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Comparative Response over Time to Surfactant-Polluted Sea Aerosol in Mediterranean Pine Seedlings

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Summary

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Seedlings of three Mediterranean pines were exposed to five spray treatments with sea water including an anionic surfactant, 5 to 500 mg/l dioctyl sodium sulfosuccinate. By comparing the response (visible injury, needle content of chloride or water, damage to stomata) at the end of the treatments with the response at 60 days after the last spraying, we aimed at investigating the recovery over time. Conversely, the phytotoxic effect of surfactant-polluted sea aerosol became more severe as time passed, even if no further exposure occurred. All the three pine species were sensitive to surfactant (*P. pinea* > *P. halepensis* > *P. pinaster*), suggesting that coastal pinewoods are an ecosystem at risk for this type of pollution.

Introduction

Surfactants contained in commercial detergents may reach the sea via waste waters and rivers. Escaping the natural biodegradation processes, surfactants are transported by winds in form of aerosol. Once deposited on the leaves, they damage vegetation, mainly in combination with sea salt (for a review see NICOLOTTI & al. 2005). All plant species appear to be sensitive to surfactant-polluted sea aerosols. Observed responses include leaf necrosis (GUIDI & al. 1986),

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chloride ion accumulation in foliar tissues (GUIDI & al. 1988), impaired water content (BADOT & al. 1995), altered leaf-atmosphere interfaces (RADDI & al. 1994).

Pinewoods (*Pinus halepensis* Mill., *P. pinea* L., and *P. pinaster* L.) are widespread across the Mediterranean basin. Our aim was to compare response and recovery of the three Mediterranean pine species after artificial spraying of surfactant-polluted sea aerosols.

Material and Methods

Thirty plants each of 5-yr-old *P. pinea, P. halepensis* and *P. pinaster* were sprayed with sea water mixed with the linear alchyl sulphonate dioctyl sodium sulphosuccinate (commercial name: AEROSOL®-OT) at the following concentrations: 0 (SW), 5, 10, 15, 30, 60, 120, 250, and 500 mg/l, namely PSW (polluted sea water) followed by the mg/l of surfactant. A second control group was treated with de-ionized water (DW). To ensure the sea water contained no surfactant, it was examined using the Methylene Blue Active Substances method (MBAS) (LONGWELL & MANIECE 1955). Spraying was performed once a week for five weeks, with an air compressor in a greenhouse at 20 ± 2°C temperature, 60% RH, 11 hours daylight. Drops measured 70 to 150 µm in diameter. In each treatment, three plants per species were sprayed to dripping point (50 ml). To avoid soil contamination, the pots were covered with a polyethylene film during spraying. Irrigation was regularly provided. In order to measure the quantity of surfactant accumulated on the needles, 20 g fresh needles from each plant were washed in a litre of de-ionized water at the end of treatments, and the washing solution was analysed using the MBAS method. Observations were carried out on current year needles, on three plants per treatment, both at the end of the five sprayings, and 60 days after the last treatment. Visible damage was assessed on 100 needles per plant, according to GELLINI & al. 1985. The quantity of chloride in needles was determined by means of the volumetric method (AMERICAN PUBLIC HEALTH ASSOCIATION 1992) on 10 g fresh needles per plant. Structural alterations to stomata were observed on 5 needles per plant, by means of a Cambridge Stereoscan 200 SEM, following the protocol by RADDI & al. 1994 for calculating a Stomatal Damage Index (SDI). Water content ($WC = ((fw - dw) / dw) * 100$) was measured on 200 mg fresh needles collected from each plant. Linear regressions were applied to test the species-specific responses over time. A canonical discriminant analysis was carried out for all the data at 60 days after sprayings. Samples were plotted in a scattergram where the axes represent the first two canonical variables. Analyses were performed by Statistica 5.1 for Windows.

