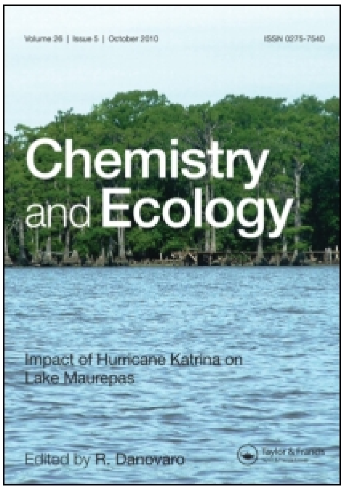

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Incipient Forest Decline in the Province of Trento (Northern Italy). Preliminary Sem Observations and Consideration of Inorganic Components of Leaves and Roots

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INCIPIENT FOREST DECLINE IN THE PROVINCE OF TRENTO (NORTHERN ITALY). PRELIMINARY SEM OBSERVATIONS AND CONSIDERATION OF INORGANIC COMPONENTS OF LEAVES AND ROOTS

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(31 July 1991)

One year old needles of both healthy and damaged silver fir and Norway spruce from the area of Trento (Northern Italy) were observed by SEM. Needles of damaged trees show quite evident structural alterations of epidermis, expecially epicuticular waxes. Roots and needles of both fir and spruce are also tested for their metal content. Evidence is reported of an increasing concentration of Fe, Mg and Ca in roots of plants grown on alkali soil. Ca, and to a lesser extent Fe, Al and Pb, appears to be more present in old needles, whereas K and Mg exhibit higher amounts in young needles. Some indications are found between damage and the content of some metals such as Mg and Mn in needles.

KEY WORDS: Forest decline, spruce, fir, SEM observations, nutritional status.

INTRODUCTION

Forest decline has been widely observed in Central Europe since the mid 70s (Schütt and Cowling, 1985). In recent years damage to vegetation has been detected and assessed by large-scale survey of several Italian areas, even for Mediterranean species (Clauser *et al.*, 1988; 1989); however, *Abies alba* Mill. and *Picea abies* (L.) Karst. appear to be the most affected species (Fassi and Falcini, 1987). It was pointed out that in the Tuscan Apennines damage to forest vegetation could be ascribed to environmental pollution (Clauser and Gellini, 1986), acid deposition being among the most significant causes (Pantani *et al.*, 1985; Barbolani *et al.*, 1988; Udisti *et al.*, 1990).

Reflecting the needle's cuticle as the interface between air and plants, it has become evident that atmospheric pollutants can interact strongly with epicuticular waxes; several experimental and in-field studies used leaf surface alteration (detectable by Scanning Electron Microscopy) as a specific diagnostic indicator of pollution damage (Rinallo *et al.*, 1986; Sauter and Voss, 1986; Gellini *et al.*, 1987; Grill *et al.*, 1987; Manes *et al.*, 1987; Sauter *et al.*, 1987; Cape, 1988; Fink, 1989). According to Huttunen (1988), tree damage could be correlated with changes in the morphological structure of needle epidermis and/or with the concentration of several chemical components in the plant (Wittenbach *et al.*, 1985; Bonneau, 1988; Rothe *et al.*, 1988; Ferretti *et al.*, 1990). Therefore, both these parameters could provide a valuable indicator of incipient tree disease in contaminated areas.

The present investigation is intended as a preliminary study, in order to evaluate both morphological changes in needle surfaces and the presence of significant factors which affect the chemical composition of leaves and roots, just at the beginning of visible injury.

This study is a part of a wider research programme in areas of the province of Trento, where forest decline has been detected. In this area, the rain, collected as bulk deposition for individual rain events, appears to be near neutral, pH ranging between 5 and 6.

