ELSEVIER

Environmental Pollution 87 (1995) 135–140

0269-7491/94/$09.50

EFFECTS OF OZONE ON ^{14}C TRANSLOCATION VELOCITY AND GROWTH OF SPRING WHEAT (*Triticum aestivum* L.) EXPOSED IN OPEN-TOP CHAMBERS

Lisbeth Mortensen[a] & Kjeld C. Engvild[b]

[a] *National Environmental Research Institute, Department of Emissions and Air Pollution, Frederiksborgvej 399, DK-4000 Roskilde, Denmark*
[b] *Risø National Laboratory, Environmental Science and Technology Department, DK-4000 Roskilde, Denmark*

(Received 24 June 1993; accepted 28 February 1994)

Abstract

Growth and yield were reduced but ^{14}C translocation velocity was not affected by increasing levels of ozone in spring wheat exposed in open top chambers to the following treatments: charcoal filtered air (CF), non-filtered ambient air (NF), or NF with addition of 30 μl litre^{-1} ozone, 8 h daily (NFO). Destructive harvests were performed at anthesis and at maturity. Parts of the flag leaf or the second leaf were exposed to $^{14}CO_2$ in small cuvettes for 5 min before, during and after anthesis. The translocation velocity was followed by autoradiography and scintillation counting of the plants frozen and lyophilized at different times after labelling. The label was transported at the same velocity in all the treatments. Ozone induced changes in carbon allocation or partitioning should probably be explained as amounts of carbon transported (mg s^{-1}), rather than as transportation velocity (mm s^{-1}). The amount translocated may be governed by source conditions under O_3 stress: reduced healthy green biomass and photosynthesis, but perhaps also by impairment of phloem loading because of membrane damage.

Keywords: ozone, spring wheat, *Triticum aestivum* L., open-top chambers, growth, yield, translocation velocity.

INTRODUCTION

Ambient concentrations of O_3 have increased considerably during the last century (Volz & Kley, 1988), and are expected to rise further in the future (Fishman, 1991). Present concentrations are known to affect plant metabolism (Heath, 1988; Runeckles & Chevone, 1991), photosynthesis (Darrell, 1989; Saxe, 1991), and carbon allocation pattern (Cooley & Manning, 1987) resulting in reduced root:shoot ratios and impaired fruit growth.

It is known that wheat is rather sensitive to O_3 while other crops like barley (Pleijel *et al.*, 1992) or *Vicia faba* (Sanders *et al.*, 1990) are less sensitive to O_3. In Europe, spring wheat cultivars have been studied in several countries using open-top chambers, and it has been found that growth (Adaros *et al.*, 1991; Fuhrer *et al.*, 1989; Temmerman *et al.*, 1992) and yield (Adaros *et al.*, 1991; Fuhrer *et al.*, 1989; Lehnherr *et al.*, 1987; Pleijel *et al.*, 1991) are reduced by O_3. Depending on O_3 concentrations and climatic conditions during the growth season, the effects of O_3 on grain yield are more pronounced than the effects on straw yield thus resulting in decreased harvest index (Adaros *et al.*, 1991; Fuhrer *et al.*, 1989; Lehnherr *et al.*, 1987; Pleijel *et al.*, 1991).

Carbon allocation pattern in a plant might be changed if O_3 interferes with the carbon translocation system. The plant could transport photosynthetic products to sinks at lower velocities. In wheat, the carbon allocation to the roots has been found to decrease in response to O_3 (Liljeroth *et al.*, 1990). This response has also been seen in Douglas fir (Gorissen *et al.*, 1991) and cotton (Oshima *et al.*, 1979). However, the studies do not provide any information on translocation velocity. This aspect was studied in Loblolly pine (Spence *et al.*, 1990) using the short-lived isotope ^{11}C. It was found that the amount of ^{11}C transported to the roots was reduced by 45% in O_3-treated trees, but the translocation velocity was only reduced by 11% which was not significant.

In the present study, we exposed spring wheat in open-top chambers to different concentrations of O_3, in order to investigate whether the translocation pattern of photosynthate in O_3-fumigated plants is different from control plants exposed to charcoal-filtered air, and if it is correlated with growth and injury symptoms.