Results and Discussion

To observe the species-specific responses over time, the values recorded two months after the last spraying were correlated with those measured immediately after it (Fig. 1). Except for needle water content – which remained constant - all parameters worsened as time passed. This is evidence that surfactants continue to act even after exposure and confirms that a condition of persistent pollution in coastal regions can severely affect plant function. Such a “delayed-action” effect may be due to the progressive melting of surfactant deposits on needles because of air humidity, and/or to a cascade of metabolic perturbances determined by alterations at stomatal level and increased chloride content in the tissues. *P. pinaster* was the species in which this worsening was less marked, especially as far as structural stomatal damage was concerned (Fig. 1).

The canonical discriminant analysis tended to differentiate among the treatments as surfactant concentrations increased (Fig. 2 down), even if the proportion of cases not classified correctly was only 18% of the total. The first two roots explained a 99% of variability when the group variable was the treatment. The stepwise method suggested the responses most important to discriminate among treatments were chloride for root1, and chloride and water content for root2, thus confirming the important role of chloride in determining the phytotoxicity of surfactant-polluted sea aerosols (GUIDI & al. 1986, 1988).

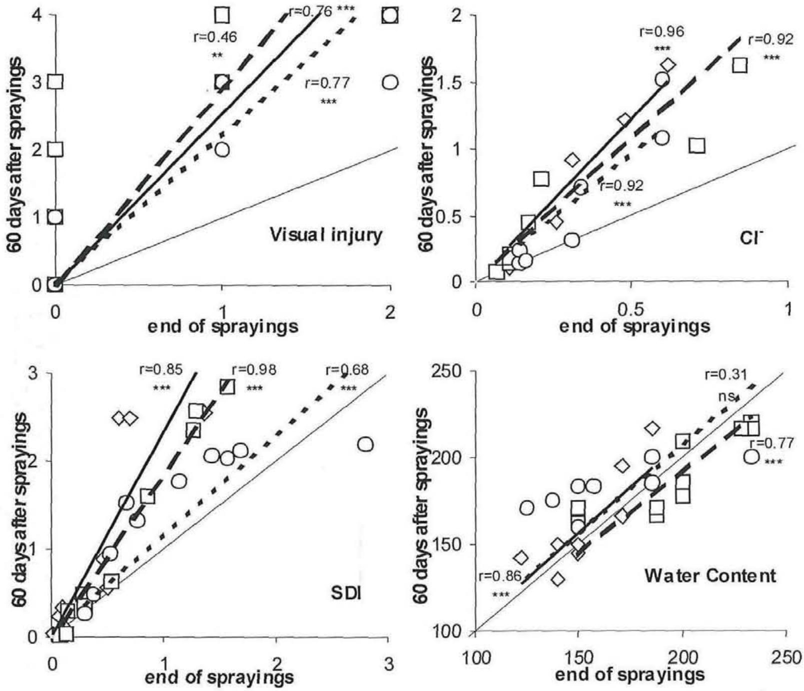

Fig. 1. Linear regressions of the visible injury (a.u.), chloride content (% dw), Stomatal Damage Index (a.u.) , and water content (% dw) values at 60 days after the sprayings versus the values at the end of the sprayings, in needles of *Pinus halepensis* (◊ ——), *P. pinea* (□ – – –), and *P. pinaster* (○ - - - -). The thin line indicates no change (1:1). n = 10

Neither unpolluted sea water (SW) nor fresh water (DW) caused any damage to any of the species (Fig. 2 down). This observation confirms that marine aerosol becomes toxic because of surfactants (DOWDEN & LAMBERT 1979, GELLINI & al. 1985, 1987, BADOT & GARREC 1993). In all species, the threshold for the onset of damage was around 30 mg/l (Fig. 2 down). This value corresponded to the accumulation of 1.95 mg/l surfactant on the needles. In fact, the quantity of surfactant sprayed (x) and the amount deposited on the needles (y) showed a linear correlation ($r > 0.99$) according to the equation $y = 0.065x$, without species-specific differences. Twenty years ago, concentrations of MBAS measured in Mediterranean aerosols could reach levels of 18-29 mg/l (BUSSOTTI & al. 1983), whereas today peaks of 0.96-1.30 mg/l have been recorded (NICOLOTTI & al. 2001). Frequent wind storms may lead to accumulate surfactants deposited on foliage, which reach levels of 1.5-2.0 mg/l on the needles of Mediterranean pines (NICOLOTTI & al. 2001). This suggests that European legislation on surfactants is still insufficient to protect Mediterranean pinewoods from this form of pollution.

