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Acute exposure to ozone inhibits rapid carbon translocation from source leaves of Pima cotton

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Abstract

Tropospheric ozone (O_3) causes significant disruption of growth and yield in upland and Pima cottons. Pima cotton (*Gossypium barbadense* L.) was exposed to brief pulses (0.75 h) of a range of O_3 concentrations (nominally 0.0, 0.2, 0.5, and 0.8 μl l^{-1}) to investigate effects on phloem translocation of ^{14}C-labelled recent photoassimilate. The initial phase of rapid efflux from source leaves was monitored with a Geiger-Müller Tube as activity remaining in the leaf as a function of time. Visual inspection of unprocessed efflux curves revealed disruption of efflux by O_3. Single exponential decay functions were fitted to these efflux curves to extract first order rate constants for phloem loading and longitudinal transport of labelled carbohydrates. A single compartment model was applied, with and without an asymptote of non-transported carbohydrate, to calculate leaf sugar contents. The effect of O_3 in retarding efflux of label, decreasing the rate constant, and increasing calculated soluble sugar pools, was consistent regardless of the method of analysis. Following incorporation of the asymptote, calculated rate constants and sugar pools were similar to values from the literature and to preliminary measurements of sugar contents in O_3-treated cotton leaves. Total carbohydrate transported from source leaves was reduced both by O_3 effects on assimilation (up to 20%) and by O_3 effects on efflux (up to 70%), but was clearly dominated by the impact on phloem translocation. These rapid efflux kinetics likely reflect oxidant damage at the plasmalemma or plasmodesmata of mesophyll or phloem companion cells. Evaluation of effects of O_3 on tonoplast function and consequences for carbohydrate translocation await a more complete compartmental efflux analysis.

Key words: Pima cotton, ozone, carbon translocation, phloem loading, compartmental efflux analysis.

Introduction

Ozone

The impact of oxidant air pollution, particularly ozone (O_3), on the phytosphere is substantial, widespread and increasing (Penkett, 1988; Taylor *et al.*, 1994). Impacts on crop plants (Heck *et al.*, 1988) and native species (Davison and Barnes, 1998; Turcsanyi *et al.*, 1999) range from minimal visible symptoms to substantial inhibition of productivity, including the potential reduction of species biodiversity (Bishop and Cook, 1981). Despite the environmental and economic significance of its effects on terrestrial ecosystems, the mechanism of action of O_3 remains poorly understood.

Carbon assimilation and allocation

In Pima cotton, biomass allocation to the root system is reduced in a dose-dependent manner by O_3, with profound functional consequences (Grantz and Yang, 1996). In many species (Reiling and Davison, 1992), including upland (*Gossypium hirsutum* L.; Oshima *et al.*, 1979) and Pima cottons (*G. barbadense* L.; Grantz and Yang, 1996; DA Grantz, S Yang, unpublished results), exposure to O_3 causes a shift in the allometric coefficient. This indicates an alteration in relative growth rates of roots and shoots and not simply a change in the instantaneous root/shoot biomass ratio associated with an O_3-altered rate of plant development. The mechanism of such O_3-effects on allocation is not well understood.

Ozone reduces photosynthetic carbon assimilation (A), with associated reductions in chlorophyll concentration,

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carboxylation efficiency, photosystem II activity, and both amount and activity of Rubisco (Farage *et al.*, 1991; Pell *et al.*, 1997; Kangasjarvi *et al.*, 1994; Dann and Pell, 1989; Reich, 1983). These changes are consistent with the observed acceleration of leaf senescence and reduced green leaf area duration (Pell *et al.*, 1997; Alscher *et al.*, 1997) and could reduce the availability of carbohydrate for export from source leaves to developing sink tissues (Anderson *et al.*, 1991). Alterations of carbon allocation between competing sinks could be mediated by such reductions in source strength (Minchin *et al.*, 1993), resulting in the reduced partitioning of photosynthate to roots (Bennett *et al.*, 1979; Oshima *et al.*, 1978, 1979), or stolons (Wilbourn *et al.*, 1995; Barnes *et al.*, 1998) that is observed in many (Cooley and Manning, 1987; Darrall, 1989; Taylor and Ferris, 1996) but not all (Barnes *et al.*, 1998; Reiling and Davison, 1992), plant species following long-term exposure to O_3.

Consistent with this hypothesized etiology, O_3 effects on radish (*Raphanus sativus* L.) were first evident as a reduction in leaf biomass and were only then followed by a reduction of allocation to the root system (Walmsley *et al.*, 1980). Such altered allocation, possibly to support repair metabolism in the shoot, could maintain assimilatory capacity in leaves (Wolfenden and Mansfield, 1991) with consequent improved plant competitive performance.

In some cases (Tingey *et al.*, 1973; Bennett *et al.*, 1979), foliar pools of soluble sugars were reduced by exposure to O_3. More commonly, carbon export has been found to be reduced, and foliar pools of soluble sugars increased, by O_3. Retention of newly assimilated carbon in source leaves is associated with reduction in sugar content of sink tissues (Balaguer *et al.*, 1995). Increased tissue sugar concentrations (manipulated in a variety of ways) have been shown to reduce mRNA for the small subunit of Rubisco (Krapp *et al.*, 1993). Thus the inhibition of carbon assimilation following exposure to O_3 could reflect the O_3-induced build-up of sugars rather than direct oxidative damage. Accumulation of soluble sugars has been observed in Pima cotton subjected to both chronic (Grantz and Yang, 1996; DA Grantz, S Yang, unpublished results) and acute (DA Grantz and S Farrar, unpublished results) exposure to O_3. This build-up of sugars suggests that the availability of carbon in source leaves is not limiting export to sink tissues. A primary O_3-impact on phloem transport is suggested.

