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Original article

The role of marine salt and surfactants in the decline of Tyrrhenian coastal vegetation in Italy

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Summary — The decline of coastal vegetation is a phenomenon affecting some areas of the Mediterranean region and Australia; it is due to the presence of surfactants in marine aerosols, a consequence of sea pollution by detergents. This paper gives some observations made at various sites along the Tyrrhenian coast in Italy. The authors show that the presence of surfactants in the environment correlates with the presence of sea salt, and that the impact of surfactants on vegetation is local and occurs only in association with strong sea winds. The study of the synergistic effect of surfactants and sea salt on the crowns of trees exposed to aerosols suggests that the surfactant can cause direct damage, while the absorption of sea salt is enhanced by the presence of the surfactant only when exposure to aerosol is prolonged, or if it is administered in very high concentrations.

***Pinus pinea* L / marine aerosol / surfactant / NaCl / leaf absorption / crown damage**

Résumé — Le rôle du sel marin et des agents tensio-actifs dans le dépérissement de la végétation côtière tyrrhénienne en Italie. *Le dépérissement de la végétation côtière est un phénomène qui concerne un certain nombre de zones de la Méditerranée et de l'Australie ; il est dû à la présence d'agents tensio-actifs, engendrés par la pollution hydrique de détergents dans les aérosols marins. Notre recherche fait le point de 10 années d'observations d'un certain nombre de localités italiennes du littoral tyrrhénien. Il en ressort que la présence d'agents tensio-actifs dans l'environnement dépend de la présence de sel marin ; leur impact sur la végétation est local et il est limité aux périodes de vent de mer fort. L'étude de l'action synergique des agents tensio-actifs et du sel marin sur les houppiers des plantes exposées aux aérosols suggère l'existence d'un dommage direct dû à l'agent tensio-actif en question, tandis que l'absorption de sel marin n'est favorisée par la présence de l'agent tensio-actif que dans des conditions d'exposition prolongée à l'aérosol ou s'il est administré en fortes concentrations.*

***Pinus pinea* L / aérosol marin/ tensio-actifs / NaCl / absorption foliaire / dommages au houppier**

INTRODUCTION

Since the early 1960s the vegetation in a number of coastal areas has been affected by a kind of decline which, in terms of both quality and intensity, is very different from the normal damage caused by salt. In actual fact the spontaneous coastal vegetation has adapted to the action of salt, so that natural marine sprays only cause rather limited damage, consisting primarily of changing the shape of the crown or in the death of external branchlets. More severe damage can be caused occasionally by violent sea storms (Franzén, 1990). The environmental factor usually blamed for this type of decline (besides salt) is the pollution of the sea by synthetic surfactants and oil, *ie* organic substances that accumulate primarily in the spray.

The first studies on this topic date back to the 1960s (Lapucci, 1968; Gellini and Paiero, 1969; Lapucci *et al*, 1972) and concentrated primarily on the death of the coastal vegetation in the forest at San Rossore (Pisa), while Gisotti (1979) and Gisotti and De Rossi (1980) studied the conditions of the forest at Castelporziano (Rome). A second set of research studies followed in the 1980s (Gellini *et al*, 1981, 1982, 1983, 1985, 1987; Bussotti *et al*, 1984; Guidi *et al*, 1988; Innamorati *et al*, 1989; Grossoni *et al*, 1990), also focusing on San Rossore. In the meantime studies were also carried out in Australia (Pitman *et al*, 1977; Dowden *et al*, 1978; Grieve and Pitman, 1978; Truman and Lambert, 1978; Dowden and Lambert, 1979; Moodie *et al*, 1986), in France (Devèze and Sigoillot, 1978; Sigoillot *et al*, 1981; Sigoillot, 1982; Garrec and Sigoillot, 1992; Badot and Garrec, 1993) and, more recently, in Spain along the coast near Barcelona (Astorga *et al*, 1993). In all the areas studied the damage is located near urban zones or near wastewater collector tanks and extends inland for a stretch of a few hundred metres or at most 1 km. After especially violent wind storms the damage can occasionally reach zones that are many kilometres from the coast (Grossoni *et al*, 1990; Raddi *et al*, 1992). However damage of this type is always extremely localized. According to the majority of the authors mentioned above this damage is primarily due to the fact that the crown absorbs an excess of sea salt, which then accumulates in the leaf tissues. In fact, the damage always appears to be associated with high quantities of Na^+ and Cl^- in the leaves. In coastal species, in normal conditions, the absorption of sea salt is limited by the normal defence mechanisms of the leaves, but in the cases examined here the absorption is enhanced by the presence of surfactants (Greene and Bucovak, 1974). Based on our findings, and also referring to the vast literature that exists on the subject, we cannot rule out that surfactants may exert a direct effect on chloroplasts and other cellular organs (Itoh *et al*, 1963; Ogawa *et al*, 1966; Deamer and Crofts, 1967; Helenius and Simmons, 1975) or on the epicuticular wax structures (Gellini *et al*, 1985; 1987; Noga *et al*, 1987; Wolter *et al*, 1988).

