

The effects of ozone and acid mist on Scots pine saplings

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Summary. It has been suggested that the forest decline (“Neuartige Waldschäden”) seen recently in parts of West Germany is due to the direct effects of ozone combined with acid mists, rather than soil-mediated effects of acid deposition. It has been proposed that ozone (a) makes the needles of affected conifers more susceptible to leaching by acid mist and (b) damages the photosynthetic apparatus, giving rise to diminished carbohydrate reserves which reduce the ability of affected trees to replace the leached nutrients. This nutrient deficiency (especially of Ca and Mg) is a characteristic symptom of the *Waldschäden*, which progresses through growth decline, needle loss, and eventually death. Parts of this hypothesis were tested in a preliminary experiment in which 3-year old *Pinus sylvestris* (Scots pine) saplings were exposed to 4 different O₃ levels, with and without acid mist (pH 3) treatment, for 56 days between July and September, 1983 in outdoor ‘solar dome’ fumigation chambers. The visual symptoms observed at >100 µg m⁻³ were more characteristic of the chlorotic mottle seen on O₃-affected trees in the USA than the general chlorosis of affected stands in Germany. O₃ at mean concentrations of >200 µg m⁻³ for 56 days reduced the fine root biomass and accelerated the senescence of older needles, in keeping with field effects observed in Germany. However, these O₃ levels increased, rather than decreased, the concentrations of most elements in the needles. Acid mist had no effect on needle concentrations, and there was no O₃-acid mist interaction. O₃ up to 300 µg m⁻³ also had no effect on the amount of ions leached from the needles, whereas acid mist increased the leaching of some ions, and again there was no interaction. The only nutritional effect of O₃ was to reduce the foliar uptake of NO₃⁻ from the acid mist solution. An aphid infection part way through the experiment caused a large increase in leaching, particularly of K, and affected the intermediate O₃ and water-sprayed plants most. Caution is needed in extrapolating these results to the field, as the experiments were of short duration on young trees with fully-formed needles, growing in a soil better supplied with nutrients than field soils. Nevertheless, these preliminary results do not support the hypothesis of an O₃-mediated increase in foliar leaching as the major cause of forest decline nor were the symptoms of O₃-injury on Scots pine comparable with those reported in the field.

In recent years a widespread and serious forest decline (“Neuartige Waldschäden”) has become apparent in West Germany and some adjacent countries (see e.g. BELF 1982; Binns and Redfern 1983; VDI 1983). A number of tree species are affected, the major ones being silver fir (*Abies alba*), Norway spruce (*Picea abies*). Scots pine (*Pinus sylvestris*) and beech (*Fagus sylvatica*). According to official figures some 2.5 × 10⁶ ha or 34% of the forest area of the German Federal Republic were affected by 1983 (BELF 1983). Symptoms vary with species, but commonly include needle loss, especially of older needles, leading to thin crowns; colour changes – yellowing of Norway spruce needles indicative of Mg deficiency, yellow, brown or grey needles on Scots pine; death of fine roots; and growth reductions. The earliest and most severe injury occurs on dominant or exposed trees, on exposed branches, or in exposed locations. A colour atlas of symptoms with brief descriptions has been published (Bauer 1983). Several hypotheses have been put forward as explanations of the decline – all involve air pollutants as a primary cause. An early hypothesis that soil acidification due to acid precipitation was the major cause (see Ulrich and Pankrath 1983) has not withstood experimental testing. For example, dieback occurs indiscriminately on adjacent limed and unlimed plots in the Black Forest (Aldinger 1983) in spite of an increase in pH in the limed plots.

More recently hypotheses involving ozone have received some support. Arndt et al. (1982) suggested that the symptoms of silver fir dieback resembled the effects of O₃ on other conifers. Prinz et al. (1982) pointed out the remarkably high O₃ levels in some high-altitude forests in Southern Germany (maximum hourly value, 1980, 358 µg m⁻³, summer monthly means 120–152 µg m⁻³). They exposed Norway spruce and silver fir to concentrations of up to 600 µg m⁻³ O₃ and observed visible injury on spruce similar to that noted in O₃-sensitive pines in N. America (*Pinus strobus* and *P. ponderosa*) – i.e. flecks of yellow known as chlorotic mottling (e.g. Jacobson and Hill 1970). This symptom is not seen in trees affected by the German forest decline syndrome. They therefore proposed a hypothesis in which O₃ increased the permeability of cell membranes, resulting in an increase in leaching of ions (especially Mg), thereby accounting for the observed Mg deficiency. As leaching agent they proposed acid fog, which has been observed at a pH as low as 2.5 in Bavaria (Schrimpff 1983).

