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Temporary Disturbance of Translocation of Assimilates in Douglas Firs Caused by Low Levels of Ozone and Sulfur Dioxide[1]

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ABSTRACT

Douglas firs (*Pseudotsuga menziesii* [Mirb.] Franco) are suffering strongly from air pollution in western Europe. We studied the effect of low concentrations of ozone (200 micrograms per cubic meter during 3 days) and sulfur dioxide (53 micrograms per cubic meter during 28 days) on translocation of assimilates in 2 year old Douglas firs. The trees were exposed to the pollutants and afterward transferred to a growth chamber adapted to the use of $^{14}CO_2$. Root/soil respiration was measured daily. The results showed a significant decrease of the $^{14}CO_2$ root/soil respiration during the first 1 to 2 weeks after exposure to either ozone or sulfur dioxide. The ultimate level of $^{14}CO_2$ root/soil respiration did not differ significantly, which suggests a recovery of the exposed trees during the first weeks after exposure.

The influence of air pollutants on growth and vitality of trees has received considerable attention in recent years, especially in relation to massive forest die-back in many countries. Effects of high concentration-levels of ozone and sulfur dioxide were mainly studied in relation to above ground reactions, *e.g.* photosynthesis, respiration, opening of stomata, and metabolism (7, 9). Ozone in a range of concentrations inhibits photosynthesis and increases respiration in the above-ground parts (3, 26). Adverse effects on photosynthesis following exposure to SO_2 have also been reported (14). Bell (2) proposed a threshold concentration of SO_2 of 100 to 200 $\mu g/m^3$ to obtain growth reduction in long-term experiments. This was confirmed by Black and Unsworth (3), who concluded that inhibition of photosynthesis thus occurred at realistic SO_2 pollution levels.

Hanson and Steward (10) discovered that ozone affected starch hydrolysis in beans and recently, swollen thylakoids were observed in chloroplasts after exposure to ozone indicating that transport of assimilates might be retarded (30).

Teh and Swanson (29) suggested that the process of translocation of assimilates is more sensitive to SO_2 than photosynthesis and several authors have reported effects of ozone and sulfur dioxide on translocation of assimilates in bean leaves or grass (2, 22, 23). The relevance of above ground effects of air pollutants on the activity and growth of the roots and vice versa is still obscure. Yet, the "whole tree approach" is essential for a realistic understanding of the effects of air and soil pollutants on tree growth and the establishment of workable parameters for the assessment of tolerance levels for air pollutants.

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The impact of air pollutants on translocation of assimilates and consequently on the whole carbon cycle in forest ecosystems has been poorly investigated. Freer-Smith (8) reported a decreased root/shoot ratio using birch trees exposed to about 110 μg SO_2/m^3 during 9 weeks, but data concerning coniferous species are not available. However, it is obvious that translocation of photosynthetically fixed carbon to below ground plant parts is essential for the activity and the growth of roots and the release of root products which provide a source of carbon and energy required by soil microorganisms such as parasites and saprophytes but in particular for mycorrhizae (16, 17). Therefore, translocation of photosynthates is a key issue in the functioning of both natural and arable ecosystems.

In this study, the effects of a short-term fumigation with ozone and a long-term treatment with sulfur dioxide on uptake and distribution of ^{14}C-photosynthates in Douglas firs were investigated by growing exposed young trees in a $^{14}CO_2$ labeled atmosphere.

MATERIALS AND METHODS

Two year old Douglas firs (*Pseudotsuga menziesii* [Mirb.] Franco) were potted in perspex columns (length 60 cm; diameter 9 cm). A description of these columns and the growth cabinet, specifically designed for $^{14}Co_2$ studies, is given by Merckx *et al.* (18).