The aim of this report is to give the results of some research work carried out over a vast area of the Tyrrhenian coastland in Italy, including the 2 estates belonging to the presidency of the Italian Republic (San Rossore (Pisa) and Castelporziano (Rome)) and the pinewood of Cecina (Leghorn), where severe damage to the vegetation has been observed. The report will also illustrate the results of a number of experiments the aim of which was to measure the toxicity of the various components of polluted sea spray, both in isolation and in synergy.

MATERIALS AND METHODS

Determination of surfactants and chlorides in sea aerosol

The determination of surfactants and chlorides (the latter are useful as indicators of the presence of sea salt) in aerosols was carried out at San Rossore, Cecina and Castelporziano, primarily in 2 matrices, rainwater and deposits on the vegetation, which normally contain such pollutants when situated near the coast.

Rainwater samples were collected both near the coast and 2–3 km inland. Samples of bulk deposits were taken from pine trees (*Pinus pinea* L) from the section of the crown exposed to the sea and also from the opposite side. Table I shows the characteristics of the different samplings. Rainwater samples were collected on a weekly basis, while the deposits were gathered only after strong sea wind events.

In order to extract the deposits from the surface of the needles, 20 g of fresh needles, measuring about 12.5-14 cm in length and giving an overall surface area of about 850 cm^2, were rinsed in 200 cm^3 deionized water for 20 min. The solutions thus obtained were then analysed.

Anionic surfactants were measured as MBAS (methylene blue active substances), according to Longwell and Manièce's colorimetric method (1955); chlorides were measured by potentiometric titration with 0.1 N silver nitrate.

Analytical values are given in ppm for rainfall and in mg/kg of needles (fresh weight) for deposits. The correlation between MBAS and Cl^- in the different matrices was calculated according to Kendall's non-parametric test (rank correlation), and the software used was Statgraphics.

Experimental tests on the toxicity of the aerosols

Tests were performed by spraying the crowns of young trees of *P pinea* L, *Quercus ilex* L, *Pittosporum tobira* L and *Acer opalus* Mill with solutions containing surfactants and NaCl in varying concentrations, thus simulating the composition of sea aerosols. The efficacy of this treatment was assessed by ascertaining the percentage of damaged leaves (*ie* leaves with yellowing) and the quantity of NaCl absorbed through the leaves shown by the increase of the Cl^- ion.

Twenty grams of needles (about 150 needles) from *P pinea* trees, or 20 leaves from broadleaves, were collected from each treated tree and the same sample was used to calculate both the percentage of damaged needles and the content of Cl^-. For each tree, the sampling was repeated twice

In order to measure chlorides in the *P pinea* needles, the needles were rinsed before testing for about 5 min in deionized water. The chloride extraction was performed according to the method described by Grieve and Pitman (1978); the analytical method used is potentiometric titration with 0.1 N silver nitrate. The absorbed salt is expressed as mg of Cl^- per gram of dry matter. The significance of the differences has been tested by Student's *t* test.

Table I. Sampling characteristics and number of samples gathered.