Experiments showed some increase in Mg and Ca leach-

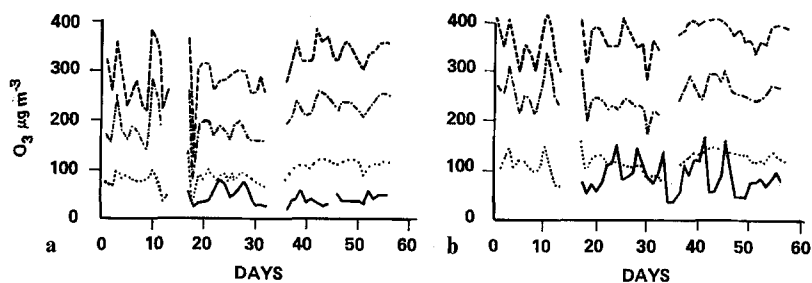


Fig. 1 a, b. Ozone levels inside and outside fumigation chambers. (a) Mean daily O₃ levels; (b) Maximum hourly O₃ levels. (—) ambient; (···) low; (---) medium; (-·-·) high O₃ levels. Gaps are due to monitoring or dosing equipment failure

ing from spruce by acid spray at 600 µg m⁻³ O₃ concentrations though the effect was not large (Krause et al. 1983).

The object of the preliminary experiment described here was to investigate whether some of the processes postulated by Prinz et al. (1983) could be demonstrated in young Scots pine. Though young nursery trees may react differently from older trees in the field, such experiments are useful in distinguishing important processes and damage mechanisms. In addition, there is little information on the effects of O₃ on European tree species and a description of the symptoms observed in experimental O₃ exposures can aid their identification in the field (coloured photographs of the O₃-induced symptoms are available from the authors).

Methods

Fumigation

Eighty-eight 3 year-old Scots pine saplings (*Pinus sylvestris* L.) were obtained from the Forestry Commission's Alice Holt Research Station. The experiment started on 19 July 1983, so the trees had completed the annual growth flush. The trees were growing in potting compost in 130 mm diameter plastic plant pots and were of varied genetic origin, ranging in height from 22 to 39 cm. Four plants were harvested as described later and the remainder divided into four height categories and distributed in eight experimental treatments so the distribution of height in each treatment was approximately the same. The plants were transferred to the "solardome" experimental fumigation rig, which consisted of four hemispherical greenhouses (35 m³ volume) supplied with charcoal-filtered air at four air changes per minute and a horizontal velocity over the plants of about 0.5 m s⁻¹. Ozone was generated by AR-type O₃ discharge valves, which produced 40 l min⁻¹ air with 1% O₃. Injection into the airduct to each chamber was regulated by a 0–5 l min⁻¹ mass-flow controller and the excess O₃ was fed through Teflon lines to a large charcoal filter. The air from each chamber was drawn to the Dasibi O₃ monitor by a 15 l min⁻¹ pump in a gas-handling unit. This unit sampled each of the four chambers and ambient air for 6 min every half-hour. A solenoid in the O₃ line closed automatically whenever the mains supply failed, the airflow in the main ducts dropped by >50% or the O₃ concentration exceeded 400 µg m⁻³.

