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**Original article**

# Water relations of spruce seedlings sprayed with a surfactant

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**Summary** — This work assesses whether the water relations of Norway spruce (*Picea abies* Karst) seedlings are affected by repeated treatments with ABS (sodium dodecylbenzensulphonate). Beginning in August 1987 4-yr-old spruce seedlings were sprayed weekly with a 50 g $m^{-3}$ ABS solution. Two experiments were performed in 1988 and 1989, during which water potential, transpiration rates and stomatal conductance were measured on treated and control plants, which were either well-watered or exposed to periods of drought of different duration. Net photosynthesis and specific leaf area of needles were also measured. ABS did not alter transpiration or stomatal conductance associated with xylem water potential and had no effect on net photosynthesis and specific leaf area.

***Picea abies*** **/ Pinaceae / Norway spruce / surfactant / pollution / transpiration / stomatal conductance / water potential**

**Résumé — Relations hydriques des plantules d'épicea commun pulvérisées avec un agent tensio-actif.** *Ce travail a été conduit afin d'évaluer si des traitements avec des solutions d'ABS (dodecylbenzensulphonate de sodium) influencent les relations hydriques des plantules d'épicea (*Picea abies *Karst). Pour cela, des plantules âgées de 4 ans ont été pulvérisées, toutes les semaines à partir d'août 1987, avec une solution d'ABS à la concentration de 50 g* $m^{-3}$*. Deux expériences ont été réalisées, en 1988 et 1989, pendant lesquelles on a mesuré le potentiel hydrique, la conductance stomatique et la vitesse de transpiration des plantules, les unes irriguées, les autres soumises à des périodes de sécheresse de durées différentes. On a mesuré aussi la photosynthèse nette et la surface foliaire spécifique des aiguilles. L'ABS n'a pas alteré la transpiration et la conductance stomatique associées avec le potentiel hydrique et n'a pas eu effet sur la photosynthèse nette et la surface foliaire spécifique.*

**Picea abies** ***/ Pinaceae / épicea / tensio-actif / pollution / transpiration / conductance stomatique / potentiel hydrique***

## INTRODUCTION

In recent years it has been proposed that air pollutants may directly affect leaf metabolism and vitality, playing a role in the so-called "forest decline" phenomenon (Schütt and Cowling, 1985; Karhu and Huttunen, 1986; Sauter and Voss, 1986). Indeed, alterations of leaf surfaces by atmospheric pollutants have been demonstrated in conifer and broadleaf trees. These findings concern both field observations and experiments carried out under controlled conditions, the most frequently described effect being the degradation of the epicuticular waxes in the stomatal antechamber (Percy and Riding, 1978; Cape and Fowler, 1981; Huttunen and Laine, 1983; Crossley and Fowler, 1986; Rinallo *et al,* 1986; Sauter and Voss, 1986; Sauter *et al,* 1987; Barnes *et al,* 1988; Raddi and Rinallo, 1989; Rinallo and Raddi, 1989a). The effect of pollutants on wax morphology of conifer needles has been reviewed by Riederer (1989).

The physiological importance of surface waxes on leaves is well known (Hall and Jones, 1961). The observed morphological alterations may support the idea that air pollutants have the capacity to alter the transport properties of the cuticle and impair the gaseous diffusional processes. For instance, in conifers the wax-filled stomatal antechamber contributes two-thirds of the resistance to the water vapour diffusion (Jeffree *et al,* 1971) and it is reasonable to suppose that this resistance may increase after the degradation of the so-called structural waxes into a more amorphous and less porous material.

Recently, alterations of the epicuticular waxes following treatments at low concentration with a surfactant (sodiumdodecylbenzensulphonate, ABS), which is an important component of synthetic detergents, have been described in broadleaf seedlings (Rinallo and Raddi, 1989b). ABS was also demonstrated to have a role as water and air pollutant and is supposed to greatly contribute to the decline of forest coastal vegetation in Tuscany, Italy (Gellini *et al,* 1983, 1985).