Location	*Period of sampling*	*Rainwater collected at the coastline*	*Rainwater collected inland*	*Deposits on exposed needles*	*Deposits on sheltered needles*
San Rossore	Nov 1982 May 1983	32	30	45	31
San Rossore	Oct 1990 June 1991	28	26	10	4
Castelporziano	Mar 1986 Nov 1987	24	0	17	17
Cecina	Oct 1983 Jan 1985	40	20	8	8

The following is a description of the tests carried out:

1st experiment — treatment of *P pinea* in the open field

The following sets of tests were made:

- NaCl in varying concentrations (0, 30, 60 and 120 g/l);
- NaCl in a single concentration (30 g/l) combined with an anionic surfactant (ABS = alkyl benzene sodium sulphonate) in varying concentrations (10, 50, 100, 250 and 500 mg/l);
- ABS in a single concentration (100 mg/l) combined with NaCl in varying concentrations (10, 15, 20, 30, 60 and 120 g/l).

The experiment was carried out on trees that were about 2 m high, belonging to a reforested plot within the San Rossore estate. Each test was repeated on 4 different trees; the treatment was repeated twice, in July and in September, and consisted of spraying the crown with a motorized atomizer. Each treatment lasted a few minutes, as long as was necessary to soak the crown to dripping point. Monitoring was done 1 month after the second treatment.

2nd experiment — treatment of *P pinea* seedlings growing in pots

The seedlings were sprayed with NaCl alone (30 g/l), with ABS (500 and 1000 mg/l) and with non-ionic surfactants (alkyl phenol ethoxylates, Lerolat 40 and Lerolat 300, which differ by the length of their alkylic chains) at a concentration of 1 000 mg/l. The experiment was carried out at the Faculty of Agrarian Studies at the University of Florence, on pot-grown trees measuring about 1.5 m in height, using a methodology similar to that described above. The trees were sprayed once only, in June, and monitoring was done the following month. Each test was repeated on 4 different trees.

3rd experiment — treatment in the nebulizing chamber

Tests were done using NaCl 30 g/l, ABS 250 mg/l, or NaCl 30 g/l + ABS 250 mg/l. Young trees of *P pinea, P tobira, Q ilex* and *A opalus* growing in pots (5 individuals per species) were sprayed continuously for 4 h a day for 3 d. The treatment was done in September and the monitoring the following month. This test simulated exposure conditions that are more similar to what occurs in nature.

RESULTS

Chemical analysis of rainwater samples and deposits

When interpreting the results of the chemical analysis of rainwater and deposits it is necessary to bear in mind the interactions between the matrix and aerosol composition. For example, in deposits on needles part of the surfactant binds to the epicuticular wax structures since it is lipophilic, and is therefore not removed by rinsing. However part of the chlorides absorbed by the needle are released during rinsing. In the rainwater samples there can be interferences with atmospheric dust and dry deposits of non-marine origin.

In any case, the highly significant ($P < 0.001$) correlations between MBAS and chlorides (table II) evident in needle deposits and in rainwater samples collected near the coast suggest that both substances originate from the sea. This is also confirmed by the low level of significance between MBAS and chlorides in the rainwater samples collected further away from the coastal area.

Tables III and IV show the levels of concentration of MBAS in rainwater samples and in deposits on needle surfaces. It is interesting to note that most findings are in the lower concentration classes, while there are relatively few high values and they were all recorded during episodes of strong sea winds (*cf,* Gellini *et al,* 1987).

The highest value of MBAS in rainwater collected along the coast is 29.2 ppm,

Table II. Correlation between MABS and Cl^- (coefficient τ of Kendall's non-parametric test), in different matrices and gathering condition.

Gathering conditions	*Number*	τ	*P*	*MBAS/Cl⁻*
Rainwater collected at the coastline	81	0.3021	< 0.001	0.007
Rainwater collected inland	64	0.2064	< 0.05	0.001
Deposits on exposed needles	44	0.5431	< 0.001	0.021
Deposits on sheltered needles	34	0.5925	< 0.001	0.009

Table III. MBAS content in rainfall.