Fumigation began on 19 July 1983 and continued until 12 September 1983. Ozone levels achieved in each of the four treatments were <1, 94, 203 and 304 µg m⁻³ averaged over the 56 day fumigation period*. Daily average and maximum hourly values fluctuated in a similar way to ambient concentrations (Fig. 1). Within each O₃ treatment,

* Within the accuracy of measurement, 1 µg m⁻³ O₃ = 0.5 ppb

plants were divided into two groups: acid-mist treated (10 plants) and distilled water mist (11 plants). Acid-mist consisted of a 7:3 mixture of sulphuric and nitric acids (700 µeq l⁻¹ H₂SO₄ and 315 µeq l⁻¹ HNO₃, pH 2.99). Mist was applied from an ASL "Killaspray 8" spray gun twice daily at 9.00 and 16.30 h, 5 days a week. The volume applied each time was 0.625 l to each treatment, equivalent to about 3 mm rain, enough to saturate and leach the foliage. A total of 44.4 l was applied to each treatment during the course of the experiment, equivalent to about 220 mm of rain. Throughfall was collected in a polythene funnel located below the canopy and stored in a polythene bottle buried below ground. A polythene sheet taped to the stem of each plant prevented throughfall or stemflow coming into contact with the soil, and plants were watered as necessary under this sheet with distilled water.

Measurements

A semi-quantitative visual estimate of symptom development was made at approximately 14-day intervals during the 56 day fumigation. Each plant was scored for the proportion of needles lost; dead needles still attached; needles showing necrosis of the tips, or some chlorotic mottling. The score was applied separately to current year's (new) needles and last year's (old) needles.

On completion of the fumigation, plants were harvested and separated into new needles, old needles, stems and roots. The roots were washed free of soil and the number of root tips per pot estimated in 3 plants chosen at random from each treatment. The number of root tips in a weighed sample of fine roots (<1 mm) was counted and divided by the proportion of fine roots this sample represented. The roots of all plants were separated into coarse (>1 mm diameter) and fine (<1 mm) roots, and all plant parts were dried at 60° C for at least 1 week, and weighed.

A sample of four needles per treatment was picked fresh for examination by scanning electron microscopy, washed with distilled water, fixed to stubs to show either the upper or the lower needle surface, and dried at 20° C for 24 h under vacuum. Stubs were given a thin gold coat in a Polarlon Argon Plasma sputter coater, and examined in an ISI Inc. Mini-SEM 5 scanning electron microscope.

Samples of old and new needles from each plant were finely chopped and digested in a sulphuric-nitric-perchloric acid mixture (Allen 1974). The digest was analysed for mineral elements on an inductively-coupled plasma emission spectrometer (ICP).

Throughfall was collected weekly and the pH measured immediately. Other cations were measured on an ICP, and anions and NH₄⁺ by autoanalyser methods within two weeks of collection; the samples being stored at 4° C in the meantime.

Soil pH was measured on fresh samples in 1:2.5 soil: water and 0.01 M CaCl₂ suspensions. The soil was air-dried at 30° C, weighed, and cation exchange capacity and exchangeable bases determined on a 2 mm-sieved fraction according to the procedures in Allen (1974).

Statistical analyses were carried out using the IBM APL statistical library 5796-PHW.

Results

Development of symptoms

The first symptom observed (after one week) was a severe necrosis of the apical third of the current year's needles (new needles) which occurred in 4 plants only – one in the high ozone, distilled water spray, two in high ozone, acid spray and one in medium ozone, acid spray. These symptoms persisted on these plants throughout the experiment, but appeared on no others. A much milder apical necrosis covering 5–10% of the new needles then developed slowly in the high ozone treatments (Fig. 2a), though not on all plants. Acid mist had no consistent effect on the development of this symptom.

Within two weeks of the start of fumigation, small yellowish patches (chlorotic mottle) began to appear on the new needles in the high ozone treatment. The mottle tended to appear on the apical half of the needle and spread towards the base, sometimes coalescing into irregular bands and occasionally going brown. The extent and speed of development of the mottling varied widely between plants, some plants having very mild symptoms while in others the whole plant took on a pronounced yellow cast. The effect of O₃ on chlorotic mottle of new needles only became significant in the later stages because of the wide variation in response between plants in each treatment (Fig. 2b). At the end of the experiment, chlorotic mottle was present on all plants but one in the high O₃ (300 µg m⁻³); 16 out of 21 plants in the medium O₃ (200 µg m⁻³); and just discernible on a few needles in the low O₃ (94 µg m⁻³) treatment. Acid mist had little effect on the development of this symptom (Fig. 2b).

