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Seasonal changes in water use of ash trees exposed to ozone episodes

By J. J. J. WILTSHIRE[1], M. H. UNSWORTH[2] and C. J. WRIGHT[3]

[1] *Department of Physiology and Environmental Science, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK*
[2] *Center for Analysis of Environmental Change, Oregon State University, Weniger Hall 286, Corvallis, OR97331-6511, USA*
[3] *Department of Agriculture and Horticulture, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK*

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SUMMARY

Young ash trees (*Fraxinus excelsior* L.) growing in the field were exposed to episodes of 150 nl l^{-1} ozone, or to clean air, in open-top chambers at the University of Nottingham, Sutton Bonington Campus, UK, in the summer of 1992. The episodes were for 8 h daily and for 1–4 d in succession, with a seasonal total of 27 d. From late-June until mid-September 1992, flow of water in the stems of individual trees was measured using a heat balance method. Short-term effects of ozone exposure on stem flow were not detected. However, in the longer-term, daily integrated stem flow values for the ozone treatment decreased throughout the measurement period relative to the clear air treatment. Further data analysis showed that integrated stem flow values for morning, evening and for the more stable central part of the day changed in a similar way, but this was statistically significant only for the evening period. No treatment differences, however, were detected in night-time water use values.

This response was probably mediated by changes in stomatal resistance and, if applicable to other species, has important implications for the long-term growth of trees in regions where photochemical ozone is common.

Key words: Ozone episodes, Water use, *Fraxinus excelsior*, ash, heat balance.

INTRODUCTION

Ozone generated photochemically from pollution in the lower atmosphere is known to influence leaf stomatal resistance to water vapour, and therefore water use, in many plant species (Darrall, 1989). Plant responses to ozone vary, depending on exposure concentration and duration and the plant species (Reich & Amundson, 1985). Recent research has shown that stomatal responses to ozone are also influenced by humidity (Maier-Maercker & Koch, 1991) and water stress (Pearson & Mansfield, 1993). It is difficult to study responses of trees to pollutants under controlled conditions because of their size and rooting characteristics (Pye, 1988), and assessment of water use by the tree as a whole from measurements on individual leaves is difficult because of sampling problems. We report here the use of an established open-top chamber facility (Wiltshire, Wright & Unsworth, 1992) to expose young ash trees (*Fraxinus excelsior* L.) growing in the field to ozone episodes or to clear air; whole tree water use was assessed using a heat balance technique for measuring sap flow (Baker & Van Bavel, 1987).

MATERIALS AND METHODS

The experiment was conducted at The University of Nottingham, Sutton Bonington Campus, UK (latitude 52° 50′ N) in 1992. The trees were seed-grown and transplanted directly into the soil, (a Wick/Arrow series, stony, sandy loam soil overlying Keuper marl at a depth of about 1 m) in March 1991. They were 4 years old at the start of the experiment, during which they were not irrigated, and were between 1·3 m and 2·6 m in height at the end of 1992. Open-top chambers (Wiltshire *et al.*, 1992), 3·8 m high and 3·5 m in diameter, were used to impose the treatments. The sides of the chambers could be covered or uncovered rapidly, allowing the trees to be uncovered when treatments were not being applied, thereby decreasing the cumulative thermal effects of the chambers. When covered, the chambers were ventilated by electric fans at a rate of approximately four chamber volumes of air per minute; pollutants were removed by passing the air stream through Purafil (Jones and Attwood, Stourbridge, UK) and activated charcoal, and artificially generated ozone was injected at a known concentration