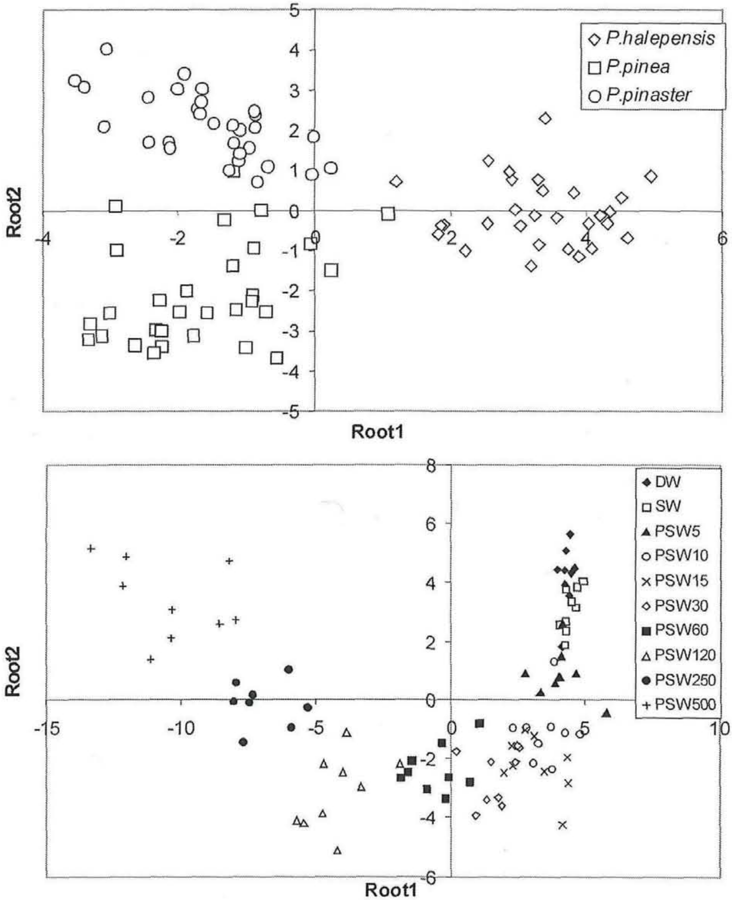

Fig. 2. Canonical discriminant analysis on all the variables and all the species at 60 days after the treatments (DW, deionized water; SW, sea water; PSW5-500, 5 to 500 mg/l surfactant in sea water).

The canonical discriminant analysis clearly differentiated the responses of the three species (Fig. 2 up), and 97% of the total cases was correctly classified. A 100% of variability was explained by the first two roots when the group variable was the species. The responses most important to discriminate among the species were SDI for root1, and visible injury and chloride content for root2, suggesting that several parameters should be analyzed when species-specific responses to surfactants are investigated. The species that displayed the most marked response to the surfactant was *P. pinea* (Fig. 2 up), thus confirming its high sensitivity to surfactant-polluted sea aerosol (GUIDI & al. 1986). Damage to stomata was initially more severe in the species that was - as a whole - less sensitive to surfactants, i.e. *P. pinaster*, which later proved to be capable of recovering (Fig. 1). Although field observations show that all species are sensitive to injury by surfactant-polluted sea aerosol, our findings suggest that sensitivity is species-specific (*P. pinea* > *P. halepensis* > *P. pinaster*). Further investigations may identify less sensitive species, suitable to be used in surfactant-polluted coastal zones.

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