MATERIALS AND METHODS

Plant growth

Seeds of spring wheat (*Triticum aestivum* L. cv. Ralle) were sown by hand, at a density of 400 seeds m^{-2} on 10 May 1991 in the soil of lysimeters below open-top chambers. This variety is of German origin and was found to be sensitive to O_3 in a screening test (Mortensen *et al.*, 1989). The plants were fertilized with 150 kg N ha^{-1} (NPK 13-6-9), treated preventively against powdery mildew with MILGO E on 11 June, and against weeds with Herbatox combi 3 on 16 June.

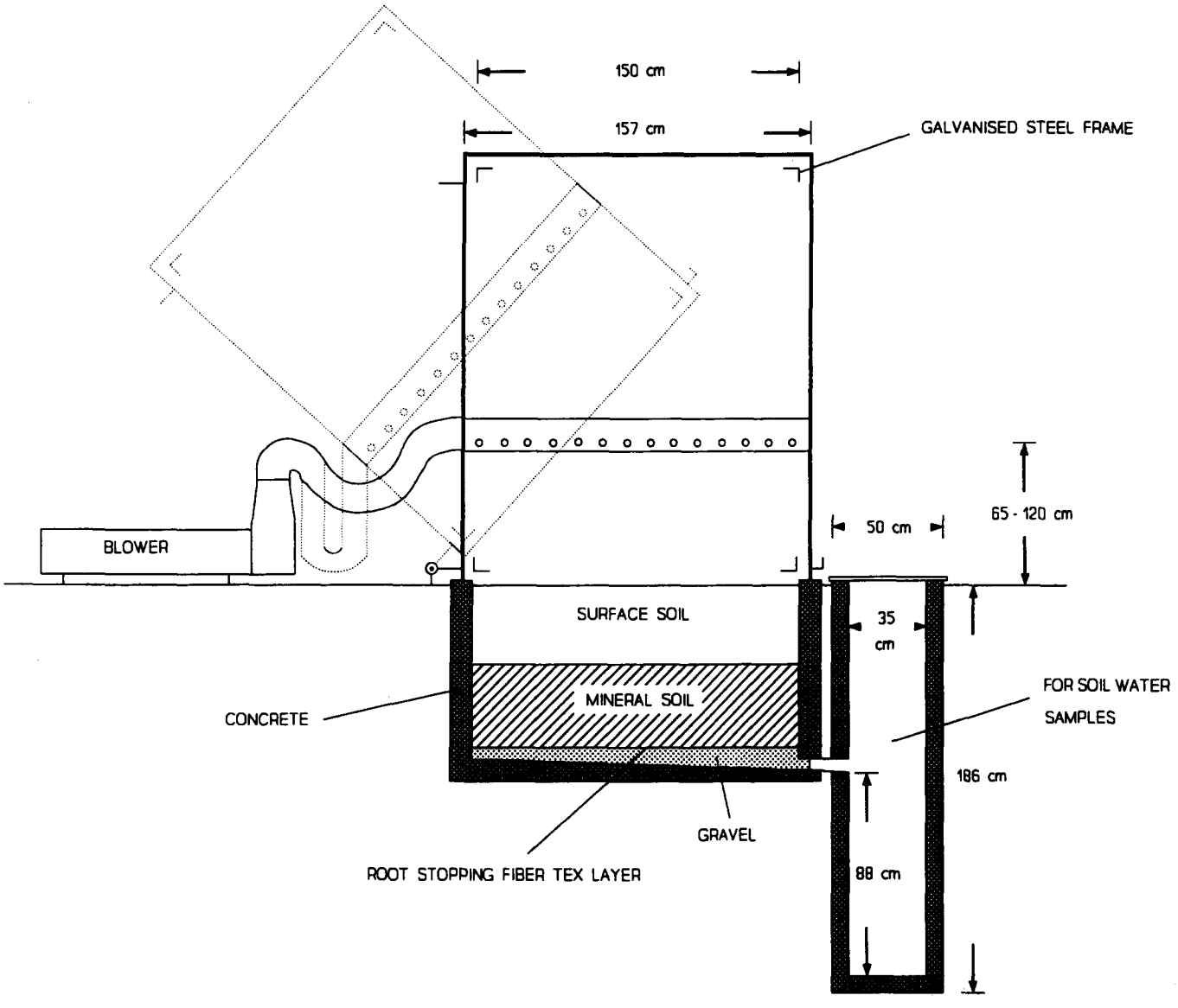

Fig. 1. Schematic drawing of an open-top chamber, showing lysimeter, well, and the blower ventilating the chamber. Access to the plants in the chamber is possible by tilting.