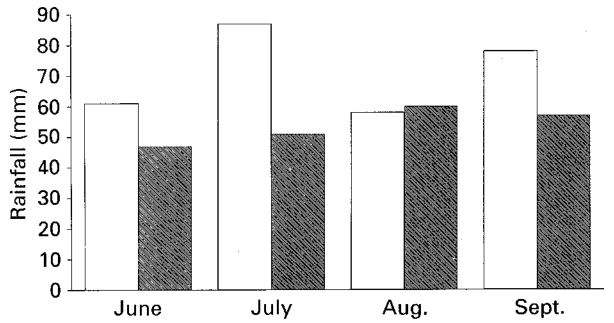

Figure 1. Rainfall data (mm) from Sutton Bonington Agrometeorological Station: monthly rainfall, June to September 1992, and long-term averages (1916–1992). □, 1992; ▩, average.

into selected filtered air chambers. Full details of chamber operation and design, and air quality and microclimate monitoring have been published elsewhere (Wiltshire *et al.*, 1992).

There were two chamber treatments with four replications of each, and three trees in each chamber. The treatments were: charcoal and Purafil filtration (CF), and charcoal and Purafil filtration with added ozone ($CF + O_3$). An additional treatment with trees in ambient air (with no chamber) was included in the study, but is not discussed here as water use measurements were not made in this treatment in 1992. When the ambient ozone concentration was greater than 50 nl l^{-1} all the chambers and fans were operated to ensure that the CF treatment did not receive high ozone, and that all chamber plots were subject to the same microclimate. All the chambers were also operated when the $CF + O_3$ plots were exposed to artificially generated ozone, but at all other times the chamber sides were uncovered. The ozone exposure concentration ($CF + O_3$) was 150 nl l^{-1}; ozone was given for 8 h d^{-1} (10.00–18.00 GMT), for 1–4 d in succession, and these episodes were given between 1 May and 31 October on days when the foliage was dry; preference was given to sunny days with low windspeeds and above-average temperatures. There were 9 exposure days before the water use measurements commenced, 14 during the measurement period, and 4 exposure days after the measurement period.

A heat balance method for measuring the flow rate of water in tree stems (Baker & Van Bavel, 1987) was used, together with a datalogger (model 21X, Campbell Scientific Ltd, Shepshed, Leics., UK), to monitor continuously the water use of one tree in each plot of the CF and $CF + O_3$ treatments, from 27 June until 17 September. Rainfall data from Sutton Bonington Agrometeorological Station for this period are shown in Figure 1. The total rainfall for the months June to September was well above the long-term average (1916–1992), and serious soil moisture deficits did not develop. Mean daily radiation and temperature were close to the long-term averages.

During heavy or prolonged rainfall, problems were experienced with rain penetration into stem flow gauges, resulting in some erratic and inaccurate data which were excluded from subsequent analysis. Reliable data were obtained for 51 complete days.

RESULTS

There was no evidence of any short-term (within days or episodes) effect of treatment on flow rate. Figure 2 gives two examples (mid and late-season) of stem flow over a 24 h period, showing treatment means (g h^{-1}) and solar radiation data from Sutton Bonington Agrometeorological Station. For each day when good data were obtained, stem flow values for each plot changed in a very similar way in response to the environment (dawn, nightfall, changes in microclimate), and this is illustrated by the examples in Figure 2. On exposure days, changes in response to the start or end of an ozone episode were not observed: Figure 2*b* is an example of an exposure day.

To investigate longer-term effects, daily integrals of flow rates for each chamber of both treatments were plotted against time for the whole measurement period. Orthogonal polynomials (linear and quadratic) were fitted. There was evidence for a linear trend ($P = 0{\cdot}0008$) but not for a quadratic trend. Linear effects between treatments were compared