For these reasons, research has been carried out to determine whether or not there is a change in the water relations of spruce (*Picea abies* Karst) seedlings subjected to repeated treatments with ABS at low concentration. In the area where the study has been performed ABS concentrations similar to those used in the present work can be detected (Rinallo and Raddi, 1989b).

## MATERIALS AND METHODS

### *Plant material and treatments*

Four-yr-old potted spruce seedlings of alpine provenance (Val di Fiemme, Trentino, Italy) were maintained in the glasshouse. From August 1987, 100 randomly sampled seedlings were sprayed weekly until the leaves were saturated to drip point with a 50 g $m^{-3}$ ABS (sodium dodecylbenzensulphonate with a non linear alkyl group) aqueous solution. Another lot of 100 randomly sampled seedlings was used as control plants.

### *Experiment 1*

The plants used in this experiment underwent a minimum of 35 and a maximum of 50 treatments with ABS solution. The needles produced in spring 1988 (current needles) had undergone between 4–12 treatments at the time of measurment.

The main objective in this experiment was to study whole plant transpiration and stomatal conductance under a wide range of plant water status, which was obtained by imposing periods of drought of different duration. In spring 1988 the ABS-treated and the control plants were divided into 2 groups (A and B) of 50 plants each, which underwent different irrigation schedules (fig 1). Irrigation was carried out by supplying the same amount of water to each pot every time.

Measurements of xylem water potential, whole plant transpiration and stomatal conductance were carried out at different dates (fig 1). Measurements were performed simultaneously on each plant, approximately every 2 h from 7:00 h am to 6:00 h pm. Three plants per group were used for measuring water potential and 3 more plants for measuring transpiration and stomatal conductance. Different series of plants, randomly sampled from group A and B, were measured at each date. On August 3 1988, net photosynthesis was also measured at 3 different times of day.

The xylem water potential was measured with a pressure chamber on 1-yr-old twigs, sampling 1 twig per plant. The whole plant transpiration was measured by weighing the plants at regular intervals to the nearest 0.1 g using a top-loading balance, after sealing the pots in plastic bags to prevent water loss from the soil. The stomatal conductance and net photosynthesis were measured respectively with a null-balance steady state porometer (Li-1600, Li-Cor Inc, Lincoln, Nebraska, USA) and with an IRGA portable open system (LCA2, ADC Ltd, Hoddeson, Herts, UK), on 3 current twigs per plant.

The leaf area of entire seedlings and measured twigs was determined at the end of each measurement day, using an optical area meter (Li-3000, Li-Cor Inc, Lincoln, Nebraska, USA).

The specific leaf area of both ABS-treated and control plants was determined as the ratio between the projected leaf area and the needle dry weight; dry weight was measured to the nearest 0.1 mg after 48 h in an oven at 60 °C.

Differences between means were evaluated by a *t*-test at the 5% level.

## *Experiment 2*

The plants sampled for this experiment had undergone > 90 treatments with the ABS solution. At the time of measurement the current needles, produced in spring 1989, had undergone at least 10 treatments with the surfactant.

The main objective of this experiment was to assess the variation of whole plant transpiration