Some rain in the district of Ala, at the southern boundary of the province of Trento, was found to contain free acidity, with pH around 4. However, independently of the pH of wet deposition, the forest canopy may act as a trap, retaining dry particles and gases from atmosphere (Halbwachs, 1989; Ojanperä and Huttunen, 1989). Prinz *et al.* (1982) refer to a combination of ozone, sulphur dioxide and acidic deposition as responsible for magnesium deficiency of spruce in North-Western Germany and this has been confirmed by other researchers (e.g. Zech *et al.*, 1983; Mengel *et al.*, 1987). Hence, it was thought interesting to evaluate the content of metals in plant tissue as well as the possible impoverishment of some nutrients due to leaching by acid deposition, in order to detect in advance the possibility of future damage.

MATERIALS AND METHODS

Needles (1 and 6–8 years old) and roots (<2 mm diameter) of both uninjured and injured trees (classes of damage: from 0 to III – see below) were collected in different forest stands of silver fir (*Abies alba* Mill.) and Norway spruce (*Picea abies* (L.) Karst.) grown on acid and alkali soil (Table 1) in the south-east side of the province of Trento (northern Italy) during September-October 1986 (Figure 1). Soil samples from the rhizosphere (indicative depth: 1 m) were also collected (see Table 1).

The assessment of tree damage has been carried out according to the methods currently in use, that is a comparison with standard trees of different classes of crown density reduction (Sanasilva, 1986). Classes of crown density reduction are defined as follows: class 0 (0–10% of crown density reduction), class 1 (11–25%), class 2 (26–60%), class 3 (>60%) and class 4 (dead tree). The overall assessment of damage (0, absent; I, slight; II, moderate; III, severe; IV, lethal) also includes discoloration (EEC Reg. 1696/87).

For SEM observations, samples of 1-year old needles were air-dried, mounted on aluminium stubs, sputtered in gold with JEOL JEE 4B and finally observed at Philips SEM 515 and/or JEOL JSM-U3 (operating conditions 15.0–15.2 kW).

For chemical analysis, needles (separated in 2 groups of different age: 1 year old and 6–8 years old) and fine roots (<2 mm diameter, collected 1.5 m from each tree and separated from the larger ones using an accurately water-washed steel blade) were washed with demineralized water to remove any dust particles present on the surface and then oven dried at 75°C for 72 hours. Wet digestion was carried out on 200 mg of plant material in 2 ml of Merck "Suprapur" nitric acid, pre-heating at 60°C for 30 min, then heating at 110°C until a clear solution is obtained. In some cases further addition of nitric acid and subsequent heating was required. After cooling the solution was quantitatively transferred in a 25 ml volumetric flask and brought to full volume with demineralized water.

Table 1 Characteristics of sampling sites. In the column "sampled tree" the numbers of the trees effectively sampled.

Sampling Site		*Soil*			*Characteristics of Samples*					
		pH	*Forest*			*Defoliation*				
Nr	*Locality*	*(range)*	*composition*		*Sampled trees*	*0*	*I*	*II*	*III*	*IV*
1	Tesero	n=4	spruce	99%	* 1		+			
	(1300 m)	4.3–4.9	larch	1%	** 0					
2	Val Cadino	n=2	spruce	77%	* 2	++				
	(1200 m)	4.4–4.7	fir	23%	** 2		++			
3	Val Cadino	n=4	spruce	97%	* 2		++			
	(1550 m)	4.3–4.9	fir	3%	** 0					
4	Centa	n=3	spruce	1%	* 1	+				
			beech	66%						
	(1200 m)	6.8–7.8	larch	33%	** 0					
5	Jungholz	n=4	spruce	30%	*3	+		+	+	
			beech	17%						
	(1250 m)	5.0–7.9	fir	53%	** 1			+		
6	Belem	n=4	spruce	60%	* 2		+	+		
	(1400 m)	4.9–8.2	fir	40%	** 1			+		

* Norway spruce, ** silver fir.