The plants were kept well watered with de-ionized water during the whole growing season. The climatic conditions during the growth season were: mean global radiation sum 15·4 ± 5·7 MJ m^{-2}, mean temperature day (6 am–9 pm/night 9 pm–6 am) 16·4 ± 4·1°C/13·1 ± 3·2°C, and mean relative humidity day/night 73·3 ± 9·6%/91·4 ± 5·0%.

Destructive harvests were performed during anthesis on 15–17 July (40 plants per OTC = 600 plants) and at maturity on 28 August (50 plants per OTC = 750 plants). At anthesis, plants growing within a distance of 15 cm from the circular chamber wall were harvested, and at maturity, plants growing between distances of 30 and 55 cm from the chamber wall were harvested.

Open-top chambers (OTC) and gas exposure

The experimental site is located in a rural area, about 40 km west of Copenhagen, Denmark (N55°41'/E12°06'). Small OTCs (diameter 1·5 m, height 1·8 m, 2 air changes min^{-1}), similar to those described by Fowler *et al.* (1987) but without baffles, were used. The OTCs are constructed of corrugated PVC Plast mounted on a galvanized steel frame, which turns on one hinge in one position (Fig. 1). The chamber was laid on its side when access to the plants was needed.

For this experiment 15 OTCs were used. The OTCs were placed on concrete lysimeters (diameter 1·5 m, depth 1·0 m, slope of bottom 8 cm), which were waterproofed with a layer of two-component epoxy. A plastic well (diameter 0·34 m, depth 1·90 m) drained each lysimeter. The lysimeters were filled with 10 cm gravel, a root stopping layer, 50 cm of screened mineral soil, and 40 cm of screened soil from the plough layer. The soil is a loamy clay taken from a nearby agricultural field. The lysimeter base allows watering of the plants from below using de-ionized water.

The blowers ventilating the chambers were equipped with coarse and fine particulate filters. The fumigation air was distributed from the blowers into the chambers through perforated polyethylene ducts, attached to the chamber walls at 1·2 m above soil level.

Plants were exposed to one of three air treatments:

charcoal filtered air (CF), non-filtered ambient air (NF), and NF with addition of 30 nl litre^{-1} O_3 8 h day^{-1} (9 am–5 pm, GMT+1) (NFO). Each treatment was replicated five times. Addition of O_3 started on 1 June, but due to equipment failure it was not effective before 23 June and it ended at final harvest. O_3 was produced by a silent discharge O_3-generator using dry pressurized air, and was distributed through flowmeters and Teflon tubes to the chamber blowers. Control experiments comparing the effects on plants of O_3 produced by electric discharge and strong UV light revealed no discernible differences (Mortensen, in preparation).

Gas and climate monitoring

Chamber air was sampled from a central position 10 cm above the canopy through 8 mm Teflon tubes which were flushed continuously with 2 litre air min^{-1}. To avoid condensation in the monitoring tubes, they were insulated and heated to 5°C above ambient. The concentrations of O_3, SO_2, and NO_x were measured sequentially in every NFO chamber and in one CF and NF chamber, using a Monitor Labs UV-absorption O_3 monitor (model 8810), a UV-fluorescence SO_2 monitor (model 8850), and a NO_x monitor (model 8840). Data from the five NFO OTCs were averaged to give the treatment mean.

In this system, monitors are calibrated after standardized methods before and after the experiment. Zero and span values are checked routinely every night and week, respectively. Gas sampling and data gathering are performed automatically using a PC based system, developed at NERI (Hansen, 1992). All raw data are collected automatically and transmitted every 6 h via the telephone net to the main frame at NERI and stored in a database.

Ambient climatic data were measured in a nearby permanent meteorological mast operated by Risø National Laboratory.