Carbohydrate translocation

A reduction of translocation of recent photosynthate to roots has been observed following exposure to O_3 (Spence *et al.*, 1990; McCool and Menge, 1983; Gorissen and van Veen, 1988). Translocation of ^{13}C in *Phaseolus vulgaris* was rapidly inhibited by O_3 (Okano *et al.*, 1984). Uptake and accumulation of ^{11}C in stem tissues was inhibited by O_3 in loblolly pine (*Pinus taeda* L.). The total amount of ^{11}C translocated was reduced substantially (about 45%; Spence *et al.*, 1990), while rates of export from source needles and velocities of transport along the stem were not significantly affected. The velocity of translocation along the stem was reduced in wheat (about 11%) by O_3 (Mortensen and Engvild, 1995), although this effect was not statistically significant.

Another oxidant air pollutant, SO_2, has been investigated in this regard (Gould and Mansfield, 1988). Phloem loading of ^{11}C-labelled photoassimilate was rapidly inhibited by 10 $\mu l\,l^{-1}$ SO_2 in *Phaseolus vulgaris* L. (Minchin and Gould, 1986). Exposure to 0.1 $\mu l\,l^{-1}$ SO_2 had no effect in this species on assimilation rate but substantially reduced export of ^{14}C from source leaves (Noyes, 1980) while efflux in the same species was generally inhibited more than assimilation at each concentration of SO_2 (Tch and Swanson, 1982). The mechanism of action of O_3 and SO_2, however, may differ significantly.

Benedict and Kohel (1975) found that about 90% of ^{14}C-labelled photoassimilate in fruiting upland cotton in the field was lost from source leaves within 22 h, following a 5 min pulse. Ashley (1972), in contrast, observed that after 2 h upper main stem leaves retained about 60% of the original label, and after 24 h they retained about 40%. These latter values are consistent with published information on C_3 dicotyledonous species (Hofstra and Nelson, 1969*b*). Neither efflux kinetics nor compartmental analyses have been presented for leaves of upland nor Pima (*G. barbadense* L.) cotton, while effects of O_3 on such kinetics have not been characterized in any species.

The present communication tests the hypothesis that an early stage in the etiology of O_3 phytotoxicity involves inhibition of translocation of newly assimilated carbon. By focusing on the rapid phase of efflux from source leaves, the emphasis is placed on structures such as the plasmalemma and plasmodesmata of mesophyll and phloem companion cells. These are the most likely membrane systems to be exposed to O_3 or products of O_3 degradation in the intercellular spaces of leaves.

Materials and methods

Plant growth

Seeds of Pima cotton (*Gossypium barbadense* L.; cv. Pima S-6; JG Boswell Company, Corcoran, CA, USA) were germinated in darkness on blotting paper wetted with distilled water. After emergence of the radicles to several cm in length (2–3 d), seedlings were removed from the blotting paper, the seed coats removed with forceps, and the stems enclosed at the crown in half-split discs of soft foam (closed cell camping mat; No. 8 cork borer; about 1 cm diameter × 0.6 cm thick). The discs were suspended in similarly-sized holes in hard polystyrene foam rafts floated in nursery troughs (7 dm^3) on continuously aerated half-strength Long Ashton solution. Twelve plants were grown

in each $15 \times 60 \times 14$ cm deep trough. Midday leaf osmotic potentials were 1.17 ± 0.04 MPa.

Plants were grown under artificial illumination (HQI/NDL/250W; Sylvania HIS-TD) at 28/22 °C (day/night) at a PPFD of 1 mmol $m^{-2} s^{-1}$ (18/6 h photoperiod). Plants were grown until the second true leaf was fully expanded (about 15 d after placement in troughs), at which time the plants were used for experiments.

Gas exchange and exposure to O_3

The second true leaf was trimmed with surgical scissors parallel to the mid-vein of the middle lobe, along a line 1 cm on each side of the vein, for a distance of about 4 cm. Then a cut at right angles to these incisions was made to the edge of the leaf, so that a strap of leaf containing a major vein was inserted into the well-stirred leaf cuvette ($7 \times 19 \times 2.5$ cm) and suspended between two nylon mesh screens about 0.3 cm above a Geiger-Müller Tube (Mullard, ZP 140; Alrad Instruments, Newbury, UK) mounted in the bottom of the cuvette. Modification of the leaf was required to reduce total transpiration into the cuvette and to increase ventilation between the leaf and the Geiger-Müller Tube, both to prevent condensation. This had no apparent effect on A per unit leaf area, nor on ^{14}C-efflux. Leaves were sealed into the cuvette at least 2 h into the photoperiod, after acclimatization to the constant, controlled temperature of the measurement facility (21 °C) overnight. Ozonation (0.75 h duration) was begun when carbon assimilation rate attained steady-state and the leaf had been sealed in the cuvette for at least 1 h. Introduction of ^{14}C-label into the gas stream was begun 15 min after termination of ozonation. Assimilation rate was recorded following the attainment of steady-state conditions and again following ozonation. Carbon assimilation rate was depressed by O_3 and did not recover substantially during the subsequent few hours of these experiments.