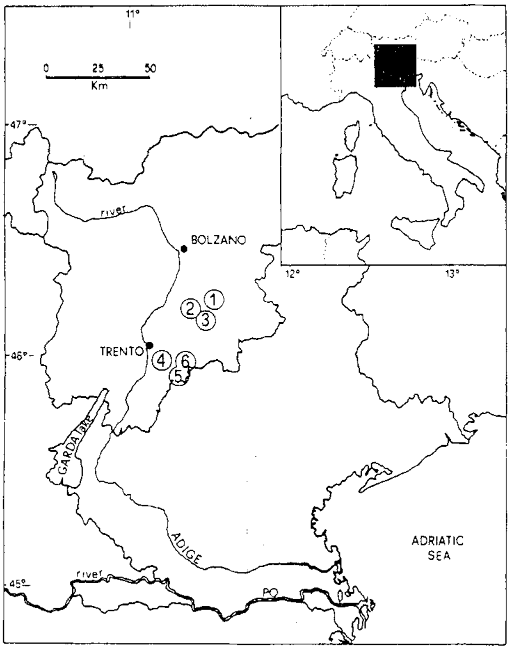

Figure 1 Location of sampling sites.

Metal determinations were performed by atomic absorption spectrometry (Perkin – Elmer mod. 2380), using a flame atomizer for sodium, potassium, calcium and magnesium or electrothermal atomization for heavy metals. A Metrhom mod. 605 pH meter with a combination glass electrode was used to measure the pH of soil (mesh 2 mm) in a 1:2.5 dispersion in water.

RESULTS

SEM Observations

In healthy trees, SEM observations show the intricate matrix and the fine organization of the epicuticular waxes; the normal shape of the waxy microtubules of the pre-stomatic chambers in one year old needles of both fir and spruce is also evident (Figure 2). Stomata show no structural alteration but, at higher magnification, the micro-morphology of the epistomatic waxes, consisting of a dense agglomeration of fine crystalloids, is clearly defined (Figure 3).

In contrast, the same-aged needles from damaged trees show a quite different condition; in spruce, epicuticular waxes appear to be disrupted, and the pre-stomatic waxy microtubules are degraded, melted and amorphously re-aggregated or frequently eroded. In Figure 4 the complete dissolution of the protective structure of the stoma is clearly detectable. Similar severe changes are evident in needles of damaged silver fir trees (Figure 5).

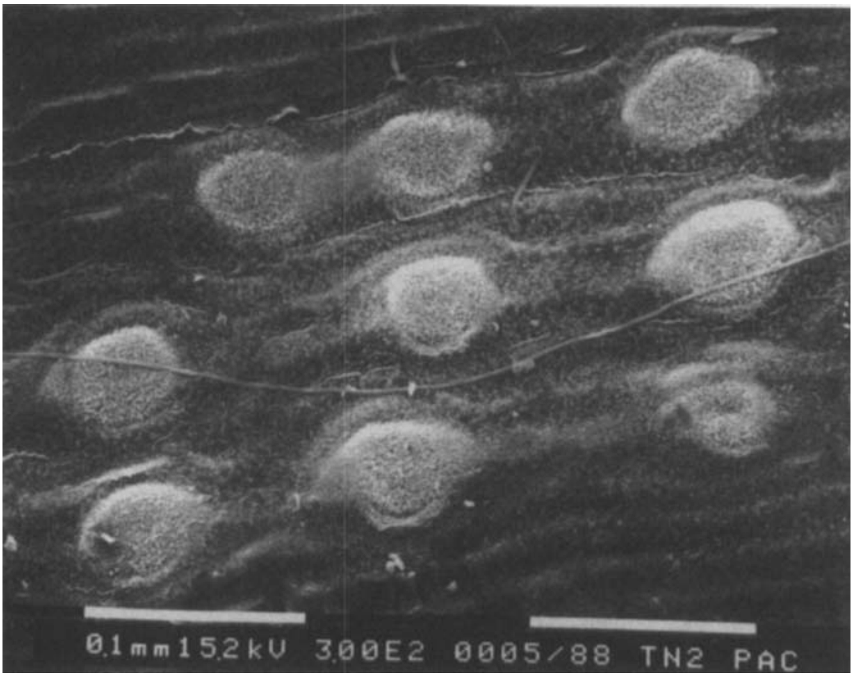

Figure 2 *Picea abies*, uninjured tree, 1-year old needle: stomata appear without alteration. Length of bar = 100 μm.