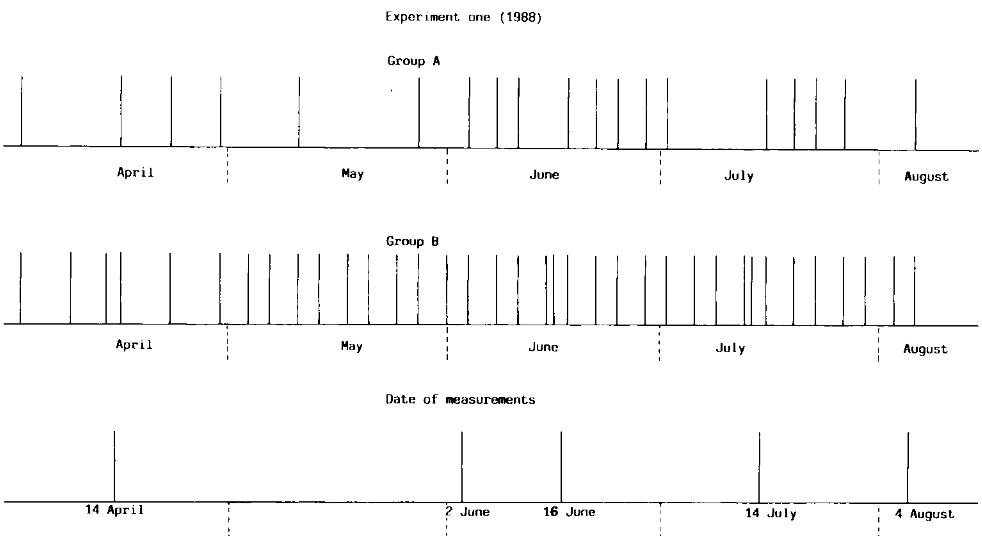


**Fig 1.** Experiment 1. Irrigation schedule for the plants in groups A and B. The vertical bars indicate the days when irrigations took place; measurement days are also shown.

and stomatal conductance in ABS-treated and control plant subjected to a condition of progressive and severe water stress. In June 1989, 8 control and 8 treated plants, both well-watered, were sampled for uniformity and subjected to drought treatment (water supply was interrupted between June 29 and July 21). The measurements were carried out as described in experiment 1, using 4 plants per group for destructive measurements (xylem water potential) and 4 other plants for non-destructive measurements (transpiration and stomatal conductance). The water potential was measured at dawn and the transpiration rate was assessed on a daily basis. Stomatal conductance was measured at irregular intervals, when photosynthetic active radiation was > 1000 $\mu molm^{-2}s^{-1}$.

## RESULTS

### *Experiment 1*

In the course of this experiment the early morning water potential (measured at 7:00 h am) ranged from a maximum between –0.5 and –0.7 MPa on April 14 to a minimum between –1.8 and –2.6 MPa on July 14, and the plants experienced daytime water potentials as low as –3.6 MPa. Daily patterns of water potential, transpiration rates and stomatal conductances did not differ between the ABS-treated and the control plants.

The physiological effect of the applied treatments may be better evaluated by considering the relationship between the measured variables. For instance, the variation of the whole plant transpiration as a function of the changing plant water status can be assessed by plotting the transpiration rates against the xylem water potential. This is shown in figure 2.

The response of transpiration to water potential follows a characteristic pattern (fig 2). Transpiration rate was not affected by the water potential when this was between –0.4 and –1.6 MPa. Indeed, within this range of water potential the transpiration rate varied from 10–90 mg $m^{-2}$ $s^{-1}$, with most variation probably being determined by the changing micrometeorological conditions within the glasshouse, where the relative humidity varied from 48–95% the air temperature from 11–32 °C and the solar radiation from 100–750 W $m^{-2}$ during the measurement days. The water potential threshold which caused transpiration to decline may be set between –1.4 and –1.8 MPa. Indeed, below –1.8 MPa no transpiration rates > 10 mg $m^{-2}$ $s^{-1}$ were measured. It is worth noting that no different behaviours were displayed by ABS-treated and control plants (fig 2).

The stomatal conductance of current needles showed a wide range of variation, from zero to 0.9 cm $s^{-1}$. In order to relate the stomatal function to plant water status, conductance was plotted against the xylem water potential (fig 3). From this figure, it is evident that the stomatal conductance was not affected by the water potential when this was > –1.4 MPa, and decreased to zero when the water potential fell between –1.4 and –1.8 MPa. Even in this case, the ABS-treated and control plants behaved in the same way.

The experiment described above was repeated using spruce seedlings from an Apennine population (Campolino, Tuscany), with basically the same results (data not shown).

