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Original article

The behavior of oaks in response to natural and induced exposure to the surfactant ABS

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Summary — *Quercus pubescens*, *Q frainetto*, *Q ilex* and *Q robur* were sprayed with ABS (sodium dodecyl benzenesulfonate, a surfactant often found in marine aerosols) over 2 growing seasons to test the effect of the surfactant on the leaf wax structure. Though all species were affected, they differed in their tolerance to ABS. The effect of ABS was also tested on the pollen quality of *Q ilex*. Pollen from declining and apparently healthy individuals was germinated in cultures with different concentrations of ABS. A negative effect on pollen germination and pollen tube length was noted. The reduction in pollen viability could affect the genetic resources of holm oak populations in the future.

ABS / oak spp / pollen / wax structure

Résumé — Réaction des chênes à l'exposition naturelle et simulée à l'ABS. Des arbres appartenant à *Quercus pubescens*, *Q frainetto*, *Q ilex* et *Q robur* ont été aspergés d'ABS (dodecylbenzènesulfonate de sodium, détergent souvent trouvé dans les aérosols marins) pendant 2 saisons de végétation, afin de tester l'effet du détergent sur les structures des cires foliaires. Alors que toutes les espèces étaient atteintes, des différences spécifiques ont été observées. L'effet de l'ABS sur la qualité du pollen de *Q ilex* a également été étudié. Le pollen issu d'arbres dépérissants et apparemment sains a été germé dans des cultures ayant des concentrations variables d'ABS. L'ABS exerce un effet négatif sur la germination du grain de pollen et le développement du tube pollinique. La réduction de la viabilité pollinique peut affecter les ressources génétiques du chêne vert dans le futur.

ABS / chêne / pollen / cires foliaires

INTRODUCTION

That the effect of air pollution on forest trees varies both among and within species has been well established (Karnosky *et al*, 1989), but there are still gaps in our knowledge about specific pollution effects on the genetic resources of forest trees. The oak forest ecosystem in Europe has recently begun to show signs of 'oak decline' (Donaubauer, 1987), the most notable symptoms of which are leaf yellowing and curling, leaf stunting, precocious leaf and twig shedding, failure of the buds to

break, changes in the branching habit and abnormally intense flowering (Gellini, 1989). Along the Tuscan coast (where a large part of the vegetation consists of oak), several symptoms of decline have been attributed to wind-borne surfactants from the sea (Gellini *et al*, 1983, 1985). The present study seeks to assess the effect of natural and artificial exposure to ABS on the leaf waxes of 4 oak species and on the pollen quality of *Quercus ilex*.

MATERIALS AND METHODS

The leaf waxes of 5-year-old seedlings of *Quercus pubescens* Willd, *Q frainetto* Ten, *Q ilex* L and *Q robur* L were sprayed with ABS (sodium dodecyl benzenesulfonate) and examined over 2 growing seasons (1990–1991). Ten seedlings per species (5 for the spray treatment and 5 controls) were maintained in a plastic chamber where, during each growing season, they were treated twice weekly for 10 weeks with 50 mg/l ABS in a fog-type spray. This concentration corresponds realistically to the mean found in rainfall on the Tuscan coast (Bussotti *et al*, 1983). Prior to treatment each year, the leaves were marked and, after each series of treatments, 2 leaves per tree were collected and air dried, as described by Karhu and Huttunen (1986). From the middle of each leaf, 2 disks of about 50 mm^2 each were removed, 1 for upper surface and 1 for lower surface examination. Samples were sputtered with gold palladium film and examined by scanning electron microscopy (SEM) at 20 kV.