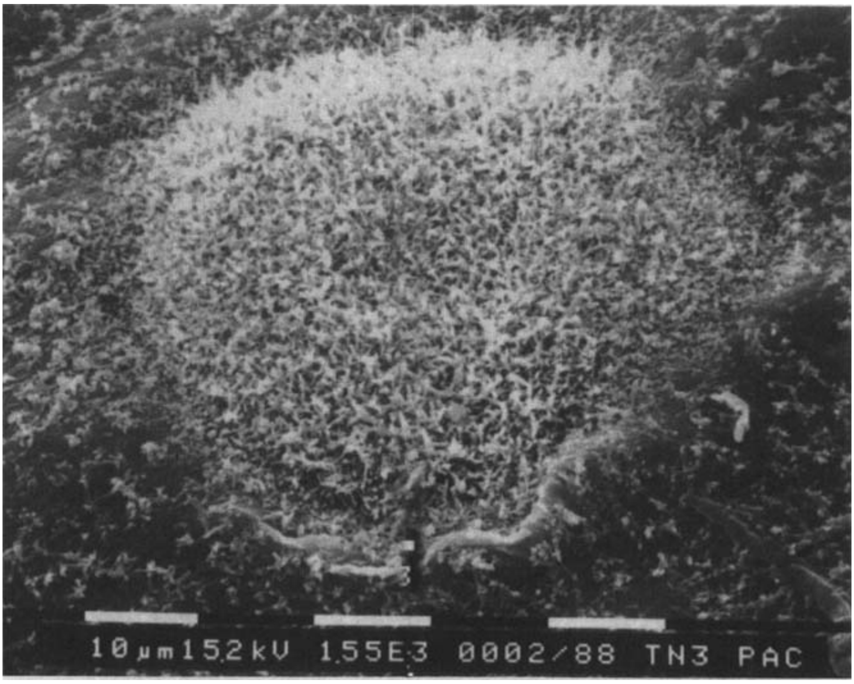

Figure 3 *Picea abies*, uninjured tree, 1-year old needle (higher magnification of Figure 2): the dense agglomeration of the epistomatic waxy microtubules. Length of bar = 10 µm.

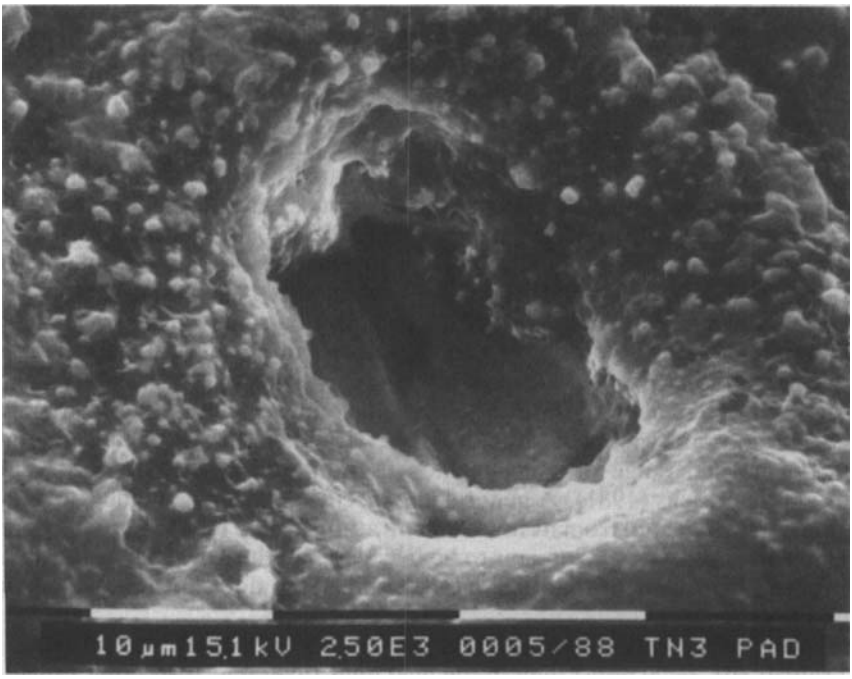

Figure 4 *Picea abies*, injured tree, 1-year old needle: the complete dissolution of the epistomatic protective structure. Length of bar = 10 µm.

