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How Reproducible are the Reactions of Stress Indicators for Sitka Spruce Treated with Acid Mist?

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Summary

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Results of previously published experiments based on open-top chambers and field exposure systems with seedlings, mature grafts and physiologically mature trees (<13m tall) of Sitka spruce exposed to pH 2.5 mist are discussed briefly for the purpose of identifying reproducible bioindicators of acid mist induced perturbation, i.e. physiological disturbance causing stress (reduction in performance). None of the bioindicators studied, i.e. visible injury, foliar nutrient concentrations, ion leakage with and without freezing, and growth responded consistently to treatment. Substantial treatment by environment interactions were detected in treatment effects on visible injury and foliar nutrient concentrations. But the enhancement of ion leakage from unfrozen shoots and changes in foliar nutrient concentrations in treated compared with untreated shoots reliably indicated acid mist-induced stress.

Introduction

Since 1990, the Institute of Terrestrial Ecology (ITE) has investigated the effects of acid mist on a range of variables; visible injury to current year shoots, ion leakage before and after freezing and foliar nutrient concentrations for spruce grown in both open-top chambers (OTCs) and the field. Work in the dual environments of the relatively controlled OTC and the semi-controlled field situation, employing similar treatments and experimental protocols, has provided a unique opportunity to: (1) evaluate interactions between the experimental treatment

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and environmental conditions and (2) identify reproducible bioindicators from these variables.

Results are described from the following experiments with Sitka spruce (effect of spraying frequency SHEPPARD & al. 1992; effects on mature grafts I (frost hardiness and nutrients) SHEPPARD & al. 1994; effect of soil type LEITH & al. 1995; effects on mature grafts II (visible injury) SHEPPARD & al. 1995c; effects on mature trees SHEPPARD & al. 1995a; effects on mature trees (hypothesis) SHEPPARD & al. 1995b). This paper draws on these results to specifically assess the suitability of the measured variables as bioindicators - variables whose response to treatment is independent of the environmental conditions in which the experimental plants are grown.

Methods

Controlled environment experiments - OTCs

Typical treatments included the following ions in equimolar concentrations, NH_4^+, NO_3^- ,H^+, SO_4^{2-} at 1.6 and 0.01 mol m^{-3} (pH 2.5 and pH 5 control). The volume of spray, droplet size 40-90 µm, was calculated on an area basis where 1 litre of solution provided the equivalent of 1 mm rain m^{-2}. Treatments consisted of 2 mm x 2 wk^{-1} or 1 mm x 4 wk^{-1} applications. Seedlings and grafts (physiologically mature tissue on seedling rootstock) were sprayed in open-top chambers (OTCs) (see FOWLER & al. 1989). Plants were grown in a peat-based compost fertilized with Osmocote (18% N, 6% P, 11% K: 5g $plant^{-1}$) except where the effects of the soil type on plant response were specifically examined. The soil experiment utilized the top 5 to 10 cm of sieved, unfertilized soil from two forest and one arable site (see below). Treatments commenced at budburst and were discontinued in early winter. The OTCs were normally fitted with roofs to exclude rain, but once, rain was allowed to wet the foliage (SHEPPARD & al. in prep). Frost hardiness was determined from artificial freezing tests on detached shoots, using enhanced ion leakage rates to indicate damage (MURRAY & al. 1989).

Field exposure with seedlings

Bare-rooted 2 year old seedlings were planted out in a weed free, base rich soil (as above), top dressed with Osmocote fertilizer (5g $plant^{-1}$). The seedlings at 12.5 cm spacing were divided into 4 blocks separated by temporary polythene walls and sprayed by hand. Treatments ran from budburst, with 2 mm m^{-2} pH 2.5 (1.6 mol m^{-3}) mist applied when the weather was overcast, dry and still (i.e. spasmodically). The plants received the equivalent of 106kg S, 112kg N and 3.3kg H ha^{-1}.