MBAS concentration (ppm)	*Rainwater collected at the coastline*		*Rainwater collected inland*	
	Number	*%*	*Number*	*%*
0–0.10	39	28.9	66	86.8
0.11–0.50	37	27.5	9	11.8
0.51–1.00	15	11.1	1	1.3
1.01–5.00	34	25.2	0	
5.01–10.00	6	4.4	0	
> 10.00	4	2.9	0	
Total	135	100	76	

Table IV. MBAS content in deposits on *P pinea* needles' surface.

MBAS (mg.kg fw⁻¹)	*Deposits on exposed needles*		*Deposits on sheltered needles*	
	Number	*%*	*Number*	*%*
0–1.00	13	16.0	21	35.0
1.01–10.00	26	32.2	32	53.3
10.01–50.00	27	33.3	7	11.7
50.01–100.00	8	9.9	0	0
> 100.00	7	8.6	0	0
Total	81	100	60	100

but MBAS concentration levels only reach 1 ppm or above in 32.5% of cases, and only go above 10 ppm in 2.9%. Inland, MBAS concentrations reach a maximum of 0.9 ppm and only go above 0.1 ppm in 13.1% of samples. As far as surface deposits are concerned, in needles exposed to the sea the highest value recorded is 514 mg of MBAS per kg of fresh needles, but the concentration only goes above 50 mg in 18.5% of samples. In needles from the side of the crown not exposed to the sea the highest concentration of MBAS is 53 mg per kg of fresh needles, but only 11.7% of cases have values higher than 10 mg.

This trend shows that 'noteworthy events', *ie* those with the greatest impact on the system because of the amount of salt and surfactants they transport, occur rather infrequently within the total number of samples examined. Finally, the ratio MBAS/Cl^- is interesting because it varies considerably according to the matrix in which it is measured. There are probably many factors that influence the quantities of these 2 substances (biological, metereological, chemical, physical, *etc*). The ratio that is most probably the closest to the original ratio in the aerosol is that recorded in the rainwater gathered near the coastline, *ie* the one closest to the source. Here the MBAS/Cl^- ratio is 0.007, which is about 1:143, similar to that found in aerosols by Gellini *et al* (1987) and by Loglio *et al* (1985, 1986, 1987a,b, 1989).

Experimental reproduction of the damage

In the 1st experiment *P pinea* appeared to be quite susceptible to the absorption and accumulation of NaCl. The levels of Cl^- in the pine needles are related to the concentration of salt in the solution, whether the solution also contains surfactants (fig 1b) or not (fig 1a). Salt absorption does not appear to be influenced by the presence of the surfactant (the differences between needles treated with and without surfactants are not significant, $P > 0.05$). There is no correlation between the percentage of damaged needles and level of Cl^- ($P > 0.05$). Conversely, the concentration of the surfactant plays a very important role in the appearance and extent of damage. The differences between tests with surfactants in concentration up to 100 ppm and without are very significant ($P < 0.01$). It is interesting to note that the percentage of damaged needles increases abruptly when the concentrations of ABS are 100 mg/l or higher (fig 1c).

The damage observed during this experiment consisted of patches of yellow needles. In no case did the treatment reproduce the typical drying of the top of the needle. If we compare these data with those of previous studies (*cf*, Gellini *et al*, 1985) we notice that the damage recorded in this experiment can be attributed to the action of the surfactant.

In the 2nd experiment, in which pot-grown *P pinea* were treated with NaCl and surfactants, the response was more marked (apical drying of needles) with a considerable accumulation of Cl^- in the needles at ABS doses of 500 mg/l (fig 1d). At higher concentrations of the surfactant (in this experiment we also used non-ionic surfactant) the results are more or less identical. Differences between treatments B (NaCl without surfactant) and A (control), C, D, E, F (NaCl with surfactants) are significant with $P < 0.01$.