The net photosynthesis was measured on August 3, at 3 times of day. In plants displaying a water potential > –1.6 MPa a mean value of 2.0 µmol $m^{-2}$ $s^{-1}$ was assessed. The control and the ABS-treated plants displayed net photosynthesis values in the range 2.03–2.71 and 1.19–2.17 µmol $m^{-2}$ $s^{-1}$, respectively; the observed differences were not statistically significant. The mean value of net photosynthesis fell to 0.35 µmol $m^{-2}$ $s^{-1}$ in plants exhibiting a water potential < –1.6 MPa.

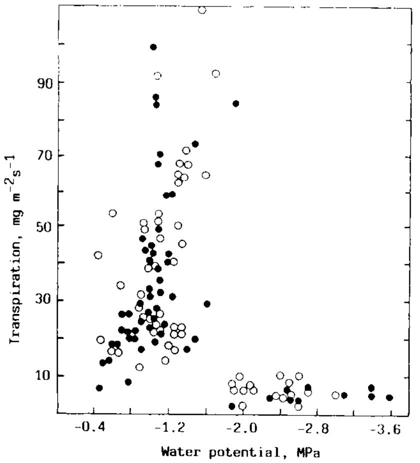


**Fig 2.** Experiment 1. Transpiration rates plotted against the xylem water potential. Solid and open circles indicate ABS-treated and control plants, respectively.

The specific leaf area of current and older needles was measured on samples taken on August 3 1988 from control and ABS-treated plants. Mean values for current needles were in the range 39.1–40.4 $cm^2\ g^{-1}$, and for older needles in the range 40.7–41.5 $cm^2\ g^{-1}$. No statistically significant differences were found between ABS-treated and control plants.

### *Experiment 2*

During this experiment the plants experienced a pronounced condition of water stress. Indeed, the mean value of dawn water potential dropped from –0.2 MPa on 29 June to –3.8 MPa on 21 July. The temporal variation of dawn water potential, whole plant transpiration and stomatal conductance is given in figure 4 for the ABS-treated and the control plants. During the first week of drought dawn water potential was > –1.0 MPa; afterwards, water potential decreased regularly. Similarly, the daily transpiration remained fairly constant, with values > 30 mg $m^{-2}\ s^{-1}$ during the first week, suddenly declining when the dawn water potential dropped < –1.0 MPa and falling to zero when the water potential decreased < –1.5 MPa. Stomatal conductance displayed a similar pattern, and fell to zero when the dawn water potential was < –1.5 MPa.

Even during this experiment the behaviour of the ABS-treated and the control plants was the same. Indeed, no statistically significant differences were found between the physiological values displayed by the 2 groups of plants at the various dates.

## DISCUSSION AND CONCLUSIONS

The experiments carried out made it possible to study the water relations of spruce seedlings (both control and ABS-treated plants) exposed to periods of drought. In experiment 1, transpiration rates and stomatal conductances were greatly reduced when the xylem water potential fell < –1.4 MPa. A threshold effect of the dawn water potential on daily transpiration rates and stomatal conductances was also found in experiment 2. These results confirm that in spruce species water stress becomes a reducing factor for transpiration and stomatal conductance when the water potential reaches some threshold value (Jarvis, 1976; Beadle *et al*, 1978; Havranek and Benecke, 1978).

As far the main point addressed in the present work is concerned, *ie* the assessment of a possible effect of treatments on plant water relations, it is worth noting that the variation of transpiration rate and stomatal conductance, as a function of the plant