Field exposure with mature trees

A single clone named DF, planted at 2.5m spacing on an ex-arable site (see above) was sprayed with pH 2.5 mist, 100mm per year for 3 years, nominally 2 x 2 mm m^{-2} wk^{-1} when local conditions permitted. A complete site, treatment description and results are given in SHEPPARD & al. 1995 a, b. Treatment chambers, with permanent scaffolding supporting roller blinds which were pulled down for the duration of spraying, enclosed 4 trees of equal stature. A 5% slope across the site had resulted in a differential growth and canopy structure. This was accounted for by grouping the sets of 4 trees into 5 height classes per treatment. Control trees were scattered over the plot, were not arranged in blocks of 4 and were unchambered.

Results

Effects of acid mist on sitka spruce in field and OTC experiments

Visible injury did not occur to any significant extent on seedlings exposed to pH 2.5 mist 2 x 2 mm m^{-2} wk^{-1} receiving 2 x 2 mm wk^{-1} in OTCs. Increasing the application frequency to 4 x 1mm m^{-2} wk^{-1} damaged up to 20% of needles on many of the plants. Recently transplanted seedlings exposed to acid mist, at pH 2.5 2 x 2 mm wk^{-1} misting frequency were visibly injured within 3 weeks. The 4 x 1 mm wk^{-1} treatment frequency injured each of the 5 grafts. Initially the degree of injury was related to the stage of budburst but from September onwards injury increased in all clones and at more similar rates. Characteristic visible injury symptoms, chlorosis and necrosis which progressed from the tip backward, were observed irrespective of clone in OTCs.

Visible injury was not observed on transplanted bare rooted seedlings in the field or on the 10 -13 m tall, mature trees even after 3 years' exposure to pH 2.5 mist.

Ion leakage rates from unfrozen shoots (pot grown seedlings in OTCs) were not increased in the 2 x 2 mm m^{-2} wk^{-1} acid mist treatment but were increased at the 4 x 1mm m^{-2} wk^{-1} treatment. Likewise, the 4 x 1mm wk^{-1} pH 2.5 mist treatment significantly enhanced leakage rates in shoots from mature grafts. Shoots from transplanted seedlings grown in natural soils in the OTCs also showed enhanced leakage rates with acid treatment even at 2 x 2 mm m^{-2} wk^{-1} frequency at pH 2.7.

No significant effects of pH 2.5 mist treatment on electrolyte leakage rates from unfrozen shoots taken from field grown seedlings were found. Leakage rates from treated mature tree shoots were always higher than those from the respective controls, though rarely significantly higher.

Pot raised Sitka seedlings receiving 2 x 2 mm m^{-2} wk^{-1} of pH 2.5 mist in OTCs showed no significant effect of treatment on frost hardiness. Seedlings and grafts exposed to 4 x 1 mm wk^{-1} were less hardy than controls and except for one of the 5 clones, clone DF, significantly so. The bare rooted seedlings grown in soil where treatments were "diluted" to 2 x 2 mm m^{-2} pH 2.7 also showed reduced levels of hardiness, significantly reduced on 1 of the 3 soil types.

The frost hardiness of shoots from bare rooted seedlings treated with pH 2.5 mist in the field was the same as those from control seedlings indicating no treatment effect. Results from the mature trees indicated a treatment by height interaction. The frost hardiness of shoots from the large trees was not influenced by acid mist whereas shoots from small trees were significantly less hardy than control shoots on half of the sampling occasions.

Significant changes in N, P, K, Ca and Mg concentrations were rarely observed to result from pH 2.5 mist treatment in OTCs irrespective of age/size, misting frequency, physiological status of the plant or soil type. By contrast large, significant increases in foliar S concentrations were common in both seedlings and the mature grafts. However, the proportional increase in % S in treated grafts compared with control grafts was usually less than for seedlings. Actual foliar S concentrations were also lower.