Chlorotic mottle and tip necrosis appeared also on the old needles, but after 2 weeks they started to senesce, die and fall off (Fig. 2c). O₃ increased the rate of senescence (Fig. 2c), whereas acid mist had no effect at the highest O₃ concentration, but increased the percentage needle loss at the zero, low and medium O₃ levels by 8, 19 and 23% respectively (Fig. 2c). At the highest O₃ level, 6 plants in each treatment had no old needles left at all by the end of the experiment. A small proportion of new needles also started to fall off, starting from the basal end of the stems (Fig. 2d). They were shed green, and had a tendency to regrow, complicating the scoring. Needle loss of 5–15% occurred in all treatments, but although there was a significant effect of ozone in the middle of the experiment (Fig. 2d) this had disappeared by the end.

Effects on plant structures

The effects of ozone and acid treatment on plant structures measured at the end of the experiment are shown in Fig. 3. There were no treatment effects on numbers of root tips (Fig. 3a), which were very high compared to field samples, or on coarse (>1 mm diameter) roots (Fig. 3b). There was also no effect on stem dry weight (data not shown), not

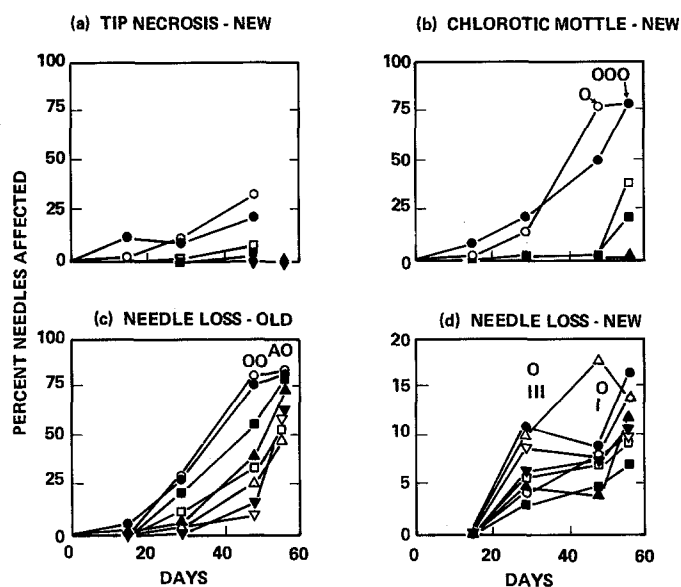


Fig. 2. Development of symptoms. Filled symbols are acid mist; open symbols water mist. O₃ concentrations (µg m⁻³): (o) 304; (□) 203; (△) 94; (▽) 0. Letters show significant differences on any given day: A, of acid mist; O, of O₃; I, interaction. Number of symbols indicates degree of significance: one symbol $p < 0.05$; two symbols $p < 0.01$; three symbols $p < 0.001$.

surprisingly because there was no stem growth during the period of the experiment. There was a significant effect of O₃ on fine (<1 mm diameter) roots (Fig. 3c) – O₃ concentrations of 200 µg m⁻³ or greater appeared to depress the weight of fine roots per plant. All plants lost old needles in the solardomes. Though there appears from Fig. 3d to be an effect of both O₃ and acid mist in reducing the weight of old needles per plant, the effects were not statistically significant owing to the variability of response between plants. There were no effects on the dry weight of new needles per plant (Fig. 3e), but when expressed on the basis of needle weight cm⁻¹ stem, acid mist produced a slight but significant reduction (Fig. 3f).

Scanning electron microscopy of needle surfaces revealed no clear differences between O₃ or acid-treated needles and controls, even when there was obvious visible injury in the form of chlorotic mottle. Although cracks appeared on top of a few cuticular ridges, these occurred in all treatments and seem most likely to be a preparation artefact. No other damage to the cuticle was apparent.

Effects on nutrient concentrations of needles

The effects of ozone and acid treatment on nutrient concentrations in new needles are shown in Fig. 4. Ozone gave rise to a highly significant increase in the concentrations of Mg, K and P, especially at the highest level, and a rising trend which failed to gain significance was also apparent for the other ions. There were no significant effects of acid mist or any significant interactions. The Mg and P levels were well above the optimum threshold of 0.5 and 1.4 mg g⁻¹ respectively (Binns et al. 1980) but K was close to the deficiency level (3.0 mg g⁻¹) in keeping with the low K status of the soil (Table 1). Comparison of these results with those from the 4 plants harvested before fumigation revealed that the fumigated plants had more Mg, Ca and Mn (up from 1.2, 0.99 and 0.13 mg g⁻¹) and less K, P and Al (down from 7.1, 1.9 and 0.08 mg g⁻¹).