Labelling with $^{14}CO_2$

Simultaneously with the measurements of leaf photosynthesis, leaves of nearby plants were incubated with $^{14}CO_2$. The incubation cuvette was constructed of a small polystyrene container (5 × 7 × 0·7 cm), fitted with two serum stoppers for injection of $^{14}CO_2$, foam rubber insulation tape with opening slits for leaves, a stainless steel wire netting, and a glass petri-dish lid clamped on the top.

Wheat leaves from two plants growing in the OTC were firmly clamped in the cuvette, such that 5 cm of the leaf was in the cuvette, with a 5 cm distance between leaf ligule and cuvette. The distal part of the leaf protruded from the other side of the cuvette. By mixing 0·2 ml of $Na_2{}^{14}CO_3$ (3·5 × 10^6 Bq ml^{-1}, 2·2 × 10^9 Bq mmol^{-1}) with 0·4 ml 1M phosphoric acid, labelled CO_2 (7 × 10^5 Bq) was generated in a syringe and injected into the cuvette. After 5 min the $^{14}CO_2$ was sucked out of the cuvette through a trap containing NaOH. To avoid condensation and excessive temperatures during incubation the cuvette was shaded from direct sunlight by an umbrella. After removal of surplus $^{14}CO_2$, the cuvette was unclamped from the leaves. During incubation with $^{14}CO_2$, the plants were exposed to ambient air as the chambers, meanwhile, were tilted on their sides.

During the days of physiological measurements the 8 h mean O_3 concentrations for the CF, NF, and NFO treatments were 16, 33, and 73 nl litre^{-1} on 5 July; 16, 30 and 57 nl litre^{-1} on 11 July and 25, 34, 67 nl litre^{-1} on July 30, respectively. The mean temperatures on these days were 25·1, 21·3, and 25·0°C, respectively.

Transport of labelled photosynthate

After incubation, the plants were left for various periods, typically 20, 40, and 60 min before harvesting (Wardlaw & Moncur, 1976; L'Annunziata, 1979). After two preliminary experiments, three experiments were performed, one before anthesis, one during anthesis, and one two weeks after anthesis. In the first experiment, leaf number two (counted from the top) was incubated and the label followed downward. In the next two experiments the flag leaf was incubated and the label followed down the leaf ligule and up to the ear. Two plants from each air treatment were harvested for each transportation period.

Sampling, autoradiography, and counting

A procedure modified from Geiger and Sovonick (1975), L'Annunziata (1979), and Wardlaw and Moncur (1976) was used for sampling, autoradiography, and counting. Plants were harvested quickly, cut into approximately 30 cm pieces, mounted on filter paper and cardboard, frozen in solid CO_2, and subsequently lyophilized. The freeze-dried material was mounted in 'Vita' wrap and autoradiographed on X-ray film. These were used for a preliminary estimate of the translocation velocity and as guides when preparing for scintillation counting.

The dry plant material was cut into 4 cm pieces, each of which were cut into smaller pieces in scintillation vials, decolorized and digested in 1·5 ml 2% NaOCl with 0·4% NaOH (Smith & Lang, 1987) overnight at room temperature. Ten millilitres of Ecoscint scintillant was added, the vials shaken well, and the samples counted in a Packard scintillation counter after a 24 h delay and with a lower energy cut off at 2·7 keV to minimize the effect of chemiluminescence.

Data treatment and statistical analysis

Average values for growth and yield were calculated for each chamber and used in a one-way ANOVA to test the effects of air treatments. If this analysis showed significance, Duncan's test was performed. The SAS-package, release 6.06 (SAS Institute Inc., North Carolina) was used for all analyses.