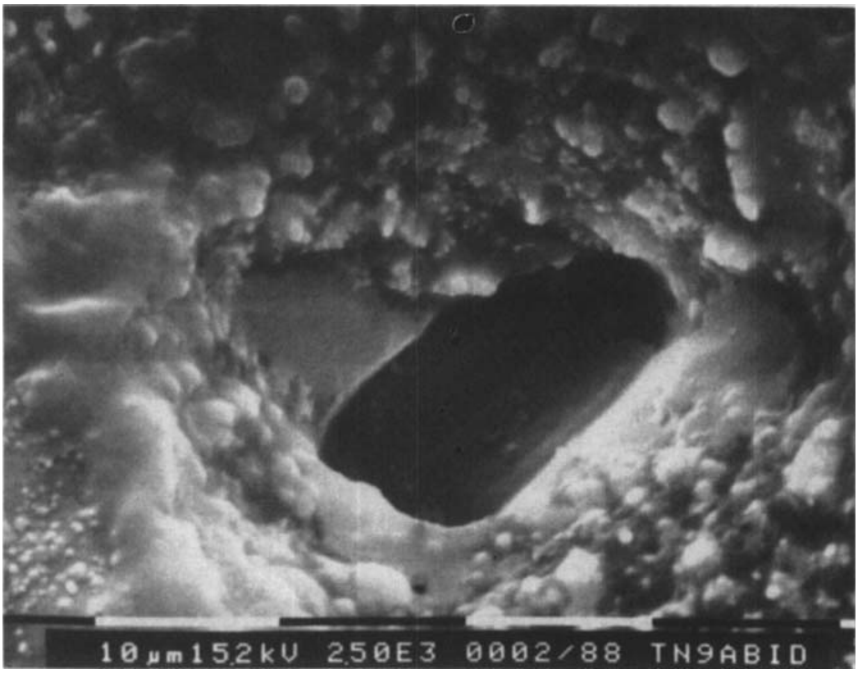

Figure 5 *Abies alba*, injured tree, 1-year old needle: is evident the analogous severe alteration, as in Norway spruce, with dissolution of the waxy microtubules. Length of bar = 10 µm.

Element Concentrations in Roots

As far as the content of sodium, potassium, magnesium and calcium in roots is concerned, no appreciable differences are revealed between fir and spruce, with average concentrations of 240±43 ppm d.w. (spruce) and 290±112 ppm d.w. (fir) (overall range: 146–402 ppm) for sodium, and 3300±1080 (spruce) and 4070±1660 (fir) (overall range 1652–6419) for potassium. On the other hand, magnesium is found in a concentration of 1903 ppm in spruce plants grown on alkali soil (range: 716–3333 ppm) whereas for acid soil the concentration is halved (average: 949 ppm; range: 645–1143 ppm). Roots of fir also show the same pattern (alkali soils average: 1277 ppm; acid soils average: 865 ppm).

A similar but less marked difference is displayed by calcium, that is ranging from 2742 to 10146 ppm (spruce: 5740±2320; fir: 7100±3430). All of these metals are within the normal range of concentration if compared to the standard values of Mayer and Heinrichs (1981). A general nonspecific deficiency of zinc (range: 11.3–23.9 ppm) was indicated from its low concentrations, while iron (349–6209 ppm) and also aluminium (82.4–1493) appear to be in excess: on the other hand, for these two latter metals it is possible that the water-washing step does not remove all surface dust.