Methods were generally those of Owera *et al.* (Owera *et al.*, 1983) except that only one of the two Geiger-Müller Tubes in the cuvette was used. The gas flow system was modified to include an ultraviolet lamp-O_3 generator (Hamamatsu Model 90-0001-01; Middlesex NJ) and sensor (Monitor Labs model 8810) and an infrared gas analyser (LI 6252, Li-Cor Inc., Lincoln NE, USA) operated in differential mode for CO_2. Ambient air (1 dm^3 min^{-1} through the cuvette) was drawn from a large roof-top reservoir to reduce fluctuations in carbon dioxide concentration. Leaf temperature was monitored with a type T thermocouple appressed to the abaxial leaf surface.

The air stream flowed constantly through a sealed aluminium chamber containing the ultraviolet lamp. Ozonation was initiated by supplying power to the lamp, and controlled manually by splitting flow between an activated charcoal filter and a bypass, located in parallel between the O_3 generator and the cuvette. Target concentrations were 0.0, 0.2, 0.5, and 0.8 $\mu l\, l^{-1}$ O_3. O_3 concentration in the gas stream exiting the cuvette increased from undetectable levels to target concentrations within 3 min, though manual control led to initial instability around these target concentrations. For this reason actual O_3 exposures are presented for individual leaves as means of 1 min integrated measurements of concentration ($n=45$) with associated standard errors. In the absence of power to the O_3 generator there was no detectable O_3.

$^{14}CO_2$ was generated by bubbling the air-stream through 5 ml of lactic acid, to which 100 μCi (3.7 MBq) of $Na_2{}^{14}CO_3$ in 15 ml Na TRIS (pH 7.0) was added using a syringe perfusion pump over 15 min. After flowing through the leaf cuvette, differential gas analyser, and O_3 analyser, the gas stream passed over soda lime to remove any residual $^{14}CO_2$.

Efflux analysis

Assimilation and efflux of ^{14}C were monitored at the source leaf by connecting the Geiger-Müller Tube to a rate meter (Model SR7; Nuclear Enterprises, Edinburgh, Scotland, UK). The analog voltage output was displayed on a chart recorder and simultaneously captured on a digital data logger (21 X, Campbell Scientific Inc., Logan UT, USA) sampling every 60 s. Data were retrieved from the digital files and normalized, taking 100% activity and time zero at the onset of sustained reduction of ^{14}C activity.

Analysis of the efflux curves (declining activity in the leaf over time) were undertaken in two stages. First a two parameter single exponential decay function was fitted (Non-Linear Statistical Utility; SigmaPlot v. 3.0; SPSS; Chicago IL) to the initial log-linear period (0.5–1.5 h) of each efflux curve, as:

$$y = ae^{-bt} \quad (1)$$

in which y is the percentage of the originally fixed ^{14}C label remaining at time (t), b is the first order rate constant for carbon efflux from the leaf (h^{-1}), and a is a fitted parameter always approximately equal to 100%. This is equivalent to the one compartment model (Evans *et al.*, 1963) and analogous to more complex models (Moorby and Jarman, 1975; Bell and Incoll, 1982; Owera *et al.*, 1983; Rocher *et al.*, 1994). The simplified single compartment protocol required minimal assumptions regarding the biology of the source leaf and few model parameters, and avoided the computational irregularities arising from the small rate constants characterizing the slow phase of efflux from these leaves over longer periods of time. Sugar contents of the source leaf laminae were calculated as:

$$Q = A/b \quad (2)$$

in which A is the net assimilation rate (g C $m^{-2} h^{-1}$) and Q is the pool size of transportable carbohydrate (g C m^{-2}), largely sucrose in cotton leaves (Hendrix and Grange, 1991).

A second stage of analysis incorporated the approximately 65% of initial activity not exported from the leaf during these experiments as an asymptote. This value, obtained visually from the chart recordings, was similar for all leaves measured. Again the log-linear initial portions of the efflux curves were fitted to a single exponential decay function analogous to Equation (1) ($y = ae^{-b*t} + 65$). Adjusted soluble sugar concentrations were then calculated by analogy with Equation (2) ($Q^* = 65 + A/b^*$).

Presentation

Data are presented as time-courses of ^{14}C activity in individual leaves (Fig. 1), as calculated efflux parameters regressed against mean O_3 concentration for each leaf (Figs 2, 3, 5, 6), and as means of 3–4 replicates of these parameters, for all leaves exposed to each target concentration, regressed against mean O_3 concentration (Figs 2, 4). Regressions were calculated using the Linear Regression Utility in SigmaPlot, follow log-transformation of the data as required (Figs 1, inset; 3A, 5, 6B).

Results

Rapid efflux kinetics

Source leaves were labelled over relatively brief periods, much shorter than those required to attain isotopic steady-state (uniformity of activity across all metabolic compartments). Monitoring of ^{14}C efflux kinetics began immediately after this brief labelling. Carbon initially in

the labile or transport pool was directly accessible to phloem loading and thus to removal of the ^{14}C from the vicinity of the Geiger-Müller Tube. The resulting initial phase of efflux of ^{14}C-label from the source leaf was rapid in the absence of O_3 exposure (Fig. 1; open triangles). The data for the first 1 h of efflux were well described ($r^2=0.98$; $n=48$) by a single exponential decay function of the form of Equation (1), with the log-linear slope (equivalent to the negative rate constant, b) revealed by linearization of the data through natural logarithmic transformation (Fig. 1, inset, open triangles).