No significant changes in N, P, K, Ca, Mg & S were seen in field grown seedlings. Foliar S concentrations were enhanced in the mature tree exposure, but by <10%, which was rarely significant. After 3 years of acid mist treatment foliar S concentrations decreased slightly. Foliar P concentrations tended to decrease as treatments progressed i.e. in the latter part of the second and third year, particularly in the bigger trees.

Assessments of growth: foliage, branch and root dry weights, root collar diameter and height were generally inconclusive for both seedlings and grafts in the OTCs (MACKLON & al. 1995). Small changes in foliar characteristics such as needle area, wax production, wettability and particularly of Ca^{2+} ion leaching from epidermal cells, were observed, but these changes were non-significant.

In field treated seedlings neither root collar diameter nor height were significantly affected by treatment. In the mature trees, growth defined as relative stem area increment (RSAI) proved to be a very sensitive indicator of perturbation and also discriminated between the response of trees of different size. Between July 1990 (spraying began May 1990) and May 1993 the large acid treated trees had 85% smaller RSAI's than their equivalent control trees while the small trees had 31% bigger RSAI's than equivalent control trees.

Discussion

The relatively controlled environment of roofed OTCs permits treatment on a very regular basis independent of constraints imposed by local weather conditions. In the field, spraying cannot be undertaken when it is raining or if it is windy, but neither of these factors restrict OTC spraying regimes. Thus in the field a high (4 x 1mm wk^{-1}) spraying frequency was rarely achieved and spraying on a regular basis on consecutive days was not possible. Furthermore, in the field rain wetted the foliage removing ions from the leaf surface. Although such a process can enhance leaching it also lowers the ion concentration on the leaf surface reducing the likelihood of injury (SHEPPARD unpubl.) Plants grown in chambers experience constant turbulence (2 - 3 m^2 s^{-1}) whereas in the field, although maximum wind speeds are often greater, frequently there was no wind to enhance rates of evaporation. Evaporation is an important factor in the development of visible injury (JACOBSON & al. 1990) since it concentrates the ion composition of the solution on the leaf surface and promotes ion exchange reactions (MILNE & al. 1988). Acid leaching of epidermal Ca^{2+} was much greater in OTC treated grafts than in the field (WULFF & al. 1996). A further factor likely to influence the response of OTC grown trees in comparison with field grown trees is that cuticles formed under glass have different properties from those formed in the field and as a result may be more susceptible to acid mist perturbation (CAPE & PERCY 1993). So, can visible injury, leaky membranes and higher foliar S concentration which are characteristic responses of OTC experiments, but absent or much less frequently observed in the field, be explained simply by such experimental artefacts as described above?

Results of acid mist experiments using red and Norway spruce, in addition to Sitka spruce, at ITE and elsewhere (JACOBSON & al. 1990, HELLER & al. 1995) suggest that visible injury is foliar mediated and is most likely to develop when: Foliage is young with incompletely formed cuticles. Plants are stressed, or growth is not the main sink for carbon. The concentration of H^+ & SO_4^{2-} ions on the leaf surface is high such as when: The exposure frequency is high. Environmental conditions promote drying. The cuticle is frequently rewetted. Plants are protected from rain. Injury is more likely when N is limiting, indicated by low foliar N concentrations, and coincides with elevated foliar S concentrations. Neither plant age, size or soil type influence plant susceptibility to acid mist induced injury. The rapid appearance of visible injury on very young needles in chambers most likely reflects rapid uptake of SO_4^{2-} and H^+ ions across poorly differentiated cuticles in response to the high frequency ion concentrating effects (SHEPPARD 1994). The strength of evidence favours the conclusion that visible injury caused by acid mist at ion strengths of 1.6 mol m^{-3} (pH 2.5) at a maximum exposure frequency of 4 x wk^{-1} is primarily due to an interaction of the experimental environment with the acid mist treatment. In the field the occurrence of the combination of environmental conditions found in chambers is unlikely, except possibly at the cloud base. Incidences of visible injury attributable solely to acid rain or cloud in the field are rare.