Data on radioactivity were used for the calculation of two translocation parameters, translocation velocity and the percentage of radioactivity transported from the labelled leaf segment per minute. Translocation velocity was calculated to the front of the radioactivity, defined as the point in the leaf where the activity was 10^{-4} of that in the $^{14}CO_2$ exposed leaf segment. The

Table 1. Mean concentrations of air pollutants (nl litre^{-1}) in the different treatments from O_3 fumigation was effective (23 June) to harvest at anthesis and maturity. CF = charcoal filtered air; NF = non-filtered air; NFO = NF + 30 nl litre^{-1} O_3 for 8 h day^{-1} (9 am–5 pm). The 8 h mean is the mean during fumigation

Harvest	Pollutant	Treatment		
		CF	NF	NFO
Anthesis	O_3 24 h mean	11	22	32
	O_3 8 h mean	16	28	61
	NO 24 h mean	2	1	1
	NO_2 24 h mean	3	5	5
	SO_2 24 h mean	<1	<1	<1
Maturity	O_3 24 h mean	12	22	32
	O_3 8 h mean	17	29	61
	NO 24 h mean	2	1	1
	NO_2 24 h mean	3	5	5
	SO_2 24 h mean	<1	<1	<1

more correct method of calculation, using the point of half maximal radioactivity at the leading edge of the radioactivity peak (Wardlaw & Moncur, 1976), was not applicable because peaks of radioactivity were often not evident.

RESULTS

The O_3 concentrations during the summer of 1991 were rather low (Table 1) in comparison to other years (Hertel & Hovmand, 1991). The concentrations of NO_x and SO_2 were often close to the detection limits of the monitors.

Raising the O_3 concentration in relation to ambient air increased early senescence and decreased the healthy leaf dry-weight plus the total biomass yield, and this was already evident at the harvest at anthesis, only three weeks after addition of O_3 commenced (Table 2). The plants showed symptoms of O_3 injury, with necroses and chloroses prominent especially on older leaves. At maturity, total biomass, grain yield and grain size decreased with increasing O_3 concentrations.

The translocation of the labelled photosynthate is shown in Table 3 and three individual measurements are shown in Fig. 2. There is no indication of differences in translocation velocity between the treatments.

Table 2. Growth parameters from harvests at anthesis and maturity. Each value is the mean of 200 or 250 plants. Different letters in a row indicate significant differences between air treatments, Duncan's test, $p < 0.1$

Harvest at:	Anthesis			Maturity		
Treatment:	CF	NF	NFO	CF	NF	NFO
Total biomass dry wt (g)	5·3^{ab}	5·5^{a}	4·8^{b}	8·4^{a}	7·8^{ab}	6·7^{b}
Healthy leaves dry wt (g)	1·0^{a}	1·0^{a}	0·7^{b}	—	—	—
Dead leaves dry wt (g)	0·2^{b}	0·2^{b}	0·4^{a}	—	—	—
Ears dry wt (g)	0·9	0·9	0·8	4·6^{a}	4·1^{ab}	3·4^{b}
Kernel dry wt (g)	—	—	—	3·7^{a}	3·3^{ab}	2·7^{b}
Weight per kernel (mg)	—	—	—	47·9^{a}	44·9^{ab}	40·2^{b}
Harvest index (%)	—	—	—	42·9	41·5	39·6

Table 3. Translocation velocity (mm min^{-1}) measured on spring leaves on three occasions

Date	Treatment		
	CF	NF	NFO
5 July	6·1	6·0	6·3
11 July	10·9	12·8	13·9
30 July	11·6	14·2	10·8

In Fig. 2, well developed translocation peaks appear with their maxima coinciding almost exactly. Since such translocation peaks were not always present in the experiments, the much less reliable leading edge estimate of translocation velocity was used. The amount of labelled photosynthate transported out of the incubated leaf segment per minute is shown in Table 4. There is no significant difference between the results of the treatments, although, a tendency that NFO treated plants transport lower amounts is suggested.

DISCUSSION

The only significant air pollutant at the experimental site where the present experiment was performed is O_3,

