids, alcohols and esters in needles of the treeline populations is observed. The relation between this fact and the high cuticular transpiration values in shoots of trees growing at the upper limit is discussed.

INTRODUCTION

The winter desiccation damage is considered to be the limiting factor in controlling the upper limit of trees on high mountains (WARDLE, 1971). As a result of low temperatures, water lost by transpiration is not adequately replaced, because already at temperatures slightly above the freezing point, the supply by roots is insufficient (EBERMAYER, 1901). This increase in root resistance to water uptake at low temperatures is due to changes in root membrane permeability (BABALONA *et al.*, 1968) and to the greater viscosity of the water (KAUFMANN, 1977). The strong heating of branches resulting from the

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high solar radiation during late winter accelerates transpiration, even the stomacal closing, via cuticle. It causes serious water stress on trees living under such conditions.

The former circunstances indicate the importance of cuticular resistance to water losses in the alpine treeline. Differences in the rate of cuticular transpiration of twigs of *Picea abies* and *Pinus cembra*, from various habitats along the altitudinal axis of Patscherkofel Mt. were found in the Alps. (BAIG *et al.,* 1974). Samples from trees growing at their upper limit shew higher cuticular trasnpirations than those of valley bottom. These differences were related to the thickness of cuticle and cutinized cell wall layer of the outer epidermal cells in a later study of the same author (BAIG & TRANQUILLINI, 1976). These structures were thinner on trees living at high altitude than those at the valley botton or at forestline. The cold and short growing season was considered the reason for this difference because, in such an environment, needles do not become fully mature.

However, the thickness of cuticle is not the only factor limiting cuticular transpiration. There is a high relationship between permeability of the membranes and their chemistry and structure (SCHÖNHERR, 1976).

Plant cuticle is composed mainly by cutin, a mesh-work of hydroxy fatty acids, and waxes (KOLATTUKUDY, 1970). There are nonlipid compounds too, such as cellulose, polyuronic acids, proteins and phenolic substances (MARTIN & JUNIPER, 1970). All this components can be classified into two fractions after a treatment of the isolated cuticles with lipid solvents (SCHÖNHERR, 1981):

Soluble cuticular lipids (SCL): waxes; easily extracted with hexane or chloroform.

Polymer matrix (MX): cutin and nonlipid compounds; the insoluble remainder non extracted with these solvents.

The extraction of cuticular waxes (SCL) of Citrus leaves cuticle increases water permeability by a factor of 300 to 400, while cuticle thickness does not decrease likewise (SCHÖNHERR, 1976). So, soluble lipids must be carefully studied in order to understand any change in functionality of this membrane.

Many studies related with the composition of cuticular lipids have been performed, some of them from a phytochemical approach (HOLLOWAY & CHALLEN, 1966); DYSON & HERBIN, 1968; TULLOCH, 1975; LAKSHMINARAYANA *et al.,* 1988) and others with regard to their physiological significance (SKOSS, 1955; GRNCAREVIC & RADLER, 1966; NODSKOV, 1974). After these studies the chemical composition of cuticular waxes can be defined as a mixture of fatty substances, mainly free fatty acids, alcohols, esters and hydrocarbons.

The relative proportions of the differents compounds may affect the permeability of cuticle. HAAS & SCHÖNHERR (1979) found permeability to be higher in Citrus laeves membranes when fatty acids and alcohols were more abundant than alkanes and other more hydrophobic compounds. This was related to the existence of polar pores in the barrier formed by SCL. SUTTER (1984, 1985) also found differences between waxes of plants growth "in vitro" and those in greenhouse. The first ones had proportionally more esters, fatty acids and primary alcohols. The study tried to explain the high mortality, owing to excessive desiccation, of plants grown "in vitro" when they are transferred to conditions of higher dryness. The reason seems to be the high permeability of cuticles when "polar" substances predominate.

The present study is aimed to apply these previous works in the understanding of trees existence at their upper limit. Fatty acids, alcohols and esters were analyzed in needles of *Pinus uncinata* taken within forest and from upper limit populations, looking for relative differences in chemical composition.

MATERIAL AND METHODS

Samples

Twigs of *Pinus uncinata* were collected in early winter of 1987 in Sierra de las Cutas (Ordesa National Park) and in Aguas Limpias River valley (Sallent de Gállego). The sampling sites were chosen because of their natural conditions. Two populations of each area were chosen, one within the forest and the other in the pine upper limit; their altitudes and other physical characteristics are presented in the table below. Twigs were taken from ten differents trees, randomly selected, of each sampling side. Needles of current year were selected and maintained in a freezer, waiting for analysis.

Analitical procedures

All of them were performed in the *Instituto de la grasa y sus derivados* (C.S.I.C.), Sevilla, under the personal direction of Prof. Dr. Vioque Pizarro and according to the methods employed by others workers cited above.

Some leaves of each sample were weighed and carried to dryness at 110 in a muff to estimate their relative water content. The remainder was weighed too and its lipids extracted three times by immersing the leaves in 150 ml. of chloroform at ambient temperature. The extracts were concentrated in a rotary vacuum evaporator and, after that, redisolved with 5 ml. of chloroform. All values of weight and relative water content are included in the table.

Preparative thin-layer chromatography was employed to obtain fractions of fatty acids, alcohols and esters, with 0,25 mm. silica gel 60 G as adsorbent. Standars employed were stearyl-erucate for esters, linoleic acid for fatty acids and linoleic alcohols for such class of compounds. 400 micro liters of each extract were applied and developed with hexane: ethylic ether: formic acid (80:20:1). Bands were visualized with iodine, scraped from the plate and the compounds were eluted with ethylic ether.