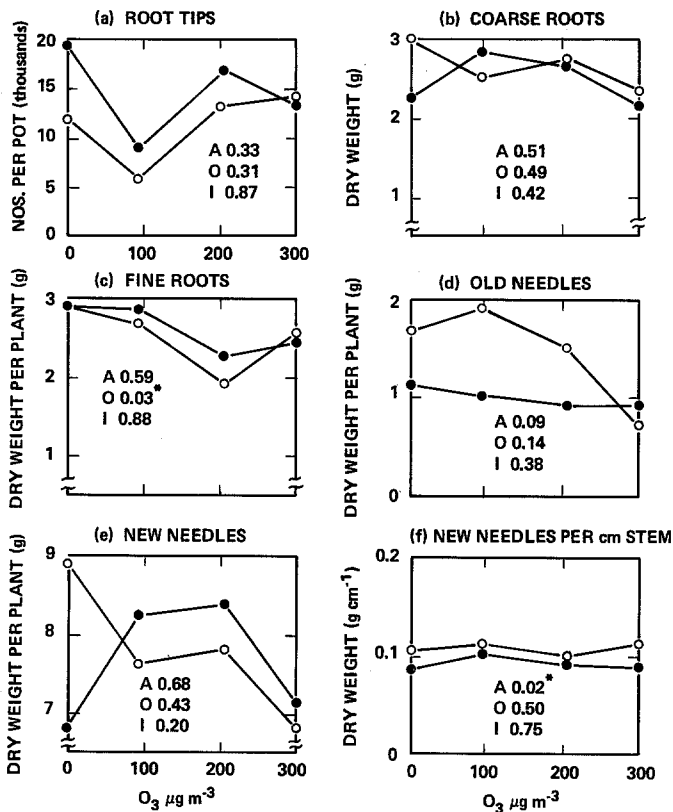


Fig. 3. Treatment effects on plant structures. (●) Acid mist, (○) water mist. A, O and I are the probabilities for acceptance of the null hypothesis that there is no effect of acid, O₃ or any interaction respectively. (*) denotes significant effects (as in Fig. 2)