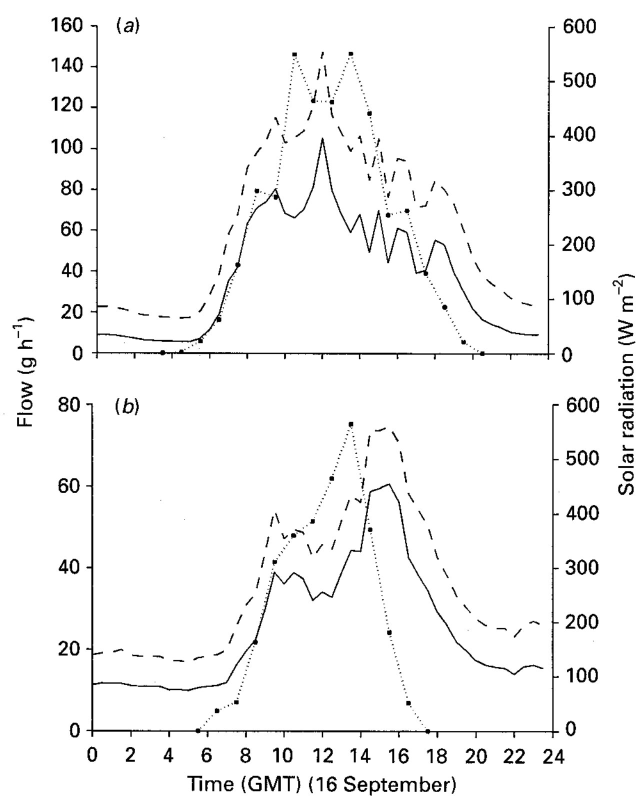

Figure 2. Mean water use (g h^{-1}), CF (dashed lines) and $CF + O_3$ (solid lines) treatments, and solar radiation (W m^{-2}) (dotted lines and square symbols): (*a*) 2 August 1992 (not an ozone exposure day); (*b*) 16 September 1992 (ozone exposure from 10.00 to 18.00 GMT).