In the bottom cap of the columns the perforated plate was covered with a gravel layer (1 cm). The remainder of the column was filled with three layers of forest soil to imitate a natural soil profile: yellow sand (C-horizont, 10 cm), humous material ($A_{1/2}$-horizont, 40 cm) and litter (A_0-horizont, 10 cm). Trees were planted with the roots in the humous layer. By gentle vibration, optimal contact between roots and soil was obtained while damage to the roots was minimized.

After planting, the trees were kept for 2 months in the dark at 2°C during the winter period. Before the experiment, the trees were acclimated to natural conditions for at least 2 months.

Treatment with Ozone and Sulfur Dioxide. *Ozone.* Six trees were exposed in growth chambers to 200 (±10) μg ozone/m^3 for a period of 3 days while six trees served as controls.

Sulfur Dioxide. Eight trees were exposed to 53 (±14) μg SO_2/m^3 for 28 d in open top chambers. Eight control trees were treated with an approximate SO_2 background concentration (24 [±8] μg SO_2/m^3) for 28 d to avoid a 'desulfurization' effect of the trees which grew up under natural conditions.

After exposure, the trees were placed in the growth cabinet and were connected for water supply and collection of root/soil-produced CO_2. The column lids were sealed airtight with Silastic (RTV Q3-7062, Dow Chemical). After closing the growth chamber unlabeled CO_2 was removed to a level of about 100 ppm

before $^{14}CO_2$ was injected into the shoot compartment. The trees were continuously exposed to $^{14}CO_2$ throughout the experiments. A preset total CO_2 level was maintained at 330 (±5) ppm either by injection of $^{14}CO_2$ or removal of $^{14}CO_2$ by a carbosorb filter. Every 6 h the columns were flushed with CO_2-free air, which was subsequently conducted through a 300 mL 0.5 N NaOH solution to trap the root/soil-produced CO_2.

Atmospheric conditions were partly adapted to the season in which the experiments were performed and are shown in Table I. Ozone and SO_2 in the growth cabinet were present at background concentrations.

Analyses. HCO_3^- and CO_3^{2-} ions in samples of the NaOH-solution were precipitated with $BaCl_2$. Total CO_2 amounts in the NaOH solution were determined by titration of the remaining NaOH with 0.5 N HCl. Total $^{14}CO_2$ amount was determined by liquid scintillation counting using Instagel (Packard). In the ozone experiment samples were taken after 1, 3, 5, 8, and 13 d, and in the sulfur dioxide experiment each day.

The ozone-treated trees were harvested 2, 6, and 13 d after they had been placed in the growth chamber. The first harvest took place as soon as $^{14}CO_2$ was detected in the root/soil respiration. The trees were subdivided into new needles (developed after the winter period), old needles, new branches, old branches/stem and roots.

The sulfur dioxide treated trees were harvested after 13 and 26 d and subdivided into needles, buds, branches/stem, and roots.

After removal of the trees from the columns root fragments were removed from the soil by hand. Roots were washed with tapwater. Dry weights of the fractions were determined after drying the ground and homogenized samples at 60°C for 24 h. To determine total C and ^{14}C a modified wet-combustion procedure was used (6, 17). Plant (30 mg) or soil (A_0-horizont 0.20 g; $A_{1/2}$-horizont 0.25 g; C-horizont 1.0 g) material was digested with a solution of 1.0 g $K_2Cr_2O_7$ in 5 mL concentrated H_2SO_4 and H_3PO_4 (3/2 v/v) at 120°C for 1 h. CO_2 evolved was trapped in 10 mL 0.5 N NaOH and processed as described above.

Total microbial biomass C and microbial biomass ^{14}C were determined using a modified chloroform fumigation incubation method after Chaussod and Nicolardot (5). A K_c-factor of 0.45 was used to convert net CO_2-C flush into microbial biomass C (13).