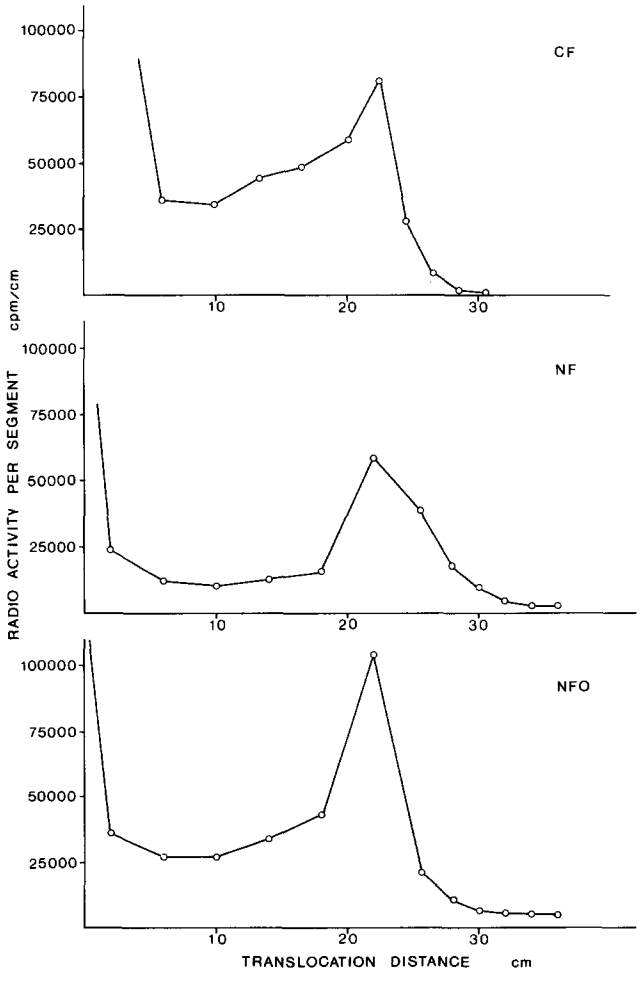

Fig. 2. Radioactivity measurements from incubated second leaf downwards given in count cm^{-1} min^{-1}. Data from an experiment performed on 5 July. Translocation time 60 min.

Table 4. Percent of ^{14}C exported from labelled leaf area (% min^{-1})

Date	Treatment		
	CF	NF	NFO
5 July	0·48	0·52	0·36
11 July	0·60	0·55	0·62
30 July	0·45	0·62	0·35

which often occurs at concentrations known to be phytotoxic (Guderian, 1988), although the concentrations vary from year to year. The O_3 level in 1991 was rather low, the seasonal 8 h mean being 29 nl $litre^{-1}$ and the maximum measured concentration being 68 nl $litre^{-1}$ in ambient air. This concentration was not high enough to cause significant reductions in any of the measured growth parameters in this present study. Significant effects of ambient air on grain yield in spring wheat were found in three out of 20 experiments performed during six years in the European open-top chamber network (Skärby *et al.*, 1993).

Adding 30 nl $litre^{-1}$ O_3 to ambient air during daylight hours led to a 100% increase in the amount of injured leaf dry-weight and a 30% decrease in photosynthesizing tissue after three weeks of fumigation. Flag leaf photosynthesis (not shown) was measured before and after anthesis and a reduction was found, especially when leaves grew older. Yield was reduced and it was caused primarily by smaller grains (Table 2). Similar results were found by Amundson *et al.* (1987), Fuhrer *et al.* (1989), Adaros *et al.* (1991), Pleijel *et al.* (1991), Ojanperä *et al.* (1992), and Temmerman *et al.* (1992).

Besides all the variables behaving in the expected manner we have also examined the translocation velocity of labelled carbon. To our surprise there were no significant differences in the translocation velocities of plants treated with O_3. We had expected the translocation velocity to decrease, because it is well known that O_3 fumigation changes allocation patterns substantially with decreasing amounts of carbon reaching the plant parts furthest away, i.e. the roots and the seeds. In the present work there is some indication that O_3 has changed carbon allocation patterns, because grain size is reduced; but the tendency is only weak. It was not possible to quantify transfer to roots.

We think it is fair to conclude from the present data that there seem to be no large decreases in translocation velocity upon O_3 fumigation. The limited number of replicates and the variability in the plants grown in open-top chambers, as well as the need to use the leading edge instead of the more exact half-maximum radioactivity, makes this conclusion somewhat tentative. However, the conclusion is consistent with the results of Spence *et al.* (1990) on translocation velocity in Loblolly pine seedlings. Spence and coworkers found a minor, non-significant reduction in translocation velocity, using ^{11}C labelling, but they did observe a large reduction (40%) in the amount transported. Our data can not be used for quantitative determination of the amount of sugar transported because the labelling procedure is not quantitative.