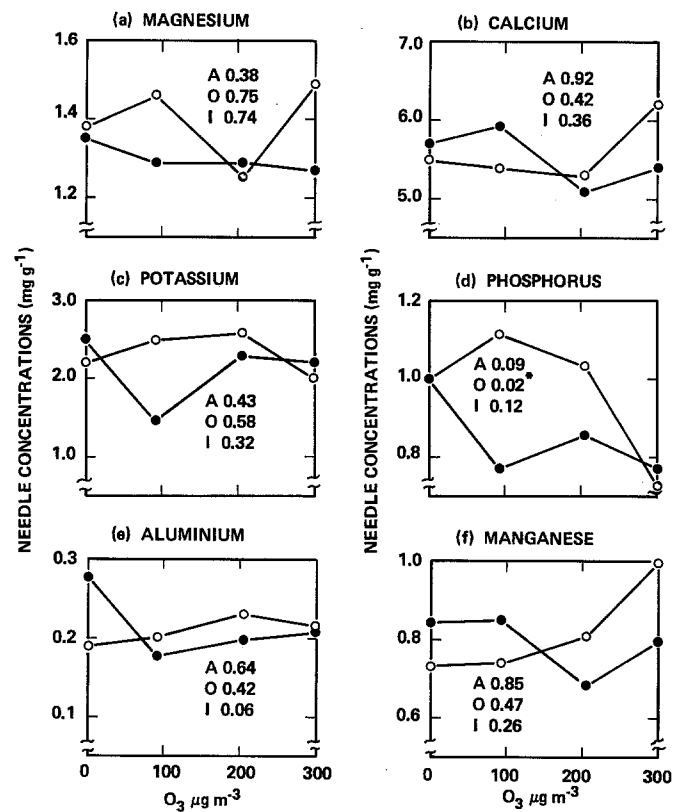


Fig. 5. Treatment effects on old needle concentrations. Symbols as in Fig. 3

The concentrations of the same elements in the old needles are shown in Fig. 5. These needles were in various stages of senescence so their concentrations were more variable, and only one effect was significant – ozone treatment significantly reduced P concentrations. Comparison of old and new needle concentrations indicated that some elements had been withdrawn prior to abscission so that P and K were below deficiency levels in the old needles whereas Mg, which was readily available in the soil, had not decreased as much. The higher Ca probably reflects a higher proportion of cell wall material in the older needles. The 4 plants harvested before fumigation started had similar Ca and Al concentrations (5.07 and 0.21 mg g⁻¹ respectively) but higher Mg, K, and P concentrations (1.48, 3.00 and 1.33 mg g⁻¹ respectively), confirming the remobilisation of these elements. However, Mn was lower in the pre-fumigation plants (0.49 mg g⁻¹).

Leaching

Interpretation of the throughfall data is complicated by several factors. The concentrations in the initial throughfall sample were much higher than in subsequent ones, presumably because of washing of earlier dry deposits from the needles as these plants had been grown outside. Secondly, after Day 27 all treatments became infected with aphids, but to a lesser degree in the high O₃ treatments, complicating the leaching pattern. Figure 6 shows the amount of selected ions leached per treatment between Days 13 and 27, representing aphid-free plants in charcoal-filtered air, and between Days 13 and 56, representing the whole experiment less the initial 10.38 l of throughfall which contained washed-off dry deposits. Before aphid infection, acid treatment increased the leaching of Mg and Al (Fig. 6a, 6e),