Table I. *Atmospheric Conditions during and after the Treatments with Ozone and SO_2*

	Exposure Room O_3	Open Top Chamber SO_2	Growth Chamber O_3	Growth Chamber SO_2
Temperature (°C)				
Day	20	18.3 (5.1)[a]		
Day shoot			20	16
Day root			18	14
Night	18	10.8 (3.2)[a]		
Night shoot			18	14
Night root			16	12
RH (%)				
Day	60	50–70	75	75
Night	80	90–100	75	75
Global radiation (W/m^2)	ND[b]	ND	95	95
Day/night (h)	16/8	16/8	16/8	16/8
Specific activity (kBq/mg C)			2.7 (1.4)	6.0 (1.2)

[a] Conditions in the open top chambers varied. *t* day and *t* night are the mean values of maximum day temperature and minimum night temperature. [b] ND = not determined.

RESULTS

Treatment with Ozone. No morphological abnormalities of the aerial parts were observed after the treatment with 200 μg ozone/m^3 during 3 d. Table II shows the total uptake of $^{14}CO_2$ and the distribution of ^{14}C over the different fractions of the trees. Total uptake was not affected by the treatment, even when the mass of the needles was taken into account as a covariable.

The old needles showed a tendency to retain more ^{14}C-photosynthetic products after treatment with ozone than the control (17.6, 31.3, and 10.9% compared to 11.9, 11.1, and 9.3%, respectively) ($P = 0.08$).

The percentage of $^{14}CO_2$ in the root/soil respiration decreased. On d 1, 3, and 5 the $^{14}CO_2$ amount in the root/soil respiration was significantly lower for the ozone treated trees ($P = 0.01$, 0.07, and 0.02 respectively; *t*-test). Note that if one compartment in Table II shows an increase another one must show a decrease.

Treatment with Sulfur Dioxide. No morphological abnormalities were found after exposure to SO_2. Table III shows the total uptake of $^{14}CO_2$ and the distribution over tree and soil. The amount of photosynthetically fixed carbon in the control trees on d 13 is much higher than in the experiment with ozone, 17263 *versus* 2921 kBq. This may be caused by a reduced photosynthesis, not caused by SO_2, during budding as described by Neuwirth (20).

No clear treatment effect (with or without needlemass as covariant) was observed for the total uptake of $^{14}CO_2$. Obvious effects on ^{14}C distribution were also lacking except for the root/soil respiration on d 13. More precise information was obtained using the daily $^{14}CO_2$ root/soil respiration data from all trees used. The cumulative amount of $^{14}CO_2$ in the root/soil respira-

Table II. *Total Uptake and Percent Distribution of $^{14}CO_2$ over Different Tree Parts and Soil after Treatment with 200 μg/m^3 Ozone during 3 d (n = 2)*

Treatment	Fraction	No. of Days in ESPAS[a] 2	6	13
		% of total uptake		
Control	New needles	38.2 (1.3)[b]	24.5 (2.5)	23.7 (12.6)
	Old needles	11.9 (0.2)	11.1 (1.1)	9.3 (3.6)
	New branches	5.2 (1.4)	5.8 (0.3)	4.6 (1.1)
	Old branches	24.8 (2.2)	20.1 (1.7)	27.2 (3.9)
	Roots	17.7 (0.2)	25.7 (2.5)	25.0 (14.4)
	Root/soil Respiration	ND[c]	11.5 (2.6)	9.2 (0.9)
	Microbial Biomass	1.1 (0.2)	0.2 (0.1)	0.4 (0.1)
	Soil residue	1.1 (1.0)	1.1 (0.0)	0.6 (0.3)
	Total uptake (kBq)	501 (41)	2541 (1639)	2921 (529)
Ozone	New needles	37.4 (6.4)	26.0 (13.7)	33.0 (16.5)
	Old needles	17.6 (0.8)	31.3 (15.9)	10.9 (1.6)
	New branches	5.3 (0.9)	6.5 (2.8)	7.2 (2.9)
	Old branches	17.9 (2.3)	26.0 (1.0)	26.9 (3.8)
	Roots	17.2 (1.9)	7.0 (6.1)	18.8 (22.9)
	Root/soil Respiration	3.4 (3.0)	2.8 (0.1)	1.5 (0.2)
	Microbial Biomass	0.8 (0.9)	0.2 (0.02)	0.4 (0.2)
	Soil residue	0.4 (0.03)	0.2 (0.1)	1.3 (1.4)
	Total uptake (kBq)	358 (143)	2622 (1305)	3054 (645)

[a] Experimental Soil Plant and Atmosphere System. [b] Standard deviations in parentheses. [c] ND = not determined.