Minor decreases in translocation velocity are, of course, not excluded by this work. It is however doubtful that plants grown in open-top chambers are sufficiently uniform for experiments of this kind. Determinations of minor changes in transportation velocity will require many more replicates being performed on uniform plants, perhaps precultivated in growth chambers. Another possibility is experiments with the very short lived isotope ^{11}C, where it is possible to use non-destructive measurements. Use of this isotope requires very specialized equipment.

The work of Spence *et al.* (1990) shows that it is not necessary to have reductions in translocation velocity (mm s^{-1}) in order to explain changes in carbon translocation patterns; changes in amounts transported (mg s^{-1}) will do that. Our results are consistent with this. These variations in amounts transported may be caused by changes at the translocation source, for example, by decreased photosynthesis and reduced green photosynthesizing tissue, or reduced loading of the phloem because of membrane injury, or by sink changes along the route, e.g. increased respiration for repairing damage.

ACKNOWLEDGEMENTS

Thanks are due to B. P. Andersen and L. Meltofte for assistance in the field and laboratory, respectively. The authors would also like to thank T. N. Mikkelsen of the University of Copenhagen for the lending of physiological equipment, G. Jensen of the Risø National Laboratory for making meteorological data available, and L. Skärby for valuable comments on this manuscript. This project was partly financed by a CEC-subcontract to Nottingham University.

REFERENCES

Adaros, G., Weigel, H. J. & Jäger, H. J. (1991). Impact of ozone on growth and yield parameters of two spring wheat cultivars (*Triticum aestivum* L.). *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, **98**(2), 113–24.

Amundson, R. G., Kohut, R. J., Schoettle, A. W., Raba, R. M. & Reich, P. B. (1987). Correlative reductions in whole-plant photosynthesis and yield of winter wheat caused by ozone. *Phytopathol.*, **77**, 75–79.

Cooley, D. R. & Manning, W. J. (1987). The impact of ozone on assimilate partitioning in plants: A review. *Environ. Pollut.*, **47**, 95–113.

Darrell, N. M. (1989). Review: The effect of air pollutants on physiological processes in plants. *Plant, Cell & Environ.*, **12**, 1–30.

Fishman, J. (1991). The global consequences of increasing tropospheric ozone concentrations. *Chemosphere*, **22**(7) 685–95.

Fowler, D., Cape, J. N., Paterson, I. S., Leith, I. D. & Nicholson, I. A. (1987). Effects of air pollution on barley growth using open-top chambers. In *Microclimate and Plant Growth in Open-top Chambers*. Air Pollution Research Report 5, 71–75.

Fuhrer, J., Egger, A., Lehnherr, B., Grandjean, A. & Tschannen, W. (1989). Effects of ozone on the yield of spring wheat

(*Triticum aestivum* L., cv. Albis) grown in open-top field chambers. *Environ. Pollut.*, **60**, 273–89.

Geiger, D. R. & Sovonick, S. A. (1975). Effects of temperature, anoxia and other metabolic inhibitors on translocation. In *Encyclopedia of Plant Physiology, New Series*, **Vol 1**, ed. M. H. Zimmermann and J. A. Milburn. Transport in Plants I. Phloem transport. pp. 256–86. Springer Verlag, Berlin.

Gorissen, A., Schelling, G. C. & van Veen, J. A. (1991). Concentration-dependent effects of ozone on translocation of assimilates in Douglas fir. *J. Environ. Qual.*, **20**, 169–73.

Guderian, R. (1988). Critical levels for effects of ozone (O_3). UNECE critical levels workshop. Bad Harzburg, Germany. 14–18, March 1988, pp. 51–104.

Hansen, L. (1992). Open-top chamber data processing. National Environmental Research Institute, Technical Report No. 41, 33 pp.

Heath, R. L. (1988). Biochemical mechanisms of pollutant stress. In *Assessment of Crop Loss from Air Pollutants*, ed. W. W. Heck, O. C. Taylor & D. T. Tingey. Elsevier Applied Science, London and New York, pp. 259–86.

Hertel, O. & Hovmand, M. F. (1991). Rural ozone measurement in Denmark, 1985–89. National Environmental Research Institute, NERI Technical Report No. 35, 23 pp.