Exposure to O_3 for 0.75 h reduced both the initial rate at which ^{14}C activity was lost, and the amount of label lost from the leaf during this experimental period (Fig. 1, and inset). O_3 had an impact at the lowest concentration tested (0.27 $\mu l\, l^{-1}$; solid triangles), a substantial impact at 0.52 $\mu l\, l^{-1}$ (Fig. 1; open circles) and nearly abolished efflux at the highest concentration tested (0.77 $\mu l\, l^{-1}$; Fig. 1; solid circles). The rate constants calculated from these data declined linearly and highly significantly ($P<0.001$; Fig. 2) with increasing O_3 concentration, from a mean value of about 0.3 h^{-1} to the very low value of about 0.05 h^{-1}. Consideration of means of leaves exposed to the same target concentrations of O_3 ($n=3$ or 4, Fig. 2; bidirectional error bars) indicated that exposure to the nominal 0.2 $\mu l\, l^{-1}$ treatment did not significantly reduce the rate constant, though exposure to the nominal 0.5 $\mu l\, l^{-1}$ and 0.8 $\mu l\, l^{-1}$ treatments significantly reduced b relative to the O_3-free control leaves. The mean rate constant of leaves exposed to the 0.8 $\mu l\, l^{-1}$ treatment also differed significantly from that of the leaves exposed to the 0.2 $\mu l\, l^{-1}$ treatment.

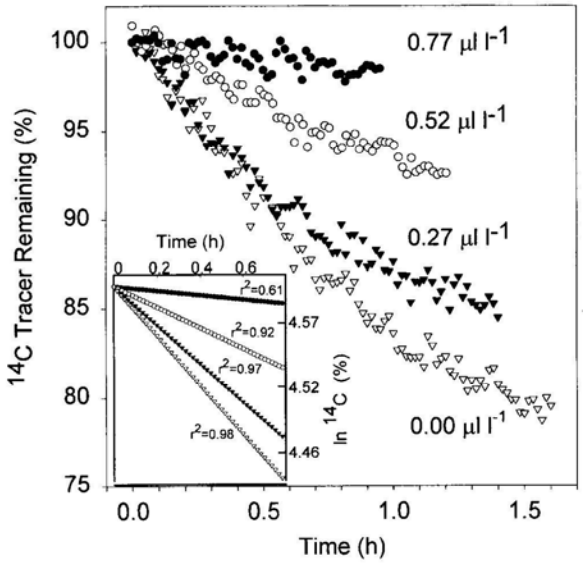

Fig. 1. Effect of exposure to O_3 (0.75 h; 0–0.8 $\mu l\, l^{-1}$) on retention of ^{14}C-labelled photoassimilate by representative illuminated, attached source leaves. Inset: ln-linear transformation of the single exponential decay functions fit to these data.

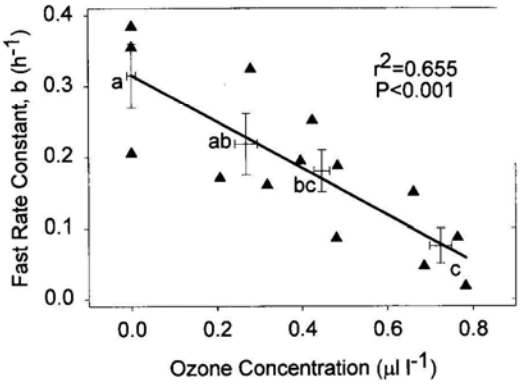

Fig. 2. Effect of O_3 concentration during exposure for 0.75 h on the rate constant (b) for the fast phase of efflux of ^{14}C-labelled photoassimilate from source leaves. Means ($n=3$–4) associated with the same letter do not differ at $P<0.05$. Regression parameters are derived for the entire data set ($n=14$). The horizontal error bar at $[O_3]=0.0$ $\mu l\, l^{-1}$ is expanded for clarity to identify the vertical midpoint.

Carbohydrate availability for sink tissues

The decline in efflux rate constant with increasing exposure to O_3 suggested substantial changes in the carbohydrate economy of the O_3-exposed leaves. The half-time for turnover of newly fixed carbon ($t_{1/2}$) increased from about 2 h in the O_3-free control leaves, to over 13 h in the leaves exposed to the highest O_3 concentration (Fig. 3A). The logarithmically transformed $t_{1/2}$ values were positively and linearly correlated with the O_3 concentration during exposure ($P<0.05$).

This increase in $t_{1/2}$ resulted in a highly significant ($P<0.001$; Fig. 3B) negative relationship between the percentage of available carbon exported in 2 h and the O_3 concentration during exposure, with export decreasing from about 50% to about 10% of label in the leaf. Expressed relative to the percentage of ^{14}C exported from control leaves, efflux declined significantly with increasing O_3 concentration ($P<0.005$), decreasing by about 25% at 0.27 $\mu l\, l^{-1}$ and by about 70% at 0.77 $\mu l\, l^{-1}$ (Fig. 4; triangles).

In contrast to this large impact of O_3 on carbon export from the source leaf (Fig. 4; triangles), the effect on carbon assimilation (A) was significant ($P<0.05$; Fig. 4; solid circles) but much smaller. A was reduced by less than 20% at the highest O_3 concentration (0.77 $\mu l\, l^{-1}$).