Table III. *Total Uptake and Percent Distribution of $^{14}CO_2$ over Different Tree Parts and Soil after Treatment with 53 $\mu g/m^3$ Sulfur Dioxide during 28 d (n = 4)*

Treatment	Fraction	No. of Days in ESPAS[a]	
		13	26
		% of total uptake	
Control	Needles	20.7 (2.5)[b]	21.5 (6.6)
	Stems	19.9 (2.8)	14.3 (3.8)
	Buds	1.6 (0.5)	1.0 (0.4)
	Roots	49.1 (3.7)	54.8 (9.1)
	Soil/root Respiration	8.3 (0.9)	7.4 (2.2)
	Microbial Biomass	0.1 (0.1)	<0.1
	Soil residue	0.3 (0.1)	0.9 (0.8)
	Total uptake (kBg)	17263 (7099)	62589 (32083)
Sulfur dioxide	Needles	21.4 (3.4)	23.3 (2.0)
	Stems	19.9 (4.1)	19.4 (3.0)
	Buds	1.3 (0.6)	1.3 (0.3)
	Roots	52.9 (6.7)	47.4 (2.8)
	Soil/root Respiration	3.9 (2.8)	7.8 (2.7)
	Microbial Biomass	0.1 (0.3)	<0.1
	Soil residue	0.5 (0.6)	0.8 (0.7)
	Total uptake (kBq)	21133 (5018)	31380 (7782)

[a] Experimental Soil Plant and Atmosphere System.
[b] Standard deviations in parentheses.

tion appeared to be significantly lower in SO_2-treated trees from d 1 until d 13 (P ~0.01) when a *t*-test is applied to the results of each day.

Each tree showed a sigmoidal curve in the course of the daily production of $^{14}CO_2$. At $t = 0$ the root/soil respiration only contained unlabeled CO_2 and after 1 to 2 weeks the daily amount of $^{14}CO_2$ reached a stable level. The mean results of the two treatment groups are shown in Figure 1. In the figure sigmoidal curves can be observed, which reach their maximum after 9 to 15 d depending on the treatment.

Using nonlinear regression analyses we have fitted Gompertz curves (4) to these daily amounts. The Gompertz curve is based on the equation

$$f(t) = A \cdot \exp(-\exp(b - k \cdot t)).$$

The curve developed by Gompertz was formerly meant as a plant growth function but appeared to describe the dynamics of $^{14}CO_2$ in the root/soil respiration of each tree in the present study quite well and made it possible to estimate the stable level for the trees harvested after 13 d.

In the equation A represents the stable level of the amount of $^{14}CO_2$ in the root/soil respiration; it was allowed to vary for all trees. The parameters k and b are form-parameters; k was held constant within the treatment groups and b was taken equal for all trees. Parameter k indicates the period in which the curve reaches its stable level; it differs for both curves significantly ($P \leq 0.001$). By that time an equilibrium is reached in the carbon flow through the trees.