Q ilex, the species selected for pollen analysis, is common along the Tuscan coast and in hilly areas further inland and for several years has exhibited abnormally intense flowering (Gellini and Paoletti, 1990). Pollen for our study was collected in the San Rossore Park from 1 healthy-looking individual that was sheltered from the sea wind by other vegetation and from 3 directly exposed and declining individuals. On these latter trees, pollen was taken both from the windward and the leeward sides for comparative analysis. Pollen viability was determined by fluorescein diacetate (Heslop-Harrison *et al*, 1984). Each test was replicated 5 times. To determine germination, pollen grains were assayed in cultures of modified Brewbaker and Kwack (1963) solution containing 200 g/l of sucrose and 0, 1, 3, 5 or 7 mg/l of ABS. Each assay was replicated 3 times. The percentages of germination was arcsin-transformed. All data were subjected to analysis of variance and to Tukey's multiple range test ($P = 0.05$).

RESULTS

ABS damaged the leaf waxes of all species, though to different degrees. Damage included: disaggregation and fusion of the epicuticular and epistomatic wax, deactivation of the stomata (through occlusion or damage to the stomatal aperture as it was plugged by migrating fused wax or through impaired function of the guard cells), lesions and cracks in the cuticle, trichome abscission and destruction, and the collapse of the secreting heads of glandular hairs (fig 1A–D). Damage decreased in the following order: *Q pubescens*, *Q frainetto*, *Q ilex* and *Q robur*. The same trees were sprayed again in 1991: initial data revealed structural degradation of the leaf waxes similar to that of the year before, but the relative tolerance among the species was unchanged.

In the culture containing no ABS, pollen germination was lower in damaged trees than in the healthy-looking tree, and in damaged trees it was lower on the windward side than on the leeward side (table I). However, the germinative capacity in the healthy-looking individuals was less than that reported for truly healthy *Q ilex* that had never been exposed to marine aerosol (54%) (Bellani *et al*, 1988). In the culture without ABS, pollen tubes were considerably shorter in damaged trees than in the 'healthy' individual tested, but there was no difference in tube length between the windward and leeward sides of exposed trees. The addition of ABS to the culture reduced germination in all trees.