The combination of O_3-induced impacts on carbon assimilation and on carbon efflux resulted in a substantial reduction of total export of carbon from source leaves with increasing O_3 concentration ($P<0.01$; Fig. 4, open circles). Total carbon available to sink tissues outside the source leaf was reduced by about 25% at the lowest O_3

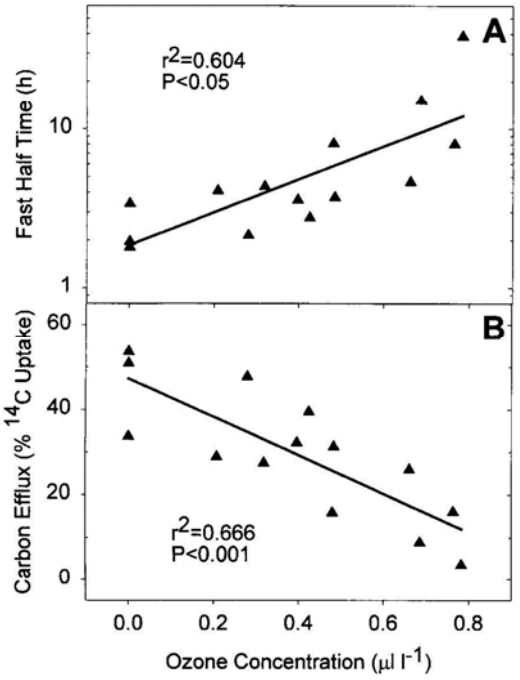

Fig. 3. Effect of O_3 concentration during exposure for 0.75 h on (A) the half-time for efflux of ^{14}C-labelled photoassimilate from attached source leaves (semi-log scale), calculated as $t_{1/2}=[(\ln 2)/b]$ using the data from Fig. 2, and (B) the export of ^{14}C in 2 h as a percentage of the total label assimilated.

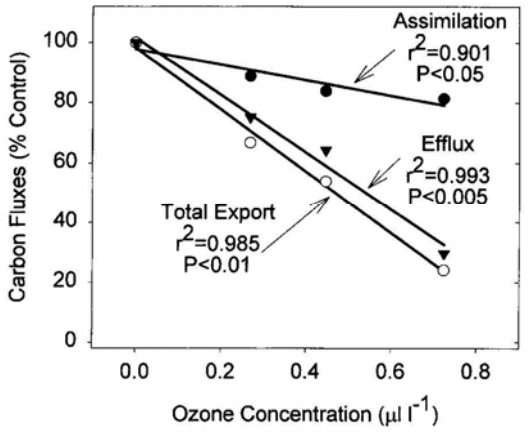

Fig. 4. Effect of O_3 concentration during exposure for 0.75 h on (solid circles) carbon assimilation, (solid triangles) efflux of ^{14}C-labelled photoassimilate within 2 h following labelling, (open circles) the combined effect on total carbon exported from source leaves at 2 h. The regression parameters are derived from mean data ($n=4$) expressed relative to O_3-free controls.

concentration tested and by up to 82% at the highest concentration. This amounts to a substantial withdrawal of carbohydrate availability for growth and development of distant sinks such as roots and reproductive structures.

Soluble carbohydrate pools

As the export of C is inhibited more than its acquisition (Fig. 4), exposure to O_3 could increase the soluble carbohydrate content of the source leaf. Calculation of the translocatable carbon pool (Q) in the source leaves (Equation (2)) following exposure to O_3 indicated a highly significant exponential increase in carbohydrate accumulation with increasing O_3 concentration ($P<0.005$; Fig. 5). Calculated carbon pools increased by more than 5-fold from about 1.5 g C m^{-2} in the control leaves to over 7.5 g C m^{-2} in leaves exposed to the highest O_3 concentration tested.

Non-exchangeable carbohydrate pool

The calculated soluble sugar contents appeared to be somewhat high, particularly following exposure to high concentrations of O_3. The simple, two parameter-single exponential decay model that was applied implicitly assumed that all newly assimilated carbon was exported from the source leaf during an adequately long photoperiod, and that the efflux of ^{14}C-label was proportional to the activity present in the single pool of soluble sugar in the leaf (i.e. first order). These assumptions are dubious and may lead to underestimation of the true rate constant and overestimation of the soluble carbon pool.

Preliminary analyses of 24 h efflux experiments suggested that about 65% of the ^{14}C activity remained in these leaves after protracted photoperiods. An alternative, single exponential decay function with three parameters, incorporating the asymptotic activity level (65%) which did not contribute to the efflux, was therefore tested.

The resulting adjusted estimates of the fast rate constant (b^*) were about 5-fold greater than the original values

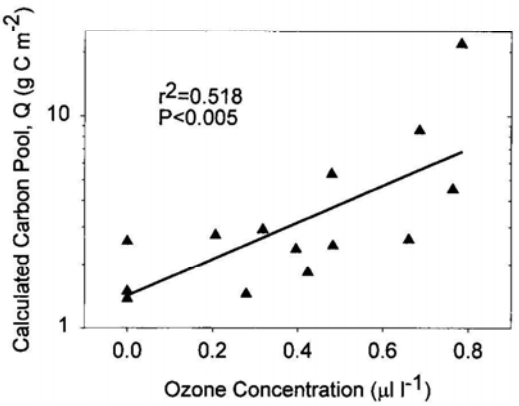

Fig. 5. Effect of O_3 concentration during exposure for 0.75 h on the leaf carbon pool (semi-log scale), calculated from mean carbon assimilation (12.2 µmol m^{-2} s^{-1}) for control leaves and treated leaves prior to exposure to O_3, the relative inhibition of assimilation following O_3 exposure for each leaf (Fig. 3), and the rate constant for ^{14}C efflux (Fig. 2).

(*b*) for control leaves and about 2-fold greater at the highest O_3 concentration. The adjusted rate constants, *b**, exhibited a highly significant, negative linear relationship with O_3 concentration during exposure ($P<0.002$; Fig. 6A), declining from about 1.5 to 0.2 h^{-1}.