In the 3rd experiment (prolonged exposure in a nebulizing chamber) dieback of the apical needles was achieved with an ABS concentration of 250 mg/l administered with 30 g/l of salt, while ABS alone only causes yellowing. The results of this experiment, illustrated in figure 2, also show the different responses to the treatment by the 4 different species tested. *P tobira* was the most resistant species, while the deciduous broadleaf *A opalus* suffered the most damage. *P pinea* and *Q ilex* gave intermediate responses: the former was more susceptible to surfactants, and the latter to NaCl. In all cases the combination of surfactant plus NaCl caused the worst damage. At the concentrations and conditions of exposure used in this experiment, the same pathological manifestations as observed in broadleaves (necrosis of the edges) were reproduced.

DISCUSSION AND CONCLUSIONS

The results obtained confirm the fact that the damage to coastal vegetation caused by marine aerosols polluted with surfactants is fairly limited in both time and space, although it can occasionally produce catastrophic results. In space, the damage is limited because large sea-salt aerosols are rapidly deposited. Apart from exceptionally violent storms the damage only affects the first few hundred metres of vegetation. In time, it appears that aerosols are produced only during some specific events in winter and surfactants in large quantities were detected only in a small percentage of the samples studied. The surfactants in the inland rainwater samples are only found in very low concentrations (always below 1 mg/l) and at these levels no synergistic action with either marine salt or other substances of anthropic origin, such as acidity or pesticides, has been demonstrated (Paoletti *et*