Fatty acids were methylated with diazomethane alcohols acetylated with acetic anhydride, prior to analysis, to obtain its esterified derivates. Quantitative estimation of resultant and natural esters were made according to the method by VIOQUE & HOLMAN (1962), by color reaction and measure of absorbance at 520 nm.

RESULTS AND DISCUSSION

Values included in the table indicate that cuticular waxes from trees growing at their upper limit have more "polar" compounds (alcohols and fatty accids) and esters, per g. of needle dry weight, than those belonging to lower localities. Measures of cuticular transpiration must be done in such populations, in order to related changes in chemical composition with differences in functionality. However, since higher values of cuticular permeability were found by BAIG *et al.* (1974) in Patscherkofel Mt., functionals differences may be expected. Therefore, these previous results may be in agreement with those of SUTTER (1984, 1985) on one hand and HAAS & SCHÖNHERR (1979) on the other: the higher the proportions of "polar" compounds, the lower the cuticular resistence.

Studies on chemical composition of cuticle may not only contribute to understand its functionality. Moreover, they could also explain onthogenetics changes along growing season, and the interactions between of biosynthetics processes and the environmental factors. As explains KOLATTUKUDY (1970), synthesis of cuticular waxes takes place in the epidermal cells as precursor of fatty acids. From these, they can derivate into hydrocarbons, by decarboxylative pathway; aldehydes, alcohols and esters by reductive pathway or remain as free fatty acids. The increment of "polar" compounds in cuticle of upper limit trees, could indicate a higher activity of reductive pathway? Is this affected by the shortness of growing season, or by differences in environmental factors such as temperatures?.

Perhaps the knowledge of these topics could contribute to the understanding of the ecophysiological reasons of the upper treeline.

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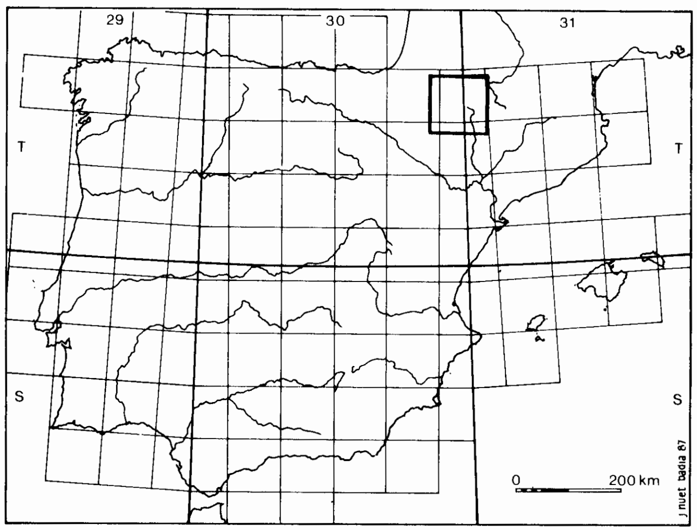
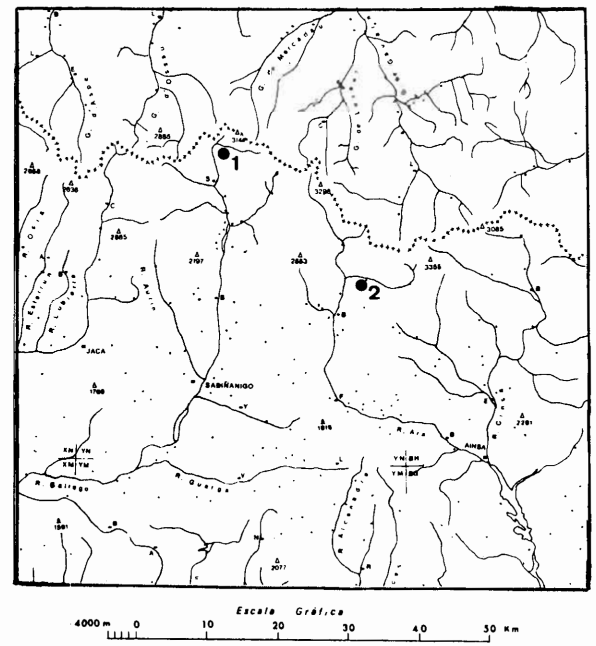
Characteristics of the sampling sites and Results of the Chemical Analysis.

	"AGUAS LIMPIAS" FOREST	"AGUAS LIMPIAS" UPPER LIMIT	"S. CUTAS" FOREST	"S. CUTAS" UPPER LIMIT
ELEVATION (m.a.s.l.)	1500	2450	2140	2200
SUBSTRATUM	GRANITIC	SLATY	CALCAREOUS	CALCAREOUS
ASPECT	E–NE	S	S	S
N.F.W. (g.)	27.15	25.92	17.93	9.23
N.D.W. (g.)	12.71	13.57	9.42	4.51
ESTERS. (µe./g.n.d.w.)	2.84	3.47	4.19	9.03
FATTY ACIDS. (µe./g.n.d.w.)	1.06	2.42	3.09	9.01
ALCOHOLS. (µe./g.n.d.w.)	2.21	3.23	4.77	10.36

Abbreviations: N.F.W., needle fresh weight; N.D.W., needle dry weight.





Location of the sampling areas:
1. "Aguas Limpias" river. 2. "Sierra de las Cutas".