The adjusted carbohydrate pool sizes (*Q**; Fig. 6B) calculated using the adjusted values, *b**, were considerably smaller than the original estimates (cf. Fig. 5), increasing exponentially and highly significantly ($P<0.002$) with increasing O_3 concentration during exposure, from about 0.3 to 3.0 g C m^{-2}.

Discussion

Ozone

The experimental protocol used in the present experiments, of acute exposures (0.75 h) to high concentrations (0.0–0.8 μl l^{-1}) of gaseous pollutants, has been abandoned recently in favour of more environmentally relevant protocols. However, acute exposures have considerable merit for physiological studies, revealing differences between species and cultivars in sensitivity to O_3 (Tingey and Taylor, 1982), and elucidating mechanisms (e.g. those underlying changes in photosynthesis; Farage and Long, 1995) that may be confounded by secondary effects and compensatory responses following prolonged exposure to more moderate concentrations. A similar conclusion regarding protocols for SO_2 exposure was reached by Minchin and Gould (Minchin and Gould, 1986).

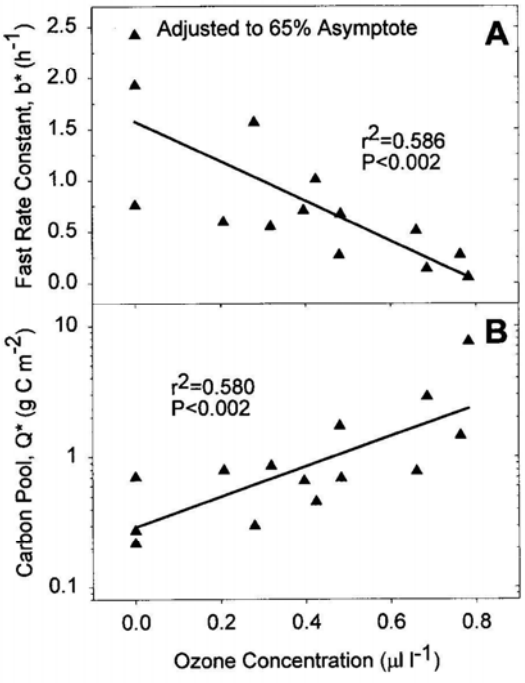

Fig. 6. Effect of O_3 concentration during exposure for 0.75 h on (A) the adjusted rate constant (*b**) for the fast phase of efflux of ^{14}C-labelled photoassimilate from attached source leaves, calculated as in Fig. 2 incorporating an apparent asymptote of 65% of photoassimilate not exported during a prolonged photoperiod, and (B) the leaf carbon pool, calculated as in Fig. 5 incorporating *b** from Fig. 6A.

The O_3 concentrations used in this study included an O_3-free control that was below background concentrations observed in natural systems, a concentration (0.2 μl l^{-1}) that is currently observed in some areas, and concentrations (0.5 and 0.8 μl l^{-1}) that are above those commonly observed. Physiological effects were observed across this range of concentrations. Plants returned to their growth environment for several days following exposure for 0.75 h exhibited no visible symptoms (0.0 and 0.2 μl l^{-1}), some discolouration (0.5 μl l^{-1}) and visible injury (0.8 μl l^{-1}).

Carbon assimilation and translocation

Carbon assimilation (*A*) of recently mature leaves of Pima cotton was reduced by up to 20% by exposure to O_3 concentrations up to 0.8 μl l^{-1}. Similar brief exposure to 0.6 μl l^{-1} O_3 of tomato (*Lycopersicon esculentum*) for 1 h reduced photosynthesis by 43%, and of bean (*Phaseolus vulgaris*) by 29%, whilst a slightly longer exposure (1.5 h) to 0.4 μl l^{-1} O_3 reduced assimilation in tobacco (*Nicotiana tabacum*) by 78% (Hill and Littlefield, 1969).

Carbon export from these Pima cotton leaves was reduced by up to 70%. However, there was little correlation (not shown) between the rate constant for efflux, *b*, and *A*, whether determined for individual leaves or as means calculated at each target O_3 concentration. Inhibition of each process by O_3 appeared to occur independently. This is consistent with the observed independence of ^{11}C-efflux and *A* when net assimilation was suppressed with CO_2-free air in the C_4 species, maize (Minchin and Gould, 1986). On the other hand, whole plant carbon allocation may be modified significantly by changes in *A* associated with altered PPFD, photoperiod, pathogens, and environmental stress (Minchin *et al.*, 1994). In cotton (Hendrix and Grange, 1991) manipulation of *A* by altering the photoperiod resulted in changes in total carbon exported from source leaves, but did not affect the relative export of newly assimilated carbon (i.e. *b*).

In tomato, O_3 increased retention of ^{14}C in source leaves (McCool and Menge, 1983). In wheat (Mortensen and Engvild, 1995) assimilation of carbon was reduced by O_3, but the velocity of movement of carbohydrate along the stem was unaltered. The velocity of phloem transport was reduced by 11% and the concentration and quantity of phloem translocate was reduced by 40% in loblolly pine following exposure to O_3, though the changes were not statistically significant (Spence *et al.*, 1990). In wheat (Balaguer *et al.*, 1995), O_3 had no effect on carbon

efflux in the light, but virtually abolished export in the subsequent dark period, resulting in enhanced retention of label in the source leaf. This is consistent with the disruption of starch remobilization and carbohydrate translocation following exposure to O_3 observed by Hanson and Stewart (Hanson and Stewart, 1970). In aspen (Coleman *et al.*, 1995) changes in assimilation dominated effects on carbon translocation, apparently determining relative sensitivity of contrasting clones to O_3.