The development of visible injury required the simultaneous occurrence of more than one influence from the following aforementioned; plant health, phenological state and environment. But physiological disturbances as indicated by enhanced electrolyte leakage rates and light microscopy (WULFF & al. 1996) were seen in apparently visibly healthy tissue. The absence of visible injury cannot therefore be used to discount stress and physiological perturbation. In these experiments and some with red spruce (CAPE & al. 1991) acid mist treatment was seen to accelerate the rate of electrolyte leakage from shoots. While an element of genetic variability was observed the environmental effect appeared to be minimal suggesting that enhanced electrolyte leakage rates may be a sensitive indicator of acid mist damage. Increased ion leakage rates reflect an increase in dead plasmolysed cells or an increase in the number of cells whose membrane integrity has been damaged, or both. Ion leakage rates in response to freezing also, were fairly insensitive to an environment by treatment interaction. There are sound reasons 'supporting' the stability of frost hardiness as a bioindicator. In response to environmental signals such as falling temperatures, northern temperate trees cease growth and reprioritize their carbon sinks. The production of cryoprotectants, sugars (CHO) and the concomitant fall in demand for S (for growth) appear to enhance the potential for acid mist perturbation (SHEPPARD 1994).

The scale of change in foliar S concentrations was apparently strongly related to plant size and in particular to the ratio of new to old foliage (SHEPPARD & al. 1994). This observation has important implications for the use of foliar nutrient concentrations as bioindicators (GRILL & al. 1995). Threshold values must be described in relation to tree size. Our studies suggest that seedlings tolerate much higher S concentrations in their foliage than mature trees. One of the drawbacks of using % element per dry weight is that it neither quantifies the amount of the element which is physiologically active, nor does it indicate its chemical form or location in the cell. The level of S enhancement in treated plants over control plants may represent a more useful bioindicator of S exposure. The proportional change in % S in foliage from a big tree, although much smaller than for seedlings due to the lower dose: foliage weight dilution ratio, may be just as biologically significant.

Measurement of growth parameters in OTC studies have often failed to detect acid mist effects except where plants were N deficient and responded positively to the additional N in acid mist (EAMUS & FOWLER 1990). The size of most OTCs and thus the numbers and size of experimental plants underlain by an indeterminate genetic component (SHEPPARD & al. 1994) means that growth responses in chambers are unlikely to yield statistically significant effects. By contrast, the rapid rates of growth of the mature trees in the field exposure provide a much greater likelihood of detecting significant effects. This was further improved in this study by the use of a single genotype. Changes in carbon partitioning i.e. the utilization of carbon by the tree either for growth of different parts (roots, stem, foliage) or maintenance and repair are an accepted response to stress (OREN & al. 1988) which is why tree rings have been used successfully to indicate previous climatic disturbances.

Conclusions and Recommendations

These observations from two contrasting experimental environments have stressed the importance of environmental and genetic influences on seedling/tree responses to acid mist treatment. Responses based around carbon partitioning, the use and distribution of assimilate in the plant e.g. for growth and frost hardiness would appear to provide the most stable bioindicators of perturbation. However, none of these bioindicators is specific to a particular treatment (cause).

Visible injury is not recommended as a reliable bioindicator of perturbation since its development is strongly preconditioned by environmental parameters. Enhancement of ion leakage from unfrozen shoots was not found to be sensitive to environmental factors but was influenced by genotype. Foliar nutrient concentrations must be interpreted with caution. OTC studies tend to emphasize foliar mediated treatment effects while in the field treatment effects acting via the soil are most likely to predominate. OTC studies therefore offer better facilities for examining mechanisms and are no substitute for field exposures when trying to predict pollutant effects on forests.

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