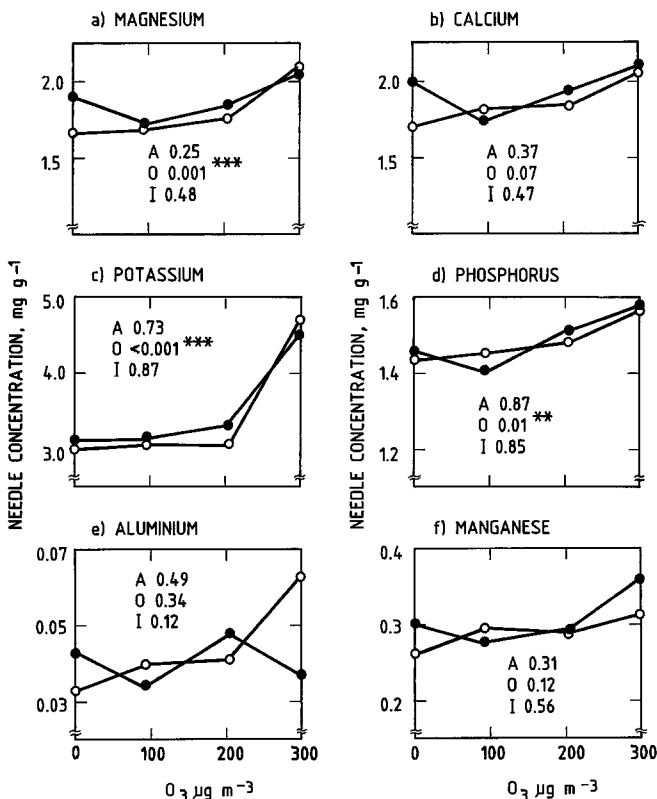


Fig. 4. Treatment effects on new needle concentrations. Symbols as in Fig. 3

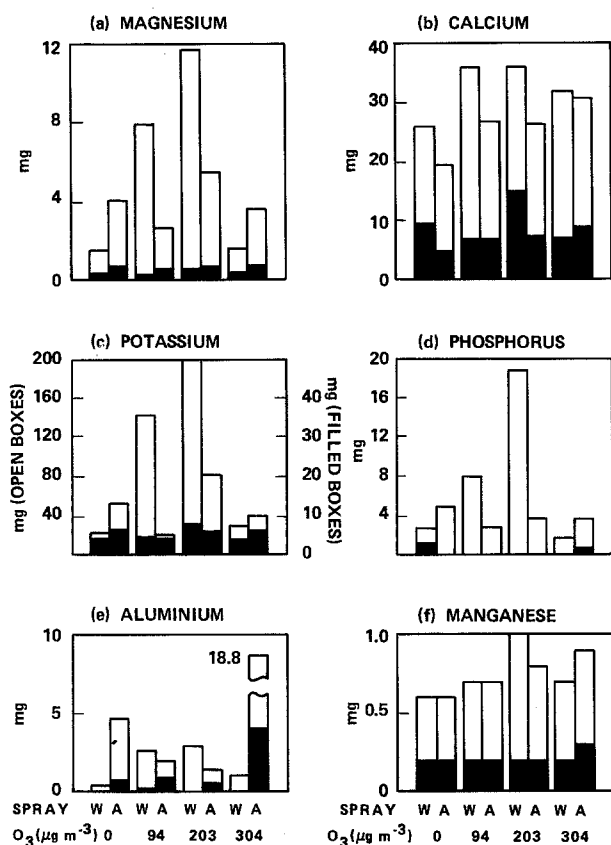


Fig. 6. Amounts of selected ions leached. Filled boxes: Days 13–27; open boxes, Days 13–56. W = water mist, A = acid mist

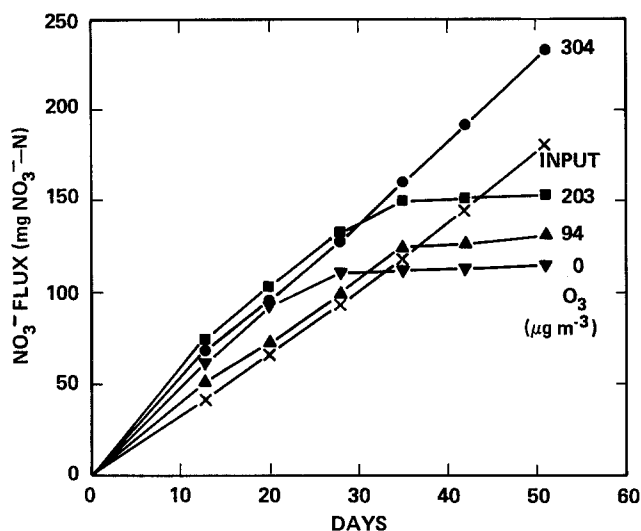


Fig. 7. Cumulative NO_3^- fluxes in throughfall under acid misted plants

Table 1. Soil analysis

	pH (water)	pH (CaCl_2)	Exchangeable Ions ($\text{meq } 100 \text{ g}^{-1}$)					% Base Saturation	
			Ca	Mg	Na	K	TEB ^a		CEC ^b
Value	4.98	4.29	10.2	6.8	0.12	0.27	17.4	29.5	59
Standard deviation	0.10	0.04	1.0	0.8	0.01	0.15	1.8	2.7	2

^a TEB = Total Exchangeable Bases

^b CEC = Cation Exchange Capacity

but O_3 treatment had no obvious effect on leaching of any ion and there was no clear interaction between acid mist and O_3 treatment. After aphid infection there was a very large increase in leaching, especially of K in the most heavily-infected treatments (intermediate O_3 , water spray), but also P and Mg. Acid treatment also increased the rate of leaching of Fe and $\text{NH}_4^+ - \text{N}$, whereas O_3 had no effect (data not shown).

The only clear effect of O_3 was to inhibit the uptake of NO_3^- from the acid mist treatment. Figure 7 shows that after an initial wash-off, NO_3^- in throughfall in the acid mist treatments paralleled NO_3^- input until Day 20, when the trees began to absorb NO_3^- in the zero O_3 treatment. The NO_3^- was almost completely removed from throughfall after Day 27 in the zero O_3 treatment and Day 35 in the 94 and 203 $\mu\text{g m}^{-3}$ treatments but was not absorbed at all in the 304 $\mu\text{g m}^{-3}$ treatment.