The relative sensitivity of A and b may depend on the stage of leaf development. In fully mature leaves of aspen (*Populus tremuloides*; Coleman *et al.*, 1995) retention of label was increased by O_3 while the movement of label to the root system was enhanced. In more recently matured leaves the retention of ^{14}C activity was not affected by O_3, though export to roots was reduced and movement to the lower stem was increased. A similar increase in ^{11}C-label in the lower stem (40%) was incorrectly interpreted as transport to the roots in loblolly pine (Spence *et al.*, 1990), since transport to the upper stem also increased (by 80%). In mature primary leaves of *Phaseolus vulgaris* (Okano *et al.*, 1984), assimilation was reduced by about 62%, but in recently matured trifoliate leaves A was reduced only by about 24%. The primary leaf exhibited greater reduction in assimilation than in export, whilst trifoliate leaves exhibited greater inhibition of export.

Rapid efflux kinetics

O_3 reduced the unadjusted rate constant for rapid efflux from about 0.3 to 0.05 h^{-1}. These values are somewhat smaller than values reported in the absence of O_3 for the C_3 species, barley (0.4–1.0 h^{-1}; Farrar and Farrar, 1986) and tomato (0.8 h^{-1}; Moorby and Jarman, 1975), and for the C_4 species *Amaranthus* (0.4 h^{-1}; Moorby and Jarman, 1975). The effect of O_3 is evident from visual inspection of the efflux traces (Fig. 1) and from the rate constants (b) calculated from unmodified data (Fig. 2). One of the early events in oxidant damage to these source leaves of cotton (at least within 1 h of exposure) is the disruption of one or more of the processes leading to removal of ^{14}C activity from the vicinity of the Geiger-Müller Tube. As this phase of efflux corresponds mostly to movement of the labile pool of carbohydrate (Moorby and Jarman, 1975; Farrar and Farrar, 1985), oxidant damage is likely to be associated with impairment of plasmalemma function along the transport pathway, i.e. through damage to cell membranes of exporting mesophyll cells, or to those of importing phloem companion cells which scavenge sucrose from the apoplast prior to transfer of translocate to phloem sieve tubes. A potentially sensitive site of action could be the plasmodesmata, whose open state is environmentally regulated. More complex efflux analyses may have the capacity to resolve further mechanistic details and these studies are currently underway.

Soluble pools of carbohydrate

The present analysis, with assumptions of a single compartment and steady-state carbon pool size, allowed prediction of the sucrose contents accessible to phloem transport. While early work treated leaf carbohydrate as a single labile pool (Evans *et al.*, 1963), later analyses have suggested two to four kinetically distinguishable compartments (Moorby and Jarman, 1975; Bell and Incoll, 1982; Rocher *et al.*, 1994). Identification of these metabolic pools becomes increasingly uncertain with their proliferation. The limitations of compartmental analysis (Bell and Incoll, 1982; Cheeseman, 1986; Farrar and Farrar, 1985; Zierler, 1981) may reside more in assumptions of first order kinetics, diurnal stability, and steady-state carbon pools, than in incomplete models of kinetically distinct compartments.

Exposure to O_3 increased the calculated transport pool of soluble sugars from about 2 to more than 7.5 g C m^{-2}. These values for control leaves are consistent with published values for sucrose contents of unexposed cotton leaves, ranging from about 0.4 g C m^{-2} (Hendrix and Grange, 1991; Miller *et al.*, 1989) to about 1 g C m^{-2} (Wong, 1990). Some hexoses are also present in cotton (0.1–0.5 g C m^{-2}; Hendrix and Grange, 1991; Miller *et al.*, 1989), mostly glucose with little fructose. In a previous study of cotton, in which leaf sugars were analysed following (chronic) exposure to a range of O_3 concentrations (Miller *et al.*, 1989), only small and inconsistent changes in glucose and sucrose were observed. The preliminary measurements from this study suggest that these leaves contained soluble sugar contents of about 1–2 g C m^{-2} (DA Grantz and JF Farrar, unpublished data).

Exposure to O_3 reduced the sucrose content of wheat leaves from about 1.2 to 0.8 g C m^{-2} (Balaguer *et al.*, 1995), despite the greater inhibition of efflux than assimilation of C, a result attributed to increased respiration in the O_3-treated tissues. A correlation was observed between carbohydrate efflux and soluble sugar content as these varied across a range of treatments, specifically excluding those treatments involving exposure to O_3. This suggests a direct effect of O_3 on translocation. In aspen (Coleman *et al.*, 1995) soluble sugars increased following exposure to O_3, but starch declined.

These calculations assume that all the sucrose in these cotton leaves is in a labile pool. In barley only about 20–30% of the soluble sugars reside in this rapidly effluxing transport pool (Farrar and Farrar, 1986). However, in many starch-storing dicotyledonous species such as sugar beet (Geiger *et al.*, 1983) 60–80% of the soluble sugars are in the transport pool. In tomato (Ho, 1976), export of newly assimilated carbon in the light

was proportional to the size of the sucrose pool suggesting that much of the leaf content of sucrose is accessible to the phloem. A prominent exception, spinach (Gerhardt and Heldt, 1984) contains up to 80% of soluble sugars in the slow pool (i.e. the vacuoles). In spinach (Servaites *et al.*, 1989) there was only a poor correlation between leaf sucrose content and carbohydrate efflux. The distribution of sucrose in cotton leaves has not been adequately investigated, and cannot be ascertained from the present experiments. However, in one experiment with cotton (Hendrix and Peelen, 1987), carbohydrate efflux in the light was strongly related to leaf sucrose content, suggesting dominance of the transport pool.