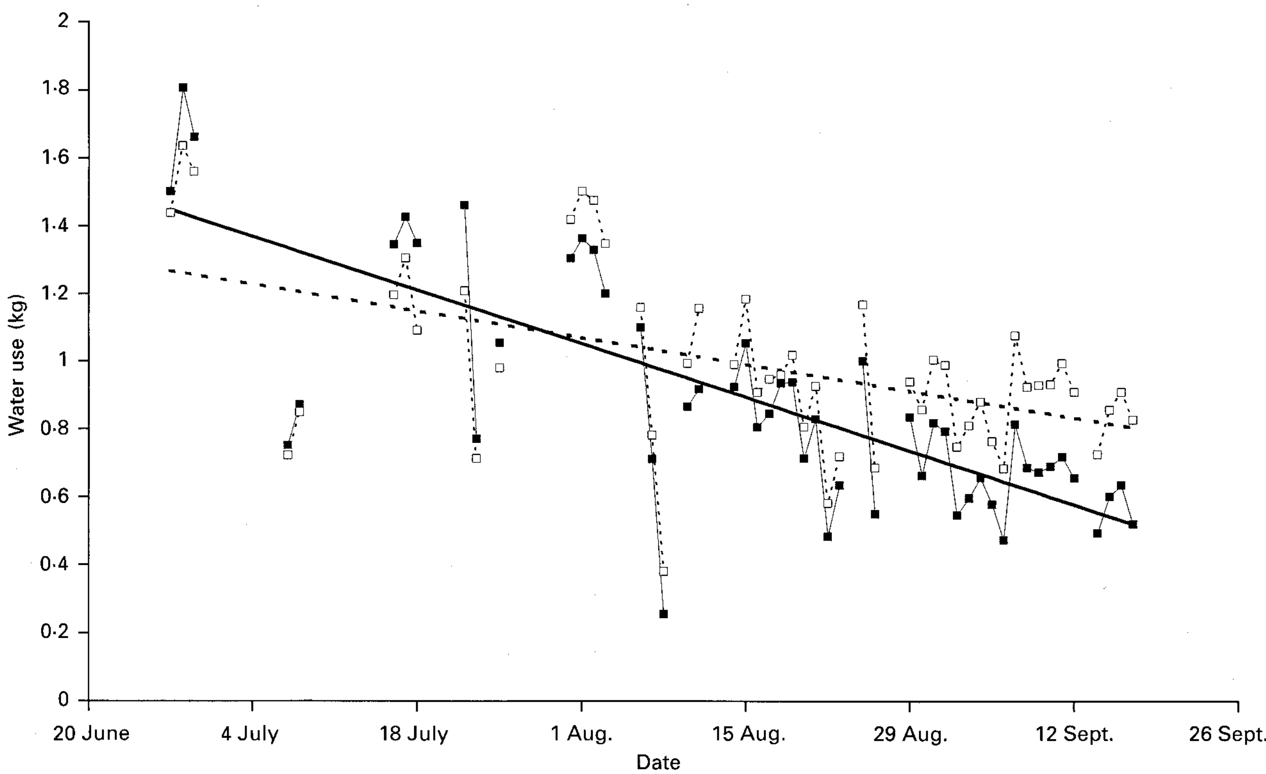

Figure 3. Mean daily water use (kg) of young ash trees: solid lines and closed symbols, trees exposed to ozone episodes; broken lines and open symbols, trees not exposed to ozone episodes. Points are not joined where data are missing. The straight lines were fitted by linear regression analysis.

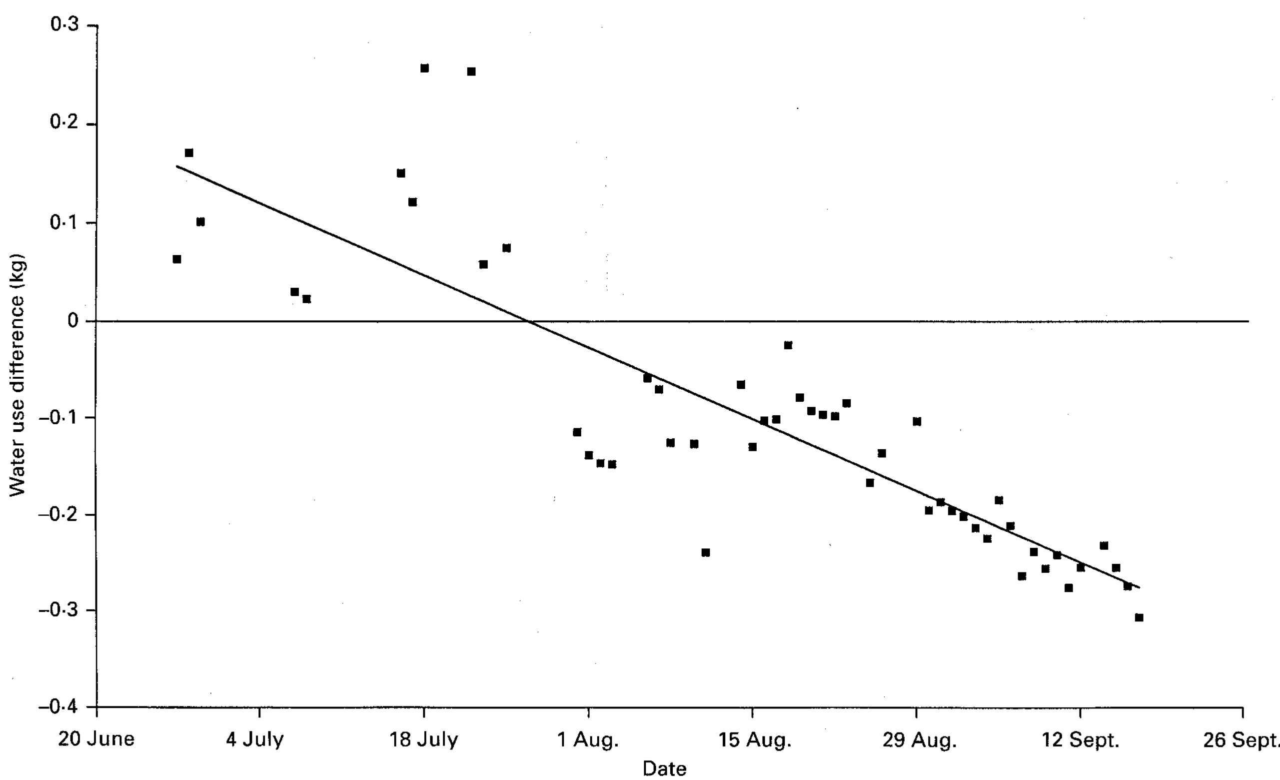

Figure 4. Mean daily water use (kg): difference between trees exposed to ozone episodes and trees not exposed to ozone episodes.