Soil

Chemical parameters measured in the soil after the experiment was complete are shown in Table 1. There were no significant differences between values from acid-sprayed and those from water-sprayed plants, so the data have been combined. Ca and Mg levels were high and K levels low compared to most forest soils. The pots contained $505 \pm 27 \text{ g}$ soil with 46% in the $< 2 \text{ mm}$ fraction analysed above. Each pot therefore contained about 24 mg exchangeable K whereas the plants contained $\sim 28 \text{ mg}$ K in the needles alone (Figs. 3, 4, 5). In contrast, the soil contained 190 mg exchangeable Mg compared to $\sim 15 \text{ mg}$ in the needles. The amount of K leached in the treatments heavily infected with aphids (Fig. 6) thus exceeds the amount in the soil exchangeable pool, which may be one reason for the low soil K.

Discussion

Conclusions concerning the field situation must be drawn very cautiously from this experiment. This was a short-term fumigation with plants rather younger than those showing symptoms in the field, and growing on a soil of higher base-status (except for K) than those commonly found in forests. The needles were fully formed at the start of the experiment, and the system was simplified so there were no gaseous pollutants other than O_3 , and no other adverse environmental influences (e.g. frost or drought). The highest O_3 concentration (304 $\mu\text{g m}^{-3}$) is higher than observed in Germany, but the medium O_3 treatment (203 $\mu\text{g m}^{-3}$) is reasonably representative of the summer months at Schauinsland in the Black Forest (Prinz et al. 1982). Nevertheless, some relevant observations can be made.

The fumigated plants showed some responses which are also observed in the field in Germany. The variability of response in most of the visual parameters measured is a

characteristic of O₃ injury (Houston 1974) and is also seen in German forests where affected trees are scattered through stands surrounded by apparently healthy ones (BELF 1982). The reduction in fine root biomass has also been reported for O₃-affected plants (Blum and Tingey 1977) and damage to fine roots has been noted in some affected German trees. Needle loss, especially of older needles, occurs in the forest decline syndrome and was markedly accelerated by O₃ in this experiment. The acid mist treatment also had a small but significant effect on senescence of new needles (Fig. 3f).

Other responses observed in this experiment have not been reported in the German forests. The main visible symptom of chlorotic mottling closely resembled that induced by O₃ in *Pinus strobus* and *P. ponderosa* in American studies (Jacobson and Hill 1970; Davis and Wilhour 1976) but has not been reported in Germany. Necrosis of needle tips was another characteristic symptom which has not been reported in the field. These results confirm that the classic pattern of O₃ damage occurs in Scots pine, and that the field symptoms observed in West Germany cannot be due to O₃ alone. No damage to the cuticle surface was observed even in needles with obvious visible injury, confirming the suggestion of Prinz et al. (1982) that O₃ acts internally (e.g. by degrading chlorophyll or damaging cell membranes). However, there was no correlation between the Mg content of a sample of needles from a damaged plant and the extent of chlorotic mottle observed on that plant.

The results do not support the hypothesis of Prinz et al. (1982) that O₃ leads to an enhanced leaching of ions from the needles, with or without acid mist. The effect of higher concentrations of O₃ was to increase the needle concentrations of most ions in the new needles (Fig. 4) and to decrease only P in the old ones (Fig. 5). O₃ treatment did not increase leaching of any ion in throughfall even before aphid infection (Fig. 6), although acid misting did so independently of O₃ treatment, in agreement with other studies (e.g. Horntvedt et al. 1980). It is possible that the aphid infection affected the final needle concentrations and for this reason and those noted above these data cannot be taken as a falsification of the hypothesis of Prinz et al. (1982). Infections of aphids and other parasites also occur in the field, and an investigation of the effect of such infections on needle nutrient status may be worthwhile.

The data of Krause et al. (1982) also do not show a good dose-response relationship between O₃ level and leaching of Mg or Ca from Norway spruce needles. Leaching was sometimes less at 600 µg m⁻³ O₃ than at 200 (Krause et al. 1982; Figs. 5, 7) and leaching in O₃ treatments sometimes less than control. The only significant effect of O₃ on throughfall in the present experiments was to reduce the extent to which NO₃⁻ can be absorbed by the needles, a phenomenon also observed with Norway spruce (Krause et al. 1984). It seems likely that factors additional to O₃ and/or acid mist will need to be invoked to explain the field pattern of damage in both Scots pine and Norway spruce. Other hypotheses which postulate an interaction of O₃ and frost (Bosch et al. 1983) or O₃ and drought (Skårby and Berglind 1983) should also be examined experimentally.

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