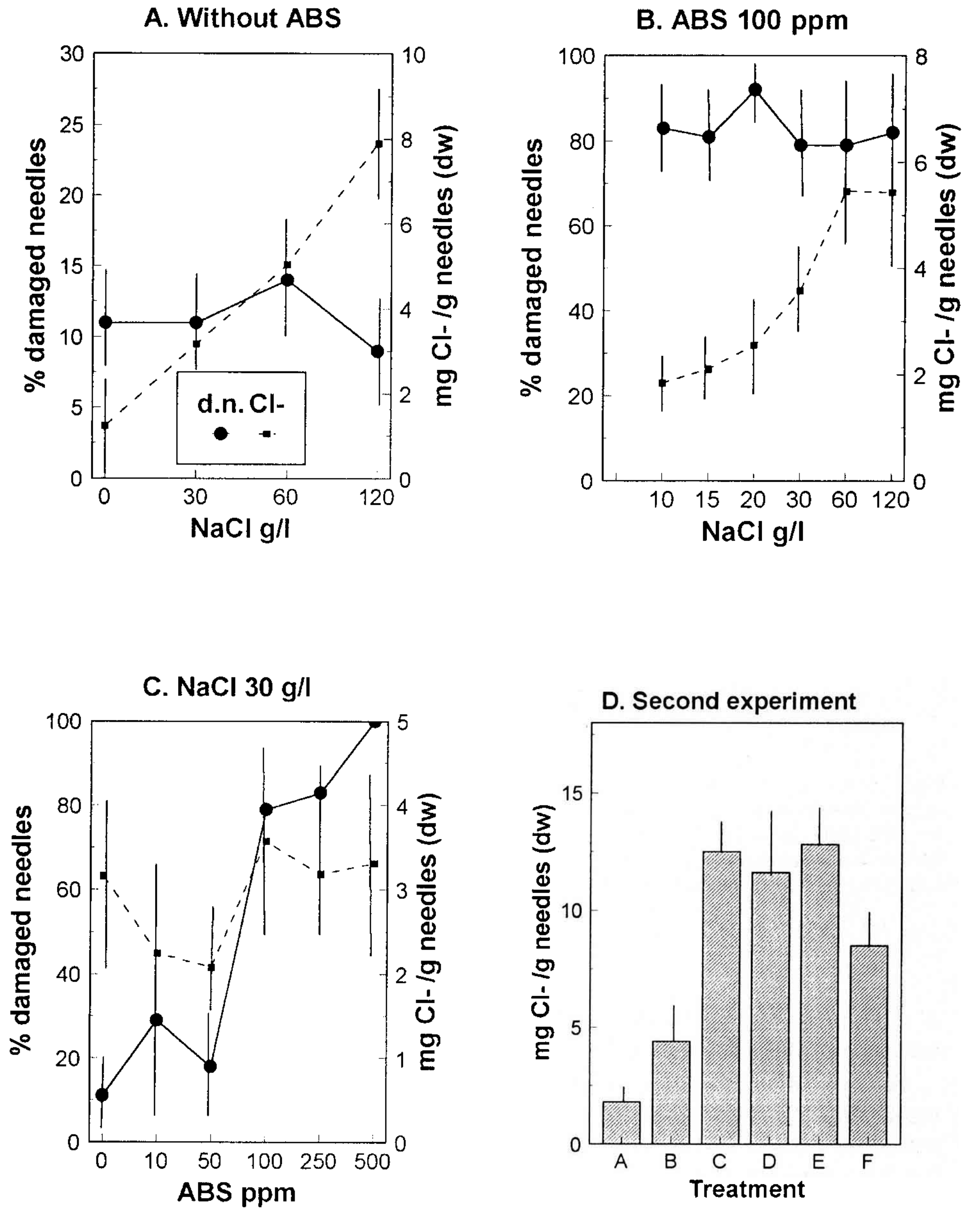

Fig 1. Results of the 1st experiment. **A–C**: The Cl^- concentrations inside the needles and the percentage of damaged needles are indicated on the ordinates. Legend: dn = % damaged needles; Cl^- = mg Cl^-/g needles (dry weight). **D**: the Cl^- concentrations inside the needles treated in the 2nd experiment: A = control; B = NaCl (30 g/l); C = ABS (500 mg/l) + NaCl (30 g/l); D = ABS (1000 mg/l) + NaCl (30 g/l); E = Lerolat 40 (1000 mg/l) + NaCl (30 g/l); F = Lerolat 300 (1 000 mg/l) + NaCl (30 g/l). Bars show standard deviation (*n* = 8).