Iron was observed in larger amounts in plant roots in alkali soil (average: 2850 ppm; range: 391–6209 ppm) than in the acid ones (average: 1080 ppm; range: 349–2671); the opposite occurs for manganese (acid soils average: 525 ppm; range: 82.4–1493, while alkali soils average: 271; range: 89.3–624).

Heavy metals like lead (range: 3.97–42.3 ppm), cadmium (0.85–2.55 ppm), zinc (11.3–23.9 ppm) and copper (17–27.6 ppm) do not exhibit any variation with respect to the different kinds of soil. Finally, no difference in metal content is apparent within the limits of these preliminary data between the roots of fir and spruce with or without visible damage.

Element Concentrations in Needles

Common metal contents in needles are reported for each tree in Table 2 and Table 3, respectively for samples of young needles (<1-year-old) and 6 to 8-year-old needles.

In general, the current year's needles are sufficiently supplied with calcium and potassium, while sodium appears to be in excess. Magnesium shows a particular behaviour: in spruce, even if the general concentration appears to be sufficient, nevertheless healthy or less injured trees (class O–I) can be distinguished from heavily injured trees (class II-III) by a lower concentration of magnesium (from 890–1593 ppm to 505–801 ppm). Since roots of trees were sufficiently supplied with magnesium (Rothe *et al.*, 1988), it is possible that magnesium deficiency may be caused by the imbalance between leaching (higher) and internal transport (lower).

A similar trend is seen also for manganese concentrations in spruce needles, ranging from 16.6 to 682 ppm: they appear to be generally low (standard value>800 ppm, Mayer and Heinrichs, 1981) and even lower in presence of strong damage (Table 2). Gärtner (1985) refers to the low level of manganese in spruce growing at Mt. Kleiner Feldberg (Taunus, Federal Republic of Germany) which results from leaching, since the canopy throughfall is 55 times higher in concentration than bulk deposition. Nevertheless, owing to the distribution of the samples (Table 1), the influence of soil pH on needle concentration of manganese cannot be excluded.

Table 2 Concentrations (ppm/d.w.) of the common metals in 1-year old needles of fir and spruce: sampling site and class of damage of each tree are also reported. Trace elements values are included in the text.

Sampling Site	*Class of Damage*	*Ca*	*Mg*	*K*	*Na*	*Fe*	*Mn*
spruce							
1	I	3360	1068	23782	233	37.8	286
2	0	2063	890	16301	181	237	284
2	0	4286	1593	25410	217	195	209
3	I	3046	928	11574	189	41.7	337
3	I	3101	1016	14421	407	38.0	206
4	0	3977	1156	806	238	533	122
5	0	6819	1027	8627	274	487	223
5	II	1298	505	7378	273	637	16.6
5	III	2355	801	4641	227	746	17.2
6	I	4116	1157	4394	279	742	682
6	II	2544	778	9687	415	828	369
fir							
2	I	6131	2544	21737	221	16.6	104
2	I	2924	802	8682	532	36.9	415
5	II	6558	2210	6941	397	959	158
6	II	7448	1101	8508	275	1073	193

Table 3 Concentrations (ppm/d.w.) of the common metals in 6–8-year old needles of fir and spruce.

Sampling Site	*Class of Damage*	*Ca*	*Mg*	*K*	*Na*	*Fe*	*Mn*
spruce							
1	I	5598	588	9259	244	134	347
2	0	18877	1009	4184	159	255	1541
2	0	13559	1353	7240	161	175	435
3	I	5811	750	7612	211	124	333
3	I	6011	1223	10792	351	62.3	304
4	0	7118	1164	3586	287	611	160
5	0	19546	983	7291	246	444	396
5	II	11201	571	3022	299	775	15.1
5	III	13789	352	2932	296	1144	17.9
6	I	6672	763	3768	243	503	690
6	II	8671	337	4793	552	294	525
fir							
2	I	14320	1182	9764	143	284	65.3
2	I	3883	688	10392	178	170	481
5	II	19850	2711	3194	266	971	273
6	II	21040	518	4723	434	902	416