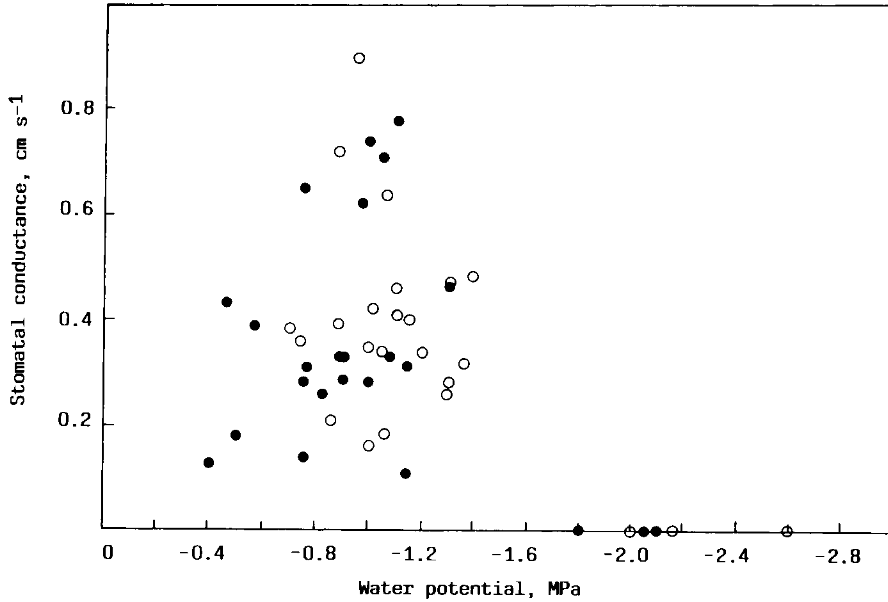


**Fig 3.** Experiment 1. Stomatal conductance plotted against water potential; solid and open circles indicate ABS-treated and control plants, respectively; each point represents the mean of 3 plants; bar indicate standard errors; their absence indicates that the standard error is smaller than the graphic symbol.

water status, was the same in ABS-treated and control plants. In particular, no lower transpiration rates and stomatal conductances were observed in treated plants. Moreover, in treated plants the stomata closed regularly in response to the decreasing xylem water potential. In other words, in the present experiment the applied treatments did not alter stomatal functionality and decrease water vapour diffusion. No unregulated water loss from the needles was also assessed, since very low transpiration rates were measured after stomatal closure. Furthermore, no evidence arose of a decreasing photosynthetic capacity in treated plants.

That prolonged treatments with ABS did not alter plant water relations may be considered as a rather surprising result. Indeed, only a few treatments with ABS, at the same concentration as those used in the present work, caused appreciable effects on wax morphology, in particular aggregations of the fibrillar waxes and fissures in the epistomatal chamber in 5-yr-old Norway spruce seedlings (Raddi, 1990; personal communication). Furthermore, after spraying young plants of *Pinus pinea* with a 40 g $m^{-3}$ ABS solution, Gellini *et al* (1983) observed that about half the needles were damaged after a single treatment with the percentage rising to 80% after the application was repeated. After 35–40 treatments with ABS solution at 50 g $m^{-3}$, a significant decrease in chlorophyll content in 1-yr-old needles of Norway spruce seedlings was also observed (Heimler *et al*, 1989).