In Figure 2 the dynamics of $^{14}CO_2$ in the root/soil respiration for the treatments is illustrated by using the Gompertz equation. The mean root/soil respiration of the control trees reaches the stable level much earlier than that of the sulfur dioxide-treated trees (9 d *versus* 15 d). The utimate level of these curves is also

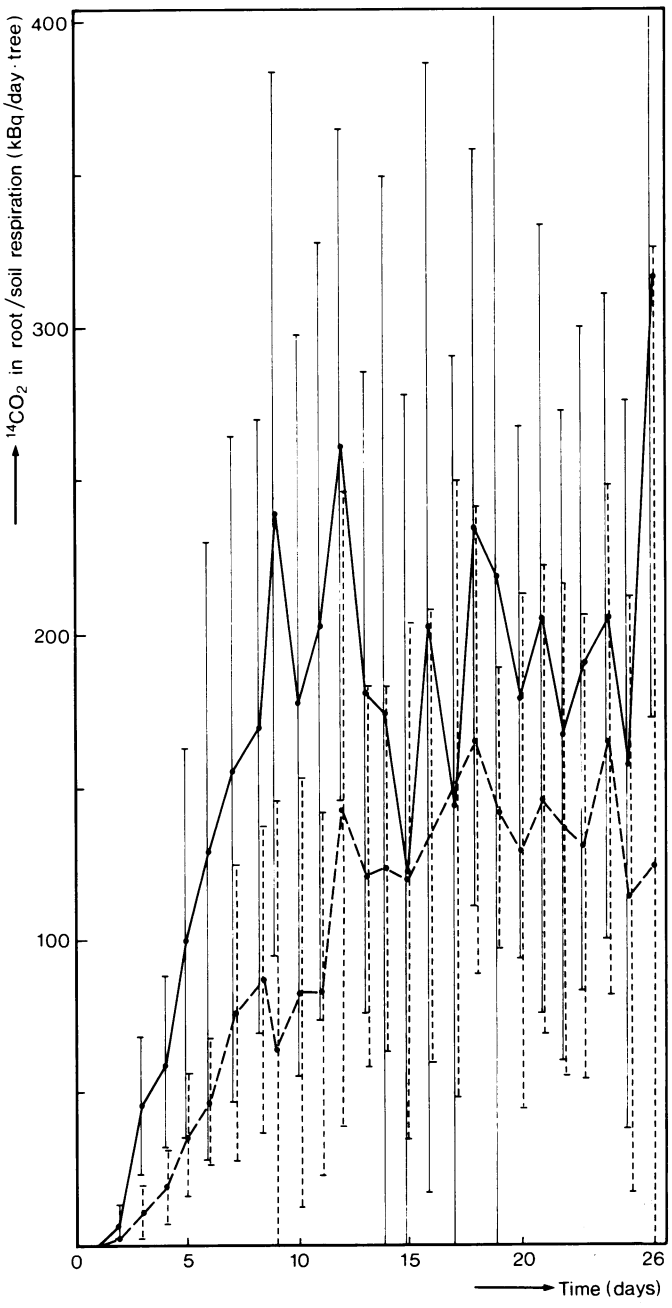

FIG. 1. Daily amounts of $^{14}CO_2$ in the root/soil respiration. The solid line represents the control trees ($n = 6$ on d 1–13 and $n = 4$ on d 14–26) and the broken line the sulfur dioxide-treated trees ($n = 7$ on d 1–13 and $n = 4$ on d 14–16).

based on estimations of the stable level of the trees harvested after 13 d. For this reason the levels can differ somewhat from the levels in Figure 1 which are based on four observations on d 14 to 26.

The heights of the levels do not differ significantly (P = 0.28, *t*-test), which suggests that the treated trees were recovering from the exposure to sulfur dioxide during the first weeks.

DISCUSSION

It is known that both sulfur dioxide and ozone can affect root growth negatively (15, 24) and can decrease root respiration as has been shown by Hofstra *et al.* (12) after exposure of beans to 300 μg O_3/m^3 for 1 to 3 d.