The characteristics of the soil appear to affect the concentrations of calcium, iron, aluminium and potassium in needles. The two former metals are present at higher concentrations in alkali soil (Ca: 1298–6819 ppm; Fe: 533–828 ppm) than in acid ones (Ca: 3046–4286 ppm; Fe: 37.8–237 ppm), whereas the inverse trend is exhibited by potassium (alkali soils: 806–9587 ppm, average 6360; acid soils: 11574–25410 ppm, average 17400) and aluminium (alkali soils: 20.7–36.3 ppm, average: 28.5; acid soils: 20.6–64.6 ppm, average: 42.6).

A comparison betweeen Table 2 and Table 3 reveals higher concentrations of calcium and possibly also iron and manganese in old needles. In agreement with Zöttl (1985), higher concentrations of aluminium (from 28.5–42.6 ppm to 70.2–97.0 ppm) and lead (from 1.52–1.84 to 2.46–2.59 ppm) are observed. In contrast, nutrient elements such as potassium, and to a lesser extent magnesium, are present in higher concentrations in young needles.

As for roots, no difference can be detected between healthy and damaged trees for concentrations of lead (ranging from 0.45 to 4.14 ppm) and cadmium (range: 0.16–1.83 ppm). Among trace elements, a general zinc deficiency (range: 10.1–19.6 ppm) was noted, confirming data from root analysis (see the section on roots, above).

DISCUSSION AND CONCLUSION

No definitive conclusions can be drawn from this preliminary study, but only some indications of the most evident phenomena which have been observed.

a) Structural changes at the needles' surface as disorganization of epicuticular waxes, disruption of stomata and dissolution of the pre-stomatic waxy microtubules are clearly detectable and widely observed in damaged trees; they appear to show the same kind of damage that other authors refer to atmospheric pollution, simulated acid rain (Rinallo *et al.* 1986; Schmitt *et al.*, 1987) and acid mist (Mengel *et al.*, 1987) exposures.

Other authors (Grill *et al.*, 1987; Bermadinger *et al.*, 1987) consider that the role of aggressive basic pollutants such as magnesium oxide dust in the erosion of the waxes that cannot be attributed to fungi or climatic conditions. Grill (1973) and Braun and Sauter (1983) pointed out the role of sulphur dioxide and photochemical oxidants + sulphur dioxide synergism in erosion of the needle's surface. Kahru and Huttunen (1986), reporting data for some conifers (*Picea abies, Pinus coulterii, Pinus attenuata* and *Pinus muricata*) of different provenances (Californian, Austrian and Finnish sites), consider that the appearance of injury is faster in urban than in rural areas, and conclude that acid deposition and ozone may play an important role in the occurrence of damage at the needle surface which predisposes the trees to insect attacks.

In experiments, ozone effects on needle surface structures and on winter hardiness of spruce were reported by Barnes and Davison (1988) and Barnes *et al.* (1988).

Stomatal disruption observed in our samples, as well as similar to that described elsewhere, is similar in fir and spruce (and also in pine and deciduous species such as oak and beech) in the Apennine region and/or as a consequence of simulated acid treatments (Gellini *et al.*, 1987).

b) Some differences found in metal concentrations of needles and roots are related to the nature of the soil, the age of needles and the occurrence of damage, and deserve closer examination. Differences are mostly evident for common metals and nutrients such as magnesium, manganese and iron, confirming data from Germany (Prinz *et al.*, 1982; Rothe *et al.*, 1988) and suggesting that nutritional imbalance plays an important role in the appearance of injury. Conversely, concentration of other toxic heavy metals such as lead and cadmium, in needles and roots can be scarcely related to the more or iess evident visible injury.

Further investigation is required, following the indications of the present study, to achieve better quantification and correlation, as well as knowledge of the general environmental status of the province of Trento, with particular regard to forest areas.

Acknowledgement

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