with the variation in these effects between replicate chambers within treatments. There was a significant difference in the slopes of the linear components ($P = 0{\cdot}0485$), showing that daily water use declined more rapidly in the $CF + O_3$ treatment than in the CF treatment. The mean daily stem flow values for

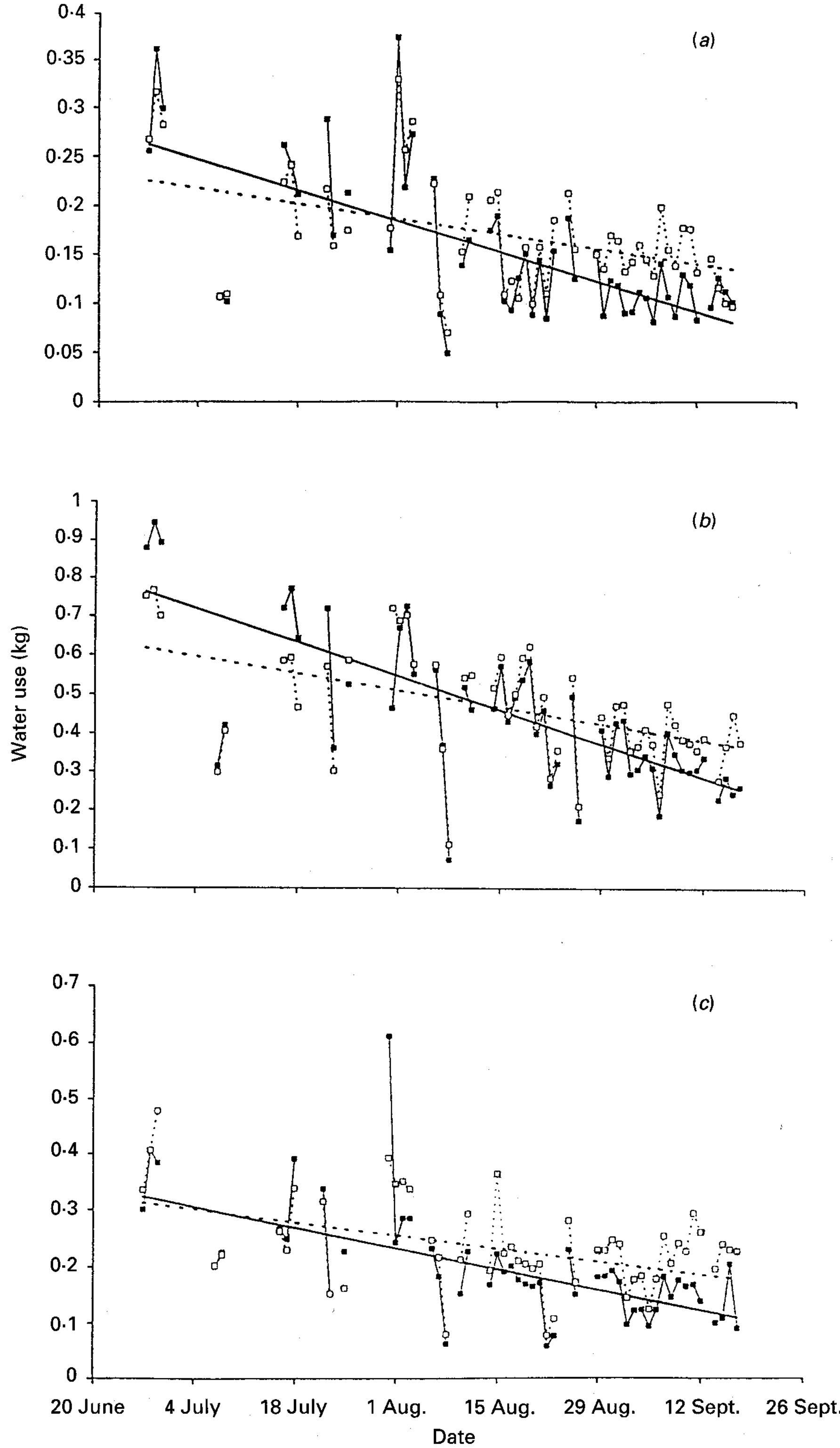

Figure 5. Mean water use (kg) of young ash trees: (*a*) 04.00 to 09.30 GMT; (*b*) 09.30 to 16.00 GMT; (*c*) 16.00 to 21.30 GMT. Solid lines and closed symbols, trees exposed to ozone episodes; broken lines and open symbols, trees not exposed to ozone episodes. Points are not joined where data are missing. The straight lines were fitted by linear regression analysis.

each treatment, and the lines fitted through data for all chambers of each treatment are shown in Figure 3. There was no significant lack of fit to these lines.

Figure 4 shows the difference between mean daily water use in the two treatments, plotted against time. This more clearly shows the trend towards decreasing daily water use in the $CF+O_3$ treatment relative to the CF treatment; it can be seen that in the early part of the season the ozone-treated trees used more water per day than the trees in the CF treatment, but later in the season the difference was reversed.

Estimates for the total water usage per tree for each treatment over the measurement period were obtained by calculating the area under the straight lines fitted through the daily water use data for each tree. Analysis of variance was then used to compare treatments. The means of total water usage per tree over this period, for the CF and CF + O_3 treatments, were 85·7 kg and 81·8 kg respectively, corresponding to 1·03 kg d^{-1} per tree and 0·99 kg d^{-1} per tree respectively: there was not a significant treatment difference.