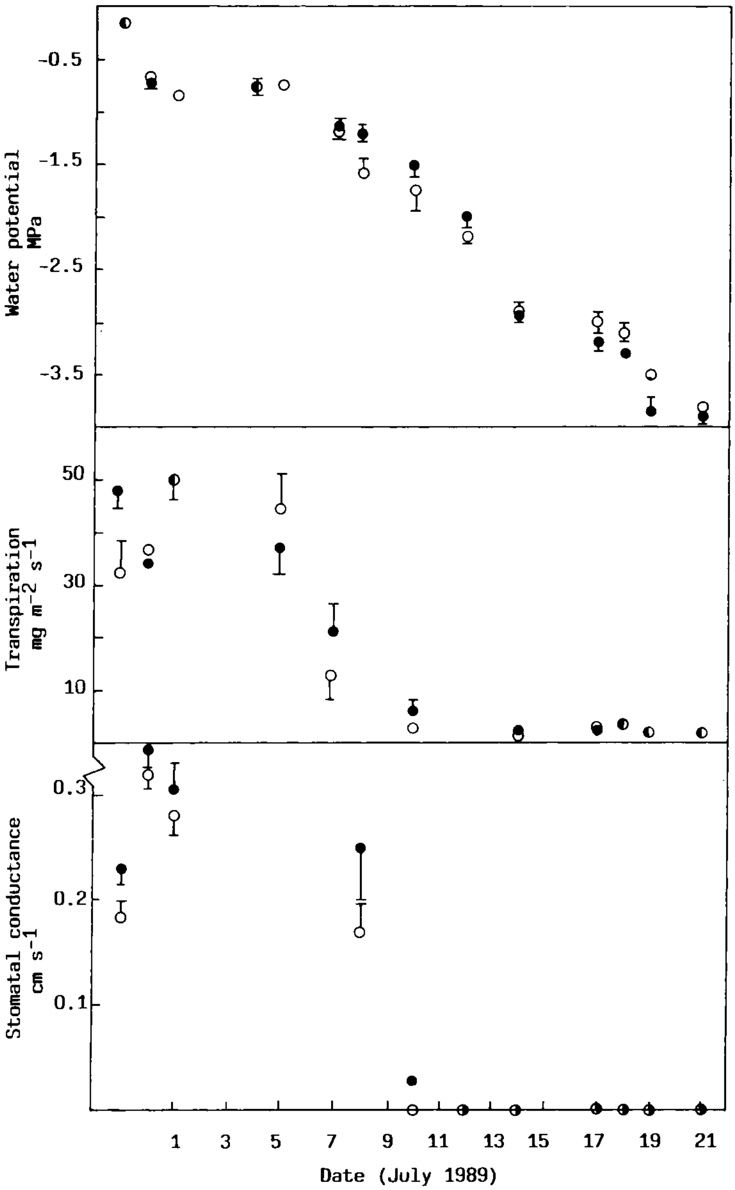


**Fig 4.** Experiment 2. Variation of dawn water potential, daily transpiration rate and stomatal conductance during the period of drought, June 29–July 21; solid and open circles indicate ABS-treated and control plants, respectively; half-solid circles indicate both ABS-treated and control plants; each point represents the mean of 4 plants; bars indicate standard errors, their absence means that the standard error is smaller than the graphic symbol.

On the other hand, the results presented here seem to be in accordance with the statement by Riederer (1989) that in conifers "positive evidence linking changes of wax morphology to reduced stomatal conductances for gases or water vapour are lacking". To account for the absence of alterations in vapour diffusional processes and stomatal regulation, is assumed that needles are able to continuously produce new wax tubes, which replace the degraded ones (Riederer, 1989). This hypothesis is supported by the observation of a short reconstitution time (6 wk) of wax deposits in the epistomatal chamber of spruce needles (Kurbasik, 1989; cited in Riederer, 1989). A partial regeneration of wax structure after replacement of an acid treatment ($H_2SO_4$ and $HNO_3$ solutions at pH 3.5) with distilled water was also observed by Rinallo *et al* (1986) in spruce and fir seedlings.

Furthermore, the lack of unregulated water losses from the needles may be considered as evidence of unchanged cuticle permeability; this may be related to the absence of damage to the intracuticular waxes (waxes embedded within the cuticle), which probably represent the main resistance to transport across the cuticle and which have, on the other hand, scarcely been investigated (Schönherr, 1982; Riederer, 1989).

However, it is worth remembering that in the present experiment the plants were protected from atmospheric depositions. The interaction between a wax-damaging substance like ABS and atmospheric deposition may produce different effects on the plant. For instance, Gellini *et al* (1983) found that the level of apparent toxicity of this surfactant is lowered to 5–10 g $m^{-3}$ when, in order to simulate atmospheric deposition from the seaside, sodium chloride is added to ABS. In general, the potential for the recovery of the degrated epicuticular waxes may be impaired by the action of other pollutants, entering the plants *via* the soil or the crown and blocking or slowing down important metabolic pathways. Further research is needed in order to test this hypothesis.

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