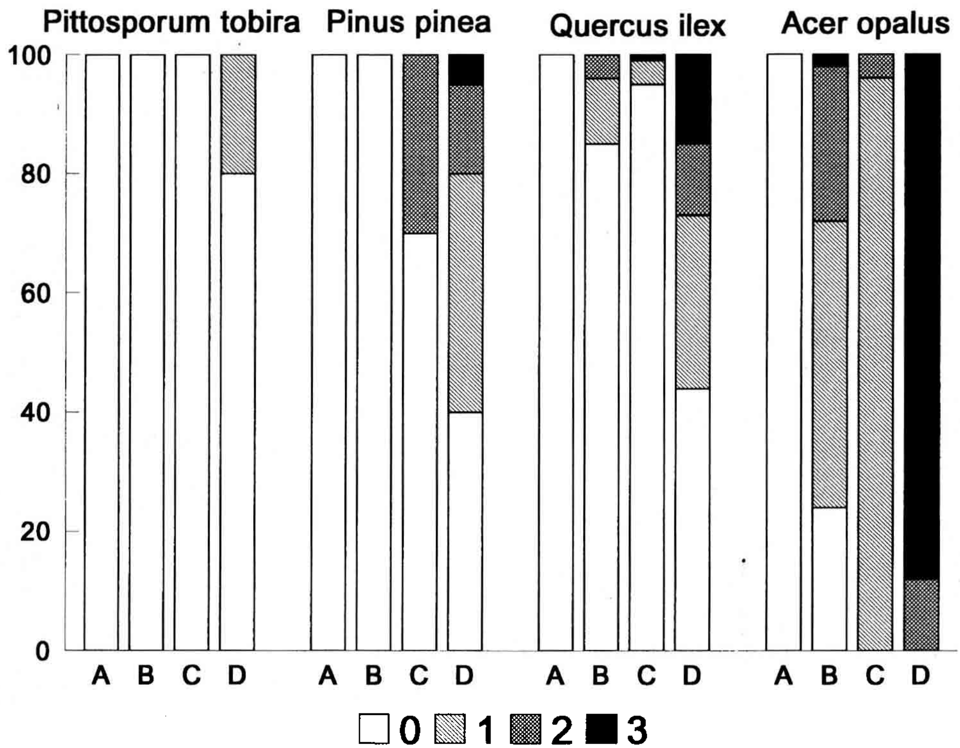

Fig 2. Results of the 3rd experiment, expressed as percentage of damaged leaves divided according to their damage class. The tests are indicated as: A = control; B = NaCl (30 g/l); C = ABS (250 mg/l); D = ABS (250 mg/l) + NaCl (30 g/l). The damage classes are indicated as follows: 0 = no damage; 1 = extensive damage affecting less than 1/3 of the leaf; 2 = extensive damage affecting between 1/3 and 2/3 of the leaf; 3 = extensive damage affecting more than 2/3 of the leaf.

al, 1989; Rinallo and Raddi, 1989; Bottacci *et al,* 1990; Paoletti, 1992).

The experiments performed confirm that the synergy brought about by the combination of surfactants and sodium chloride is the main cause of the decline of coastal vegetation. However, we still need to explain why similar treatments administered to individuals of the same species *(P pinea)* have yielded different results (*cf* Gellini *et al,* 1987; Guidi *et al,* 1988; Loglio *et al,* 1989). In our opinion these differences are not so much due to genotype differences (the stone pine is a species characterized by a considerable genetic uniformity), but rather to the different stand conditions (trees grown in pots, for example, were more suceptible), vegetational status, macro- and microclimate conditions experienced during the treatment period, as well as treatment modalities.

We would like to stress the following. When the trees were subjected to a treatment consisting of a brief exposure to simulated marine aerosol (1st experiment) the damage they suffered was always less severe than when they were subjected to more prolonged exposure (3rd experiment). This last type of treatment is more similar to natural exposure conditions. This observation is also confirmed by the results obtained by Guidi *et al* (1988) in their 'wind tunnel' experiments. From a practical point of view this means that if we want to reproduce the damage as it appears in nature using only short treatments, we must resort to concentrations that are much higher than those of natural aerosol. Empirically, we can refer to the deposits found on the needles. In order to experimentally obtain deposits that are quantitatively similar to those found in nature we need to spray the crowns with solutions containing at least 1 000 mg/l of surfactant.

The results obtained also highlight the direct action of the surfactant alone, which appears to exert its action before the synergistic effect of surfactant plus salt. This is suggested by the fact that the damage caused by fairly low doses of ABS (1st experiment), even in the presence of salt, are mainly attributable to the surfactant, and there is no correlation between the degree of leaf damage and the Cl^- levels in the leaves. Only above a certain level of concentration does the surfactant begin to act synergistically with the salt. In our experiments this threshold appears to be around 250 mg/l in the case of prolonged exposures, and 500–1 000 mg/l for short exposures. Above these thresholds the damage observed is identical to the damage found in nature and is associated with the high content of chloride in the foliar tissues, thus reproducing a type of damage similar to typical salt-induced damage (*cf* Dobson, 1991).

It is interesting to note that the response of *P pinea* to treatment does not appear to be proportional to the treatment, but rather seems to be influenced by a 'sensitivity threshold'.

Another element which can be useful in understanding our results is the time of year at which treatment was administered. Our experiments, especially those done in the field, were all carried out in the summer, for obvious experimental reasons. Treatments carried out in late autumn and winter, especially surfactant treatments, generally cause less severe damage (our unpublished data), but it is precisely in late autumn and winter that 'normal' exposure to these substances would take place.

Further observations arising from the results of this study concern the toxicity of non-ionic surfactants and the different levels of resistance exhibited by the various species. The results of the 2nd experiment suggests that (at least at the higher concentrations) non-ionic surfactants also act synergistically with salt, exactly like anionic ones. Since about 30% of all surfactants available on the market today are non-ionic (Olori and De Fulvio, 1989), it is highly likely that they have a large ecotoxic effect although they are probably not being properly monitored, since we do not possess suitable monitoring methodologies.

Finally, the comparison between the different behaviour of the species tested suggests that resistance to the action of aerosols is the result of the strength of the structures protecting the leaf, and increases with sclerophyllia and with the thickness of the cuticle.

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