It was possible that these observed changes may have been a consequence of differences associated with a particular part of the 24 h period, for example a change in the rate of stomatal opening at dawn. For this reason the data for each day were divided into four portions: night (midnight to 04.00 GMT and 21.30 GMT to midnight), morning (04.00 to 09.30 GMT), day (09.30 to 16.00 GMT), and evening (16.00 to 21.30 GMT). The precise extent of these periods was decided with the aid of 24 h plots of stem flow against time (Fig. 2 gives examples). Day and night were each defined as the relatively stable periods of water use when there was apparently little stomatal movement; morning and evening were defined as the remaining periods when there were large changes in the stem flow values associated with the beginning and end of daylight. Integrals of flow rates for each part of the day were plotted against time for the whole measurement period and statistical analyses were conducted in the same way as was described for the 24 h values. There was no significant relationship between night stem flow values and time, and so this analysis was not pursued further. Night stem flow values were also small relative to the 24 h values, and therefore if small changes did occur at night these would be unlikely to contribute significantly to the seasonal changes observed in the 24 h values.

For the morning, day and evening periods (Fig. 5) there was evidence for linear trends with time over the duration of the study ($P = 0{\cdot}0011$, $P < 0{\cdot}0001$ and $P = 0{\cdot}0023$ respectively) but not for quadratic trends. There were no significant differences between treatments in the linear components for the morning and day periods ($P = 0{\cdot}097$ and $P = 0{\cdot}127$ respectively), but the difference for the evening period was significant ($P = 0{\cdot}035$), indicating that water use between 16.00 and 21.30 GMT decreased over the duration of the study in the CF + O_3 treatment relative to the CF treatment.

DISCUSSION

Changes in stem flow in response to individual ozone episodes were not observed, so we conclude that leaf conductance to water vapour was either not changed by treatment in the short-term, or changes were too small to be detected in this experiment.