L'Annunziata, M. F. (1979). *Radiotracers in Agricultural Chemistry*. Academic press. London. 254–57.

Lehnherr, B., Grandjean, A., Machler, F. & Fuhrer, J. (1987). The effect of ozone in ambient air on ribulosebisphosphate carboxylase/oxygenase, activity decreases photosynthesis and grain yield in wheat. *J. Plant Physiol.*, **130**, 189–200.

Liljeroth, E., van Veen, J. A. & Miller, H. J. (1990). Assimilate translocation to the rhizophere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. *Soil Biol. Biochem.*, **22**, 1015–21.

Mortensen, L., Moseholm, L., Ro-Poulsen, H. & Johnsen, I. (1989). Work with open top chamber grown spring wheat in Denmark. In *The European Communities Research Project on Open-top Chambers; Results on Agricultural Crops 1987–88*, ed. J. Bonte & P. Mathy. Air Pollution Research Report 19, pp. 111–22.

Ojanperä, K., Sutinen, S., Pleijel, H. & Selldén, G. (1992). Exposure of spring wheat, *Triticum aestivum.*, cv. Drabant, to different concentrations of ozone in open-top chambers: effects on the ultrastructure of the flag leaf cells. *New Phytol.*, **120**, 39–48.

Oshima, R. J., Braeggelmann, P. K., Flagler, R. B. & Teso, R. R. (1979). The effects of ozone on the growth, yield, and partitioning of dry matter in cotton. *J. Environ. Qual.*, **8**, 474–79.

Pleijel, H., Skärby, L., Wallin, G. & Selldén, G. (1991). Yield and grain quality of spring wheat (*Triticum aestivum* L., cv. Drabant) exposed to different concentrations of ozone in open-top chambers. *Environ. Pollut.*, **69**, 151–68.

Pleijel, H., Skärby, L., Ojanpera, K. & Selldén, G. (1992). Yield and quality of spring barley, *Hordeum vulgare* L., exposed in open-top chambers. *Agriculture, Ecosystems and Environment*, **38**, 21–29.

Runeckles, V. C. & Chevone, B. I. (1991). Crop responses to ozone. In *Surface Level Ozone Exposures and Their Effects on Vegetation*, ed. A. S. Lefohn, Lewis publishers, 189–270.

Sanders, G. E., Turnbull, N. D., Clark, A. G. & Colls, J. J. (1990). The growth and development of *Vicia faba* L. in filtered and unfiltered open-top chambers. *New Phytol.*, **116**, 67–78.

Saxe, H. (1991). Photosynthesis and stomatal responses to polluted air, and the use of physiological and biochemical responses for early detection and diagnostic tools. *Adv. Bot. Res.*, **18**, 1–128.

Skärby, L., Selldén, G., Mortensen, L., Bender, J., Jones, M., Trappeniers, M., Wenzel, A. & Fuhrer, J. (1993). Responses of cereals exposed to air pollutants in open-top chambers. *Proc. of Int. Conf. Air Pollution and Crop Responses in Europe*. 23–25 November 1992, Tervuren, Belgium (in press).

Smith, I. K. & Lang, A. L. (1987). Decoloration and solubilization of plant tissue prior to determination of ^{3}H, ^{14}C, and ^{35}S by liquid scintillation. *Analyt. Biochem.*, **164**, 531–36.

Spence, R. D., Rykiel, E. J. & Sharpe, P. J. H. (1990). Ozone alters carbon allocation in Loblolly pine: Assessment with carbon-11 labelling. *Environ. Pollut.*, **64**, 93–106.

Temmerman, L. de, Vandermeiren, K. & Guns, M. (1992). Effects of air filtration on spring wheat grown in open-top field chambers at a rural site. I. Effects on growth, yield and dry matter partitioning. *Environ. Pollut.*, **77**, 1–5.

Volz, A. & Kley, D. (1988). Evaluation of the Montsouris series of ozone measurements made in the nineteenth century. *Nature*, **332**, 240–42.

Wardlaw, I. F. & Moncur, L. (1976). Source, sink and hormonal control of translocation in wheat. *Planta*, **128**, 93–100.