In grasses such as barley (Farrar and Farrar, 1986), and wheat (Balaguer *et al.*, 1995) sucrose contents in source leaves reach maximum values late in the photoperiod. In dicots, in contrast, these pools generally fill rapidly and become stable within about 2 h of the onset of the photoperiod, as in cotton (Hendrix and Grange, 1991) and sugarbeet (Geiger *et al.*, 1983). The current measurements were thus performed near steady-state, though perturbations of both A and b following exposure to O_3 may disrupt calculated sucrose contents. Because the inhibition of A was much less than that of b, such perturbation would likely cause underestimation of the O_3 effect on sucrose pool sizes determined within a few hours of exposure.

Specific leaf weight of the leaves in the present study was about 44 $g\,m^{-2}$, with water content of about 260 $g\,m^{-2}$. This did not vary with O_3 exposure. The control leaves, and leaves prior to exposure to O_3, had osmotic potentials of 1.17 ± 0.04 MPa at midday, equivalent to over 15 g $C\,m^{-2}$ if all osmotic activity is attributed to sucrose. The sugar contents that were estimated are substantially lower than these values, and thus are not constrained by osmotic considerations.

Effect of a non-exchangeable pool

While C_4 grasses such as maize and sugarcane (Hofstra and Nelson, 1969*a*; Hartt, 1965) export more than 80% of newly assimilated label in 2 h, C_3 species generally export less rapidly and less completely, typically retaining 40–50% of activity after 6 h (Hofstra and Nelson, 1969*b*). Barley exported about 60% in a 16 h photoperiod (Farrar and Farrar, 1985). Main stem leaves of cotton exported about 40% of activity at 2 h and 60% by 24 h (Ashley, 1972), consistent with the 65% asymptote observed across all O_3 concentrations in the experiments reported here.

In dicotyledonous leaves such as those of cotton, leaf expansive growth occurs from distributed meristematic areas throughout the lamina. Although the leaves used in the present study were fully expanded, some leaf thickening and small amounts of expansion may have occurred during the experiment. Incorporation of the ^{14}C-label into structural material throughout the leaf is effectively lost from the transport or storage pools and is irreversibly accumulated, as is ^{14}C used in the turnover of leaf proteins such as Rubisco in non-growing leaves.

In addition to structural material, starch is not labile during the photoperiod (Hendrix and Grange, 1991), and represents an effective removal from the soluble transport pools during the present experiments, which were conducted in the light. Starch was not determined in the present experiment, but cotton leaves may accumulate starch contents from 0.4 g $C\,m^{-2}$ (Miller *et al.*, 1989) to 16 g $C\,m^{-2}$ (Wong, 1990). Starch accumulated in the light to about 2.5 g $C\,m^{-2}$ in the experiments of Hendrix and Grange (Hendrix and Grange, 1991) similar to values observed in field-grown cotton (Radin *et al.*, 1987). Efflux of label in darkness was related to the starch content measured at the onset of the dark period (Hendrix and Grange, 1991; Hendrix and Peelen, 1987), but this correlation was disrupted by exposure to O_3. Export of label in the light was unrelated to starch content, and little starch is degraded in the light (Hendrix and Grange, 1991). In aspen (Coleman *et al.*, 1995) starch content declined following O_3-treatment.

Incorporating the cumulative asymptote of 65%, representing label retained in all these possible ways, demonstrated that increasing O_3 exposure resulted in a significant decline in the adjusted rate constant, b^*, from about 1.5 to 0.1 h^{-1}. These values are more consistent with those expected (Farrar and Farrar, 1986; Moorby and Jarman, 1975), than the unmodified estimates of b (cf. Fig. 2). However, as the absolute magnitudes depend on the value chosen for the asymptote, it is reassuring that both approaches confirm the substantial impact of O_3 on carbon efflux from source leaves. On the other hand, the values of soluble sugar content (Q^*; Fig. 6B) of 0.3–3.0 g $C\,m^{-2}$ are much more in line with expected values than were estimates of Q, providing some confidence that the choice of 65% as a non-exchangeable fraction approximates the true value.

Conclusions

In the recently fully expanded second true leaf of Pima cotton, exposure to a range of O_3 concentrations inhibited ^{14}C export more than it inhibited carbon assimilation. This effect is reflected in the short-term kinetic parameters that likely reflect plasmalemma or plasmodesmatal function in mesophyll or phloem cells in the source leaves. It is consistent with a reduction of carbon allocation to distant sinks, particularly roots, of cotton (Oshima *et al.*, 1978; Grantz and Yang, 1996), but cannot fully explain the continued transport of carbon to stems (as was also noted following exposure to SO_2 by Jones and Mansfield, 1982) which also requires vigorous export from source leaves. The reduced flux of C associated with the inhibi-

tion of phloem transport could result in apparent preferential transport to adjacent sinks (e.g. stems) at the expense of distant sinks (e.g. roots), as modelled by Minchin *et al.* (Minchin *et al.*, 1993). Further work on the mechanisms underlying whole plant carbon allocation will be required to elucidate oxidant effects on plant growth, development, and productivity fully. Effects on vacuolar sugar pools, on starch storage and remobilization, and on critical tonoplast transport processes (Farrar and Farrar, 1986) also await a more complete compartmental analysis than was attempted here. It is becoming clear that effects of O_3 on carbon transport may dominate the better characterized effects on carbon assimilation. These considerations may aid in scaling oxidant effects from tissue damage and single organ function to the productivity of whole plants and ecosystems.

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