The observed seasonal changes in water use could be caused by changes in leaf area or in leaf diffusive resistance. Studies of leaf development on individual shoots indicated that leaf area development had stopped by mid-July, soon after the water use measurements began: treatment effects on leaf number per shoot were not observed. Good estimates of whole tree leaf areas were not obtained because of practical difficulties in obtaining these data non-destructively. However, effects of treatment on the timing of leaf abscission were small, abscission of the oldest leaves being advanced by only a few days (Wiltshire, unpublished) and this was not observed until mid-October, about a month after the water use measurements had ceased. No visible foliar injury was observed during the measurement period.

The ozone-treated plants were using more water than the controls during the early part of the measurement period: it is uncertain whether this was caused by lower stomatal resistance, or by greater leaf area. During the measurement period it is unlikely that there were any changes in leaf area which were related to treatment, and therefore it seems most likely that, over this period, the decrease in water use relative to the control in the ozone treatment was caused by changes in stomatal conductance.

Evening water use (16.00 to 21.30 GMT) declined more rapidly over the measurement period in the CF + O_3 treatment relative to the control, partly accounting for the similar trend in daily water use which was observed. We can therefore speculate that more rapid or earlier stomatal closure in the evening partly accounted for the observed changes in daily water use with time. However, evening water use values (Fig. 5*c*) were much lower than the values for the middle part of the day (Fig. 5*b*), and therefore, although this seasonal trend in evening water use was statistically significant, it only accounted for *c*. 17% of the relative seasonal change in daily (24 h) water use (Fig. 3).

Effects of ozone on stomatal aperture have been reported by many authors. Darrall (1989) in her review stated that the majority of published literature shows an increase in stomatal resistance as a result of fumigation with ozone, and similar results have been reported more recently (Eamus & Murray, 1991; Farage *et al.*, 1991; Olszyk, Takemoto & Poe, 1991). However, others have reported either no effects or even decreased resistances (Freer-Smith & Dobson, 1989), or decreased plant water use efficiencies (Reich & Lassoie 1984). These apparently contradictory results may have been due to interspecific differences, or to differences in experimental conditions, including ozone concentration, and time and duration of exposure, and time of stomatal resistance measurements.

Maier-Maercker & Koch (1991) demonstrated that transpiration rates in ozone fumigated *Picea* *abies* (L.) Karst. were less than those of control plants when the air was dry, but rates were greater than in controls under humid conditions. These results highlight the usefulness of stem-flow gauges which allow continuous field measurements of water use to be integrated over periods of days, weeks or months.

Plant water status has also been shown to have a major effect on a plant's response to ozone. Pearson & Mansfield (1993) found that in well-watered container-grown beech seedlings, ozone significantly increased stomatal resistance, but when water stress was applied part way through the growing season this situation was reversed, i.e. the control trees had the higher stomatal resistances.

Our results show that seasonal water use of young ash trees may be disturbed by episodes of photochemical ozone. If water use is increased early in the summer, this could deplete limited water reserves in the soil and, in dry seasons, expose trees to longer periods of drought. However, water was not a limiting resource in 1992. An increase in stomatal resistance towards the end of the measurement period would agree well with the results of Pearson & Mansfield (1993) for well-watered container-grown trees. The early decrease in stomatal resistance suggested by this experiment would not necessarily have been observed by Pearson & Mansfield (1993) since their stomatal measurements began on 1 August.

Total water use over the measurement period did not differ significantly between treatments. However, the differences reported here are relative differences in the rates or change of water use over the measurement period, and provide evidence that, in the field, ozone causes subtle damage to physiological processes, even though this damage may not result in recognizable changes in seasonal water use, especially in a wet season.

If decreased stomatal aperture caused the observed decrease in water use later in the season, this would also reduce carbon assimilation and growth. Ash trees in England are experiencing a decline which appears to be linked with dry summers (Hull & Gibbs, 1991). Since dry years are often associated with an increased frequency of ozone episodes, the results presented here suggest that ozone could be an additional factor in the decline.

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