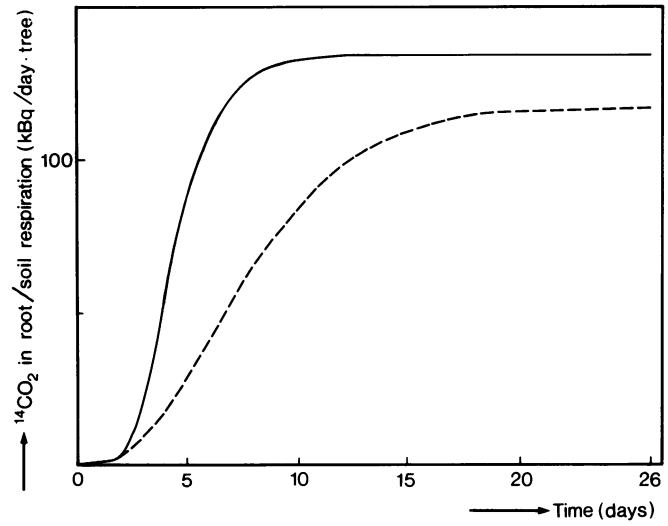

FIG. 2. Development of the root/soil respiration as described by the Gompertz equation. The solid line represents the respiration of the control trees, the broken line the sulfur dioxide-treated trees.

The present results show that short-term exposure to ozone and long-term exposure to sulfur dioxide temporarily delayed transport of fixed $^{14}CO_2$ to the lower parts of the plants, resulting in a statistically significant decrease of the root/soil respiration rate of $^{14}CO_2$ followed by a gradual recovery. A similar pattern was recently found in an experiment with a long-term exposure to 81 μg ozone/m^3 and 169 μg ozone/m^3, in which the delay of $^{14}CO_2$ respiration during the first 5 d was proportional to the concentration of ozone (A. Gorissen, J. A. van Veen, unpublished data).

An intriguing matter is the mechanism and the extent of the apparent recovery in $^{14}CO_2$ root/soil-respiration of the exposed trees when they are removed from the exposure rooms. Recovery after removal of the cause is not an adaptation; but this does not exclude occurrence of adaptation in the field.

It is unknown how an air pollutant causes delay in translocation of photosynthates. Fixation and translocation of carbon in plants depend on many processes (28). Several of these processes might be influenced by the action of air pollutants, which may result in an enhanced storage of assimilates, an increased usage for repairs and a decreased translocation.

Recent studies showed a retarded hydrolysis of starch in needles of trees exposed to ozone, which indicates an enhanced storage of photosynthetic products (30). This agrees with the observed increase of shootweight of pumpkin after exposure to 400 μg ozone/m^3 (25). An enhanced storage of assimilates can also explain our findings. During the labeling period $^{14}CO_2$ was either stored as starch (to a larger extent in the exposed trees), or diluted in the existing pool of unlabeled carbohydrates. Both would delay transport of ^{14}C-assimilates.

Physiological activity of the sink has been mentioned to be the main factor controlling translocation of photosynthates (28). The undisturbed total (labeled and unlabeled) CO_2 root/soil respiration indicates that the pollutants did not directly affect the activity of the roots and the microflora; carbon supply was still sufficient at this stage.

Inhibition of transport of photosynthetic products would not only affect the roots but also the rhizosphere microflora, in particular mycorrhiza-fungi, which largely depend upon the supply of carbohydrates by the roots (11, 21). It has been reported that the occurrence of mycorrhizae has been altered during the last thirty years both in composition and in size (1). This decrease may well be related to the increasing concentrations of air pollutants and the acidification of the soil (19). Since mycorrhizae are often essential for the supply of nutrients and water to the trees their less frequent occurrence has also been mentioned as an important factor for the forest die-back in Europe (27). Whether a disturbed translocation of photosynthates as shown in the present study is directly related to the above-mentioned phenomena has still to be proven. Extrapolation of the results to older, probably more vulnerable, trees under field conditions should be done with great care.

When disturbances of translocation do occur at low concentration levels it seems that these are rather small. However, even these small disturbances, occurring repeatedly during periods of air pollution, when integrated over the entire life of a tree, will have a significant impact on the vitality of a tree.

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