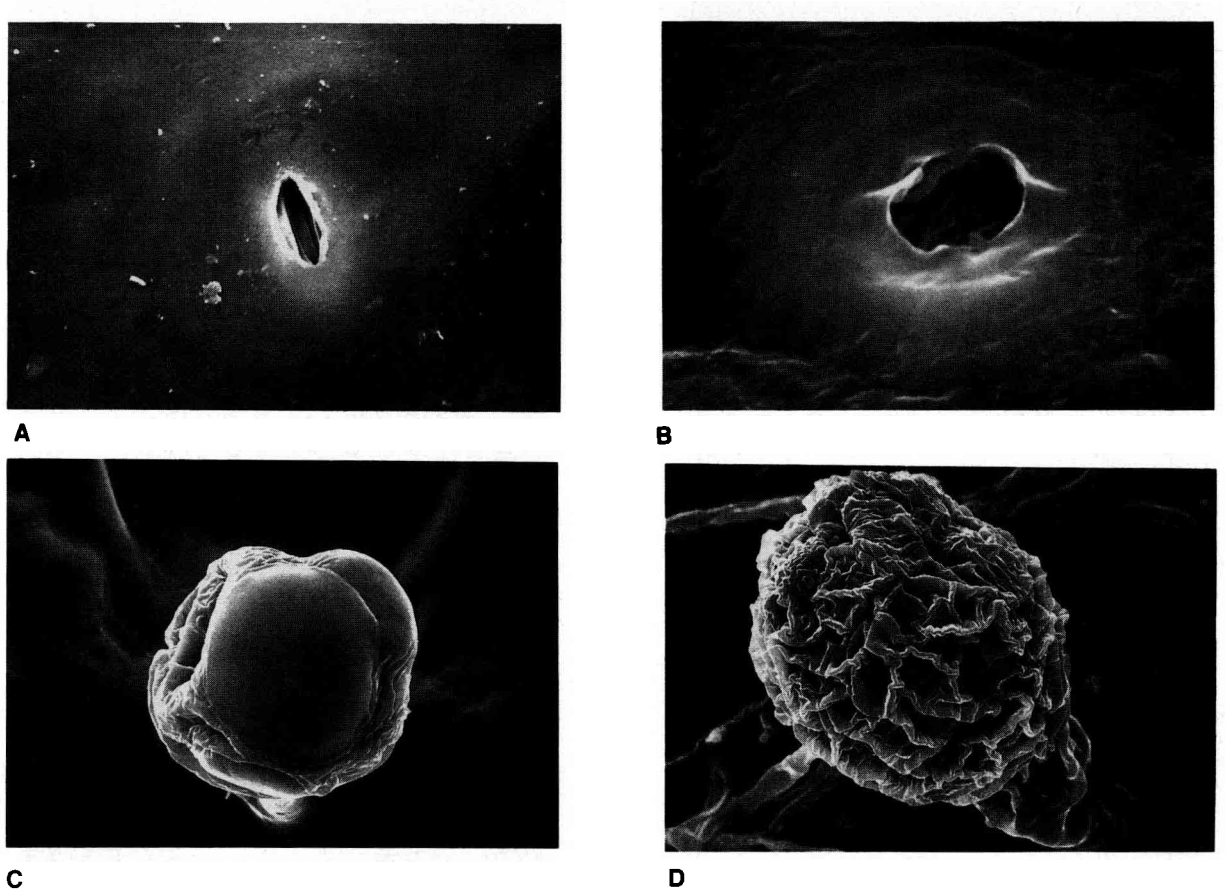

Fig 1. A–D. SEM examination of *Q ilex* leaves (bar scale = 10 μm). **A.** Lower surface. Control. Healthy stoma with normal distribution of the epicuticular wax (x 2550). **B.** Lower surface. ABS treated. Deactivated stoma, with stomal fissure impaired and slight aggregation of the epicuticular wax that gives the cuticle a wrinkled appearance (x 2550). **C.** Upper surface. Control. Normal glandular hair (x 1455). **D.** Upper surface. ABS treated. Collapsed glandular hair (x 1455).

Table I. Germination, tube length and viability of holm oak pollen at different levels of natural and artificial exposure to ABS.

Parameter	*ABS (mg/l)*	*Apparently healthy*		*Damaged*			
				Leeward		*Windward*	
Germination (%)	0	36	Aa	18	Ab	5	Ac
	1	16	Ba	3	Bb	2	Bb
	3	3	Ca	0.8	Bb	0.7	Cb
	5	1	C	0	D	0	D
	7	0	D	0	D	0	D
Length (μm)	0	223	Aa	59	Ab	59	Ab
	1	154	Ba	75	Ab	95	Ab
	3	99	Ca	84	Aa	58	Aa
	5	53	D	0	B	0	B
	7	0	D	0	B	0	B
Viability (%)	0	83	a	74	a	58	b

Significant differences for each variable obtained by variations in artificial exposure are indicated by different upper-case letters (within columns); significant differences resulting from variations in natural exposure are indicated by different lower-case letters (along a horizontal line).

The extent of reduction increased with increasing doses, until 0% germination was reached at 7 mg/l for the 'healthy' tree and 5 mg/l for the damaged trees. Increasing ABS concentrations also reduced pollen tube length in the 'healthy' tree, but not significantly in declining trees.

DISCUSSION

The results confirm that wet depositions of a substance such as ABS are harmful to oaks. The morphological and physiological alterations to the leaf waxes have biological consequences, increasing the cuticular transpiration rate and leaving the tree more vulnerable to parasites (Cape, 1983). Pollen quality is confirmed as a sensitive indicator of air pollution, whether natural or artificial. Moreover, in the ABS-containing cultures, damaged trees exhibited a lower germination capacity than healthy trees. Surfactant-induced reduction of the number and length of pollen tubes could diminish seed production and quality by weakening microgametophytic competition for ovules, or it could increase the tolerance of succeeding generations of trees by selecting for pollen grains that are surfactant-tolerant. The alterations in the leaf waxes and in the pollen noted in this study suggest that current surfactant levels in nature (Bussotti *et al,* 1983) give cause for serious concern. Pollution exerts selective pressures in favor of more tolerant species, but thereby reduces genetic diversity, which is crucial if species are to adapt to